ABSTRACT

The biosynthesis of nanoparticles by using microorganisms is developing as an ecofriendly method for nanoparticle synthesis because of its cheap, simple and non-toxic. Bacillus sp. can be used for producing iron oxide nanoparticles. In addition, it has the ability for the biosynthesis of Fe$_3$O$_4$ nanoparticles. The nanoparticles producing was evaluated by using ultra violate-visible (UV-visible) and Fourier-transform infrared spectroscopy (FT-IR) methods also the production and size of the nanoparticle was confirmed by X-ray Diffraction and Field Emission Scanning Electron Microscope (FESEM) to confirm the accuracy of iron oxide nanoparticles. pH, Temperature, and Incubation time of production of iron oxide nano-particle also studied.

Keywords: Bacteria, Drug delivery, Iron oxide, Nanoparticles.

INTRODUCTION

Nanotechnology is a multi-disciplinary kind of science that covers many areas of scientific techniques, like biomedical, pharmaceutical, agricultural, environmental, materials, general chemistry, general physics, electronics, data sciences and technology, etc.\textsuperscript{1-4} Nanotechnology is become applied now in the pharmaceutical industry, medicine, electronics, robotics, and tissue engineering. The usage of nanomaterials in the enhancement of delivery systems for various molecules, like DNA, RNA, plasmids, and proteins it is very important today and has been considered widely throughout the last years.\textsuperscript{2} Nanoparticles have been used to deliver drugs to target tissues and to increase stability against degradation by enzymes.\textsuperscript{3} Their exclusive size-dependent properties make these materials indispensable and superior in many areas of human activities.\textsuperscript{3,4} Green synthesis methods are eco-friendly approaches and compatible with pharmaceutical and other biomedical applications, as the toxic chemicals are not used in these methods.\textsuperscript{5} Iron oxide nanoparticles are most suitable for biomedical applications due to their proven biocompatibility. These particles have an ability to interact with various biological molecules in different ways due to their superparamagnetic properties, high specific area and wide choice of surface functionalization.\textsuperscript{6} The potential of drug delivery systems based on the use of nano- and microparticles stems from significant advantages such as;\textsuperscript{7,8} The ability to target specific locations in the body. The reduction of the drug quantity needed to attain a particular concentration approximately the target. The reduction of the concentration of the drug at non-target sites minimizing severe side effects. Living microorganisms, especially Bacillus sp. have a remarkable ability to form exquisite inorganic structures often in nano-dimensions. The development of these eco-friendly methods for the fabrication of nanoparticles is developing into an important division of nanotechnology, especially iron oxide nanoparticles.\textsuperscript{7,9} Microbes play direct or indirect roles in several biological activities. So use them in the biosynthesis of nanoparticles is a more demanding approach for the bio-production of nanoparticles via a highly stable, eco-friendly process with no toxic chemical and large scale production.\textsuperscript{10} Our study aims to investigate and detect iron oxide nanoparticles produced by Bacillus sp. to Sp. Bacteria.

METHODOLOGY

Bacillus Identification

Large gram-positive rods, often in pairs or chains with rounded or square ends (which may have a single endospore). Some species may be Gram variable. The identification was done by using spore stain method, which used to stain the spores of Bacillus species. Spores were in light green, and vegetative cell walls were pick up the counterstain safranin. The media used with conditions were blood agar incubated in air/CO$_2$ at 35°C-37°C for 24 – 48-hour.\textsuperscript{11}
A Study of Iron Oxide Nanoparticles Synthesis by Using Bacteria

Preparation of Supernatant Solution of *B. subtilis* 
A nutrient broth medium was prepared by dissolving 30 gm of nutrient broth in one liter of distilled water (D.W.). After that, it was put in the autoclave at 110°C with one atm for 20 min. then cooled to room temperature. Next, Freshly *B. subtilis* were grown on nutrient broth medium and left for one day at 37°C. Finally, the fresh grownup cells were centrifuged at 6000 rpm for 12 min. to be removed, then supernatant solution collected for preparation of nanoparticles.

Biosynthesis of Iron Oxide Nanoparticles
The supernatant of *B. subtilis* was used as an aqueous solution of 2 mM FeCl₃ was added of *B. subtilis* supernatant solution by (1:1) addition in a 250 mL Erlenmeyer flask (pH adjusted to 9). The solution was incubated at 35°C (200 rpm) for 48 hours (in dark condition). UV-visible spectroscopy (UV-Vis) in the range of 200–1,100 nm was made in a biotech spectrophotometer to assess the Fe₃O₄ formation.

Temperature Effect on Iron Oxide Nanoparticle Production
To study the influence of temperature on the nanoparticle production, five different temperatures (25, 30, 35, 40 and 45°C) was approved. For these conditions, the pH was constant at nine, the incubation time dated kept as 48 hours with rotation system 200 rpm. At last, the absorbance was read at 258 nm.

pH Effect on Iron Oxide Nanoparticle Production
To study the influence of pH on the nanoparticle production, seven different pH value (1, 3, 5, 7, 9, 11, and 13) was approved. For these conditions, the temperature was constant at 35°C, the incubation time dated kept as 48 hours with rotation system 200 rpm. At last, the absorbance was read at 258 nm.

Incubation Time Effect on Iron Oxide Nanoparticle Production
To study the influence of incubation time of the reaction on the nanoparticle production, five different periods (24, 48, 72, 96, and 120 hours) was approved. For these conditions, the temperature was constant at 35°C, and the pH value kept as nine with a rotation system 200 rpm. At last, the absorbance was read at 258 nm.

Characterization of the synthesized nanoparticles
Scanning electron microscopy was used to study the size, morphology, and composition of the nanoparticles. In addition, X-ray Diffraction was used to perform the chemical formula and the particle size of the nanoparticles by using Sherrer’s equation, \( D = \frac{k\lambda}{\beta \cos \theta} \) where D is the average crystallite size, k is an arithmetical factor, \( \lambda \) is the X-ray wavelength, \( \theta \) is the Bragg angle, \( \beta \) is the line broadening in radiation. The FT-IR spectra was used before and after the formation of Fe₃O₄ for the description of nanoparticles, and they were ordered at a range between 400 – 4,000 cm⁻¹.

RESULTS AND DISCUSSION

Bacteria Identification
The *B. subtilis* group are closely related and are not easily distinguishable. Cells of these organisms are less than 1μm wide, sporangia are not swollen, and spores are ellipsoidal. They are in general mesophilic with regard to temperature and neutrophilic with respect to pH for growth, while often being tolerant to higher pH levels. All species were differentiated on the genetic level and it is to be expected that when genotypic analyses are applied to a wider range of strains of the classical species mentioned above, additional genospecies will be detected. The colonial appearance in blood agar media was large (2–7mm) with a frosted-glass appearance. Variable colonial morphology foe some species was produced mucoid, smooth, and some raised wrinkly colonies.

Biosynthesis of Iron Oxide Nanoparticles
The ferric chloride solution was added to the *B. subtilis* supernatant. The color-changing (Figure 1) was confirmed the production of Fe₃O₄ nanoparticles. Where FeCl₃ was dark green in color while Fe₂O₃ was black-brown in color.

The biosynthesized iron oxide nanoparticles band was indicated by using the UV–Visible scanning (Figure 2) at 258 nm. That refers to the dispersion of particles in the aqueous solution.

Temperature Effect on Iron Oxide Nanoparticle Production
Figure 3 shows that the temperature influence on the rate of nanoparticles production were temperature less than 35°C reduced the formation of nanoparticles and this effect is increased with increasing the temperature than 35°C within a period time of 48 hours. this may be because of the deactivation or squalor of molecules accountable for the reduction of Fe³⁺. This is contentious to the synthesis of nanoparticles by chemical methods where rising the temperature of the reaction improved the rate of iron oxide nanoparticles formation.

![Figure 1: color change before (A) and after (B) the production of nanoparticles](image1)

![Figure 2: Absorption spectra of iron oxide nanoparticles](image2)
A Study of Iron Oxide Nanoparticles Synthesis by Using Bacteria

pH Effect on Iron Oxide Nanoparticle Production
The result of (Figure 4) shows the optimum pH for the production of iron oxide NPs is approximately nine, more than nine, an abrupt decrease in amount formation observed. Reach to 12 and more the formation was very low. This decline might be because of the absence or defect of the molecules or enzymes responsible for the formation of Fe$^{2+}$, ferric oxide might be formed, and this formation increased with pH increasing. The generation of the Fe$_3$O$_4$ nuclei occur simply at the medium pH is inferior to eleven, though the growth of the Fe$_3$O$_4$ NPs happens more definitely at the medium pH is upper than 11.$^{17}$

Incubation Time Effect on Iron Oxide Nanoparticle Production
The finding of the study referred (Figure 5) that the optimum incubation time for iron oxide NPs formation was approximately 48 hours. Subsequently, a decrease for the formation of NPs was noticed. Where less than that time, there was no enough amount of Fe$^{3+}$ available for NPs formation. While incubation time more than 48 hours there were enough yield of iron oxide NPs.$^{18}$

Characterization of the Synthesized Nanoparticles
The result of the FESEM image of iron oxide NPs. Where this image appears the delivery of iron oxide NPs with groups. In addition, the FESEM image has approved the formation of iron oxide NPs by Bacillus sp. The morphology of NPs was spherical with size ranged between (3.6 ~ 7) nm. Also, from image noticed that the NPs with high stability by a coating means. This coating might be by proteins other molecules generated by Bacillus sp.$^{19}$

In Figure (7,8) illustrated the FT-IR spectrum of Fe$_3$O$_4$ NPs synthesized by bacteria that determined by the band between 800–400 cm$^{-1}$ for iron oxide, in comparison with Figure 7 of FeCl$_3$ FT-IR spectrum, the strong band between 800 – 400 cm$^{-1}$ are referred to Fe-O bond stretching vibration in iron oxide. In addition, the specific vibrations for the Fe-O
bonds are allocated at 623, and 582 cm$^{-1}$ where these bands are disappearing in the spectrum of FeCl$_3$ also the band at 1585 for Fe-Cl stretching vibration is missing in iron oxide spectrum.$^{20}$

X-ray diffraction image in Figure 9 appears the biosynthesized of iron oxide NPs where the series of distinguishing peaks at 20 and Bragg reflection, as mentioned in Table 1, which comparable with pattern references magnitude of XRD infers that the biosynthesized of iron oxide NPs are cubic back in creation. In addition, the average particle size of iron oxide NPs was calculated by using Sherrer’s equation ($D = kλ/ B \cos θ$) and is about 18.17 nm, as shown in Table 1.$^{19}$

**IN CONCLUSION**

The freshly *Bacillus* *sp.* was used to generation iron oxide NPs where this bacterium has the capability to biosynthesized of Fe$_3$O$_4$ NPs. The extracellular excretion of enzymes or minor metabolites arbitrates the biosynthesis of iron oxide NPs with a faster rate and slight toxicity. The presence of O-H was depicted by FTIR spectrum, which may work as stabilizers by link the molecules onto the functional forms. The formation of iron oxide NPs was confirmed by SEM analysis, and particle size ranged between (12–32) nm that supported by XRD pattern with average particle size 11.94 nm, which also authorizes the morphology of NPs to be cubic back in creation.

**REFERENCES**

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