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Production of silver nanoparticles from the aerobic and anaerobic cultures supernatants of *Pseudomonas aeruginosa*

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Abstract

This study was designed to produce silver nanoparticles from the supernatant of *Pseudomonas aeruginosa* cultures propagated under aerobic and anaerobic conditions. Silver nitrate solution at concentration 100 mM was added to the supernatants, then thermal treatment at 80 ºC for 4 h. The heated supernatants were then burned at 600 ºC for 3h. Silver nanoparticles products were characterized using qualitative and quantitative tests. Morphological observation of the supernatants color change was made after adding silver nitrate and heating treatment.. The color of supernatant was changed into dark brown and light brown for each aerobic and anaerobic cultures supernatants, respectively. Quantitative tests included the analytical measurements for determination the λ_{max} for aerobic and anaerobic supernatants after heating treatment in range of 300-500 nm to record the silver nanoparticles production and two peaks were obtained at 390 and 420nm, respectively. FTIR analysis schemes of the supernatants after thermal treatment at 80 ºC showed differences in composition of active compounds since more groups in the supernatant of aerobic supernatant were noted. No specific active groups were observed after burning the samples. Scanning electron microscopy (SEM) images of burned supernatants were conducted and revealed the presence of small nanoparticles for aerobic treatment in comparison with anaerobes. The results of atomic force microscopy(AFM) showed that aerobic the treatment was produced a silver nanoparticles of approximate diameters of 20-50nm compared with anaerobic treatment (50-100nm).

Keywords: Characterization, Pseudomonas aeruginosa; Sliver nanoparticles; Supernatant.

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Introduction

Metal reactions by microorganisms have an important function in many biotechnological applications including the fields of biodegradation of anthrapogenic pollutants, biomining, and metal solubilization . at persent time, a few microorganisms genra have been used as biofactories for synthesis of different metallonanoparticles such as semiconductors cadmium sulfide, gold, and silver nanoparticles[1]. The problems with the chemisorption and physisorption methods for the manufacturing of nanosilver are that the process is costly and the nanoparticles may be toxic product. To ovoid these problems, development of a safe green synthesis operation for the synthesis of nanomaterials is an important feature of current nanobiotechnology research[2,3]. Biosynthesis of nanomaterials has received considerable attention due to the growing need to develop safe technologies of environmentally friend material synthesis. For example, a potential effort has been utilized into the synthesis of inorganic materials, especially metal nanoparticles by using microorganisms. Both whole live and dead microbes are gaining importance by their ability to facile assembly of nanoparticles. Furthermore, the problem, about the synthesis of nanoparticles and their uniformly stabilization can be resolved in natural conditions. Research in nanobiotechnology provides trusty and eco-friendly methodology for the synthesis of nanomaterial[1]. Inspiration from around nature comes through the activities magnetotactic microorganisms synthesizing magnetite nanoparticles (Fe_3O_4), diatoms synthesizing siliceous materials and sulfide-layer bacteria producing gypsum ($CaSO_4$) and calcium carbonate ($CaCO_3$) layers. It has been shown that the synthesis of silver nanoparticles by microorganisms, such as bacteria and fungi, now play an important role in the detoxification of toxic metals through the mineralization of the metal ions[3].

The product of sliver nanoparticle with light brown color in solution refer to the formation of silver nanoparticles within the range of 100 nm[4]. So, it was evident that the excretion of metabolites from bacteria exposed to silver ions could be play as reducing agent and reduced these ions to the silver nanoparticles. This is clearly indicating that the reduction of the ions is conducted extracellularly through reducing agents released into the solution by *E. coli*. Silver cations are very active and tend to bind strongly to electron donor groups and this indicated that *E. coli* can synthesize silver nanoparticles extracellularly[5].Many groups of microbes are known to reduce the silver ions to the spherical silver nanoparticles[6]. Klaus and coworkers have shown that the *Pseudomonas stutzeri* AG259, when placed in a aqueous solution of concentrated AgNO3, played a major role in the reduction of the silver ions and the formation of AgNPs of uniform size and shape within the periplasmic space of the bacteria[7]. Silver nanoparticles were synthesized by many types of fungi in the form of a film or produced in solution or accumulated on the surface of the cells[8]. Xia *et al*. (2023)[9] used *P. aeruginosa*, for biosynthesis of silver nanoparticles. A part from standardizing the best parameter for the synthesis of silver nanoparticles, efforts were directed towards assessing the reducing agent involved in reduction of silver ion to silver nanoparticles. The involvement of nitrate reductase as a reducing agent was confirmed by biochemical assay[10]. Caio *et al*. (2018)[3] confirmed the ability of bacteria to form AgNPs within 24 h. Sliver nanoparticles synthesis were characterized by analytical techniques UV-visible spectroscopy and XRD, and imaging techniques SEM and AFM. UV-Visible absorption scan of supernatant injected with silver nitrate revealed a peak at 450 nm indicative of the surface plasmon resonance (SPR) of silver nanoparticles. TEM image revealed the formation of well-defined AgNPs of 20-50 nm[3].

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Materials and Methods

Microorganisms: *P. aeruginosa* isolate was kindly supplied from the microbial bank, College of Biotechnology, Al-Nahrian University .The isolate was re-purified by activation in nutrient broth and streaking on nutrient agar. The pure colonies were confirmed by gram stain and light microscope. The isolate was maintained in slant and plates of nutrient agar inside refrigerator.

Preparation of inoculum and culturing: Pure and active colonies *P. aeruginosa* isolate from nutrient agar culture of 24h incubation period at 37 ˚C was used for preparation the inoculum. Five colonies were resuspended in 5ml of sterile normal saline (0.85%) in sterile screw cupped tube. Two ml of suspension were used to inoculate 150 mL nutrient broth prepared in 250 mL conical flask. The first treatment was incubated under aerobic conditions (150 rpm at 37 ˚C for 24 h) and anaerobic conditions in anaerobic jar with gas generating kit at 37 ˚C in static incubator.

Growth conditions: . The conditions of incubation were 37 ˚C for 24 h for all the experiments. Aerobic growth was conducted in a shaking incubator at 120 rpm and the anaerobic growth in anaerobic jar supplied with gas generating kit.

Synthesis of silver nanoparticles: After incubation period, the cultures of *P. aeruginosa* were centrifuged at 8000 rpm for 15min under cooling conditions. The supernatants were transferred to sterile conical flasks. Ten mL of 100mM of silver nitrate was injected by sterile syringe to each supernatant. The flasks were exposed to heating at 80 ˚C for 4h. After cooling, the solutions were distributed in ceramic containers and burned in furnace oven at 600˚C for 3h.The deposit was harvested in sterile dried screw cupped tubes.

Characterization of silver nanoparticles: Qualitative tests were performed by morphological observation of the color change of aerobic and anaerobic supernatants after mixing with silver nitrate and heating treatment. Analytical analysis of heated supernatants was conducted in UV-Visible scanning spectrophotometer (Buck, USA) and FTIR (Testscan Shimadzu FTIR 8000 series). Imaging topography was achieved for burned samples using AFM (Angstrom advanced AA2000) and SEM (INSPECT S50, UK).

Results and Discussion

Cultures of *P. aeruginosa*: Fig.1 shows the growth of *P. aeruginosa* bacterium under aerobic and anaerobic conditions. Aerobic growth was heavier than anaerobic growth because the metabolism activities were restricted under anaerobic condition.

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Aerobic growth **Anaerobic growth**

Figure 1. Cultures of *P. aeruginosa* growth under aerobic and anaerobic conditions.

Characterization of silver nanoparticles: Fig.2 shows Morphological observation of the supernatants of aerobic and anaerobic cultures of *P. aeruginosa* after adding silver nitrate and heating treatment was showed in figure 2. The color was changed into dark brown for aerobic supernatant in comparable with light brown for anaerobic supernatant. This observation confirmed the ability of aerobic supernatant to produce small size and high quantity of AgNPs[11].

Figure 2. Morphological observation of silver nanoparticle production by *P. aeruginosa* supernatant under aerobic (left flask) and anaerobic (wright) conditions.

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UV-Visible analytical test: Fig. 3 illustrates the scanning spectrum of the synthesized silver nanoparticles by the supernatant of aerobic and anaerobic growth of *P. aeruginosa*. The results indicated to the synthesis of silver nanoparticles in a range of 20 -50 nm in diameter according to the peak of absorption at 390 nm[12]. The peak of AgNPs absorbance realized between 400 -410 nm and this range may be indicating to size of nanoparticles between 50 – 100 nm or more[13]. The formation of silver nanoparticles was conducted by reducing agents present in the supernatant which were produced by the viable cells or from the analyzed cells in the culture in addition to the components of medium. These reducing agents are in a large quantity in aerobic supernatant compared with anaerobic culture. The reducing activity was enhanced by heating treatment.

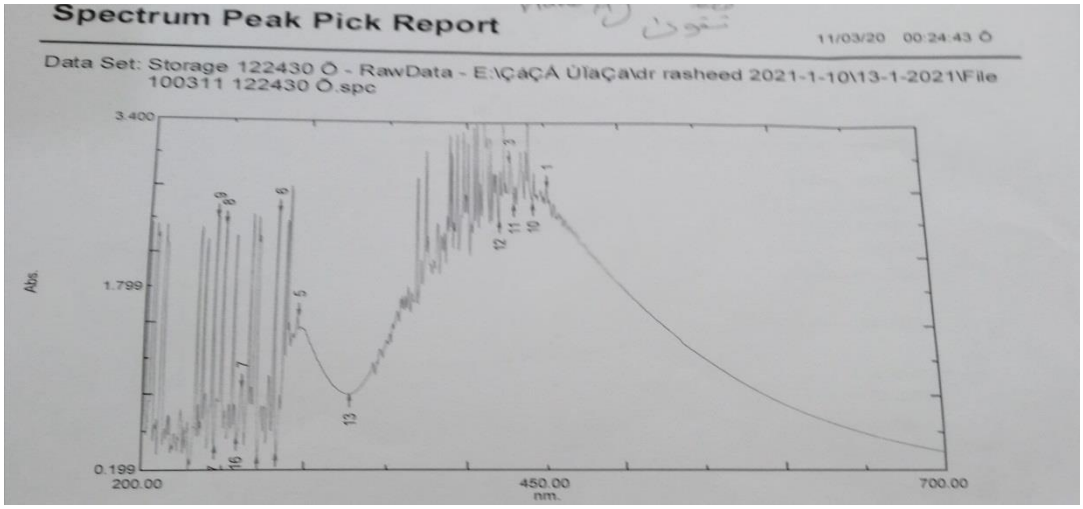

Figure 3. Scanning spectrum of synthesized silver nanoparticle by aerobic and anaerobic supernatants of *P. aeruginosa* cultures.

FTIR scanning: Fig. 4 (a,b) shows FTIR analysis of aerobic and anaerobic supernatants after heating 80 ºC. It is clear that the supernatant of aerobic culture contains higher active groups in comparison with anaerobic culture and this returned to the aerobic nature of bacteria which under aerobic respiration enhanced all metabolism pathways for active growth and biomass production, while under anaerobic conditions, the bacterium used only the oxidative fermentation pathways, so the external bioproducts will be reduced.

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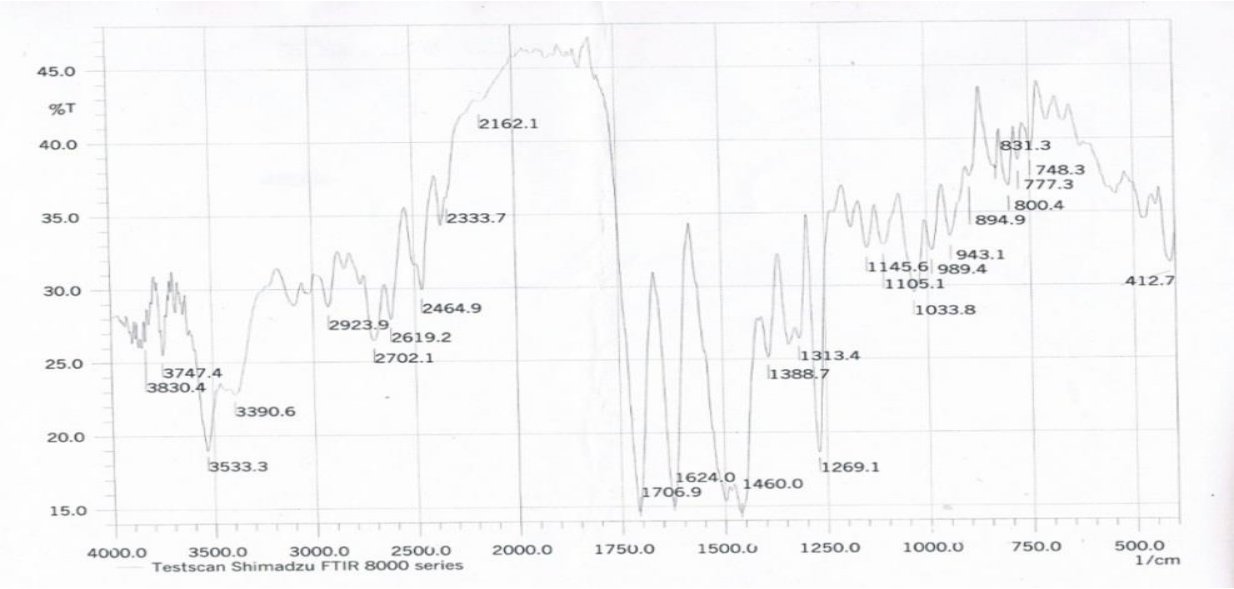
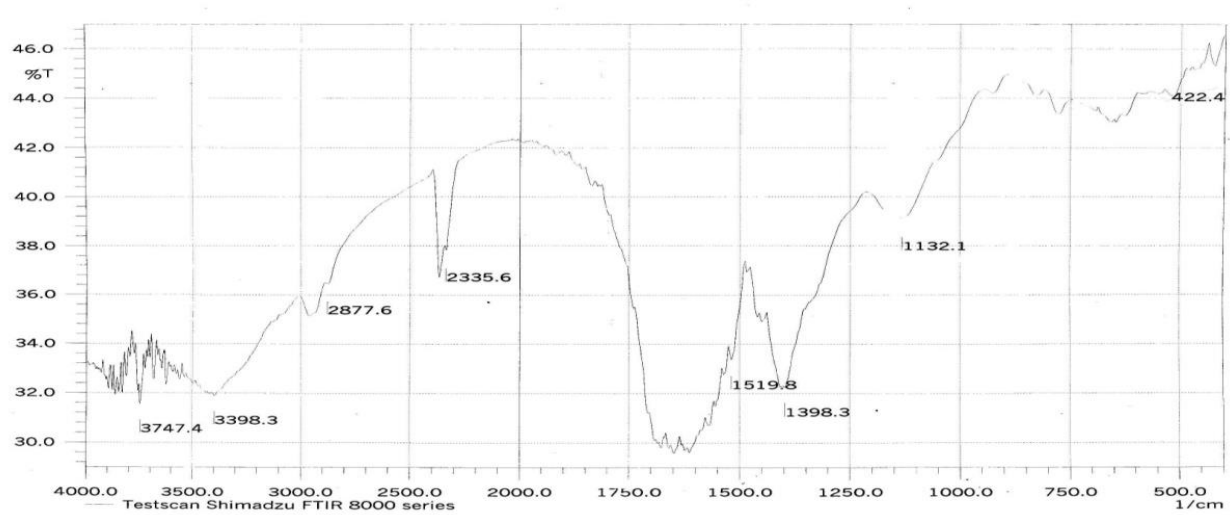


a: Aerobic



b: Anaerobic

Figure 4a, b. FTIR analysis of aerobic (a) and anaerobic (b) production of AgNPs by culture supernatants of *P. Aeruginosa*..

SEM and AFM images: Fig. 5 shows the SEM images of AgNPs of burned samples. It is clear that the aerobic supernatant (A) have a potential effect in synthesis uniform and small size production of AgNPs

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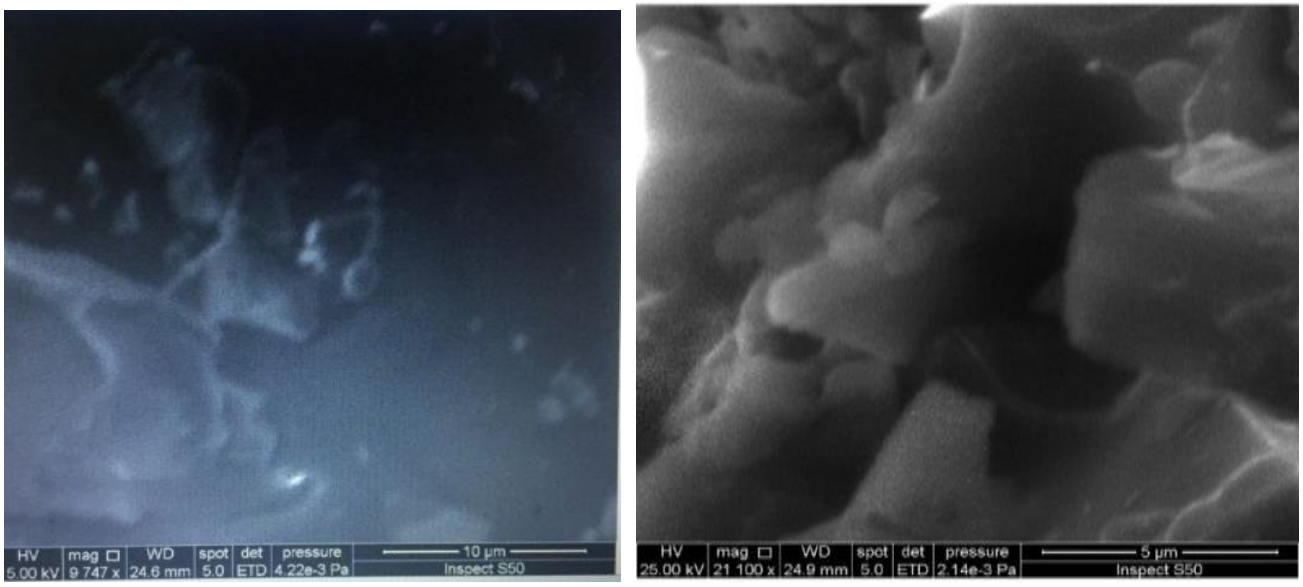
compared with anaerobic supernatant (B). AFM imaging included approximate size measurement confirmed the results of scanning UV-Vis. spectroscope in fig. 3.



Aerobic **Anaerobic**

Figure 5: SEM images of silver nanoparticles in the burned supernatants of *P. aeruginosa* culture.

Atomic force microscopy (AFM): Fig. 6 (a,b) shows the average diameter for AgNPs was 62nm.

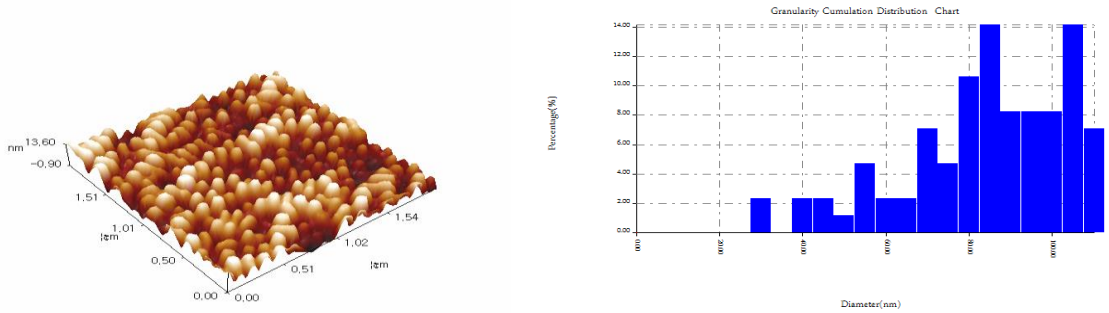


Figure 6 (a, b). AFM imaging and nanoparticles distribution curve of AgNPs prepared under aerobic conditions.

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X-Ray Diffraction (XRD) analysis: Figure 7, shows XRD analysis of silver nanoparticles synthesized by aerobic supernatant of *P. aeruginosa* culture. The unique peak is fingerprint

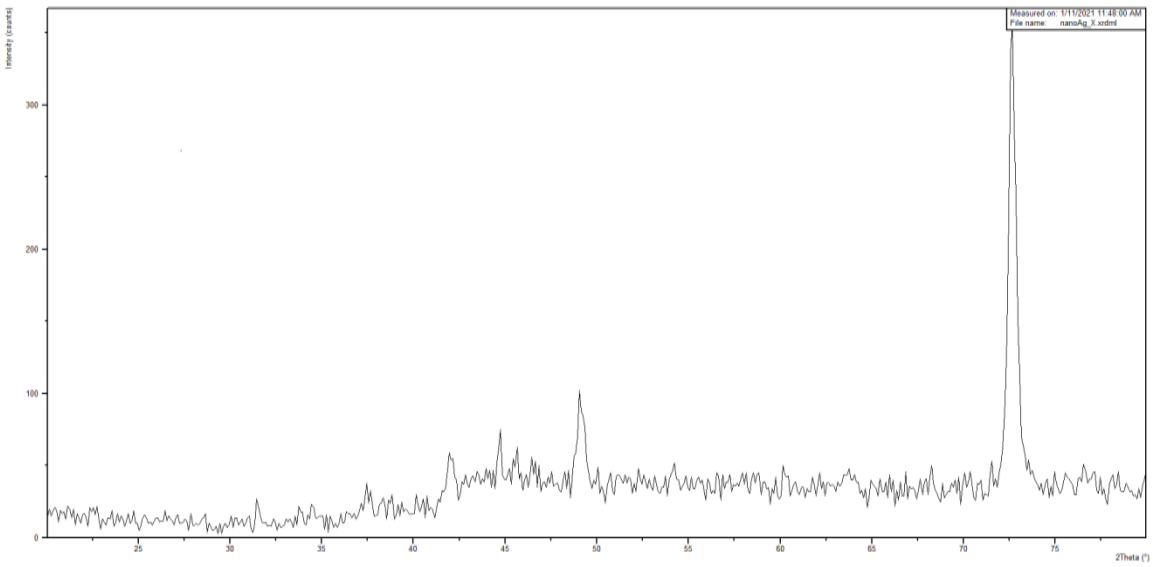

Figure 7: XRD scanning of silver nanoparticles synthesized by *P. aeruginosa* supernatant under aerobic condition.

Conclusions

Ability of production a safely nanosilver particles by using the supernatants of *Pseudomonas aeruginosa* of aerobic and anaerobic culture conditions. Aerobic culture produced small size and large quantity of nanosilver.

Acknowledgments

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Author's Declaration

- We hereby confirm that all the Figures and Tables in the manuscript are original and have been created by us.
- We have obtained ethical clearance for our study from the local ethical committee at [Al-Nahrain University/College of Biotechnology]. This approval underscores our commitment to ethical research practices and the well-being of our participants.

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- Ethical Clearance: The project was approved by the local ethical committee at [Al-Nahrain University/College of Biotechnology], ensuring adherence to ethical standards and the protection of participants' rights and welfare.

Author's Contribution Statement

[First Author]: Contributed to the conception and design of the study, conducted some experiments, data rearrangement and drafted the initial manuscript.

[Second Author]: conducted some experiments, collection a part of literature review and conducted some characteristics of the products.

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