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Yeast Mediated Synthesis of Iron Oxide Nano Particles: Its Characterization and Evaluation of Antibacterial Activity

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Abstract— *Production of nanoparticles by using microbes are a new area of interest. Saccharomyces Cerevisiae (baker's yeast) was allowed for the biosynthesis of iron nanoparticles in a culture media. Myco-synthesis of iron nanoparticles is considered to be more straight forward and easy for the stable production of NPs as compared to bacteria. Fungi have several advantages over bacteria showing (i) higher biomass and easy mode of culture, (ii) higher bioaccumulation of metabolites (iii) higher tolerance to, and uptake capability of metals. Present paper discussion "Biosynthesis, characterization and evaluation of iron nanoparticles by yeast cells". It was carried out by culturing the yeast powder and the yeast obtained from the potato water on PDA (potato dextrose agar media) and incubated for 1-2 days. After incubation, and subcultured on PDA slants under sterile conditions. Inoculum was prepared by transferring the cells from PDA slants into 250 ml flask containing a mixture of ferrous sulphate (100mM) and ferric chloride (0.001). Four different culture media was prepared in four different flasks. The flasks is then kept in the rotary shaker at 30 °C at 120RPM for 2-3days. End of the synthesis the cells and debris were removed by centrifugation/filtration. The residue was collected and dried to get the dry powder of iron nanoparticles. Progressive results and maximum yield of iron nanoparticles obtained with Trial-II. Further the biosynthesized Iron nano particles were characterized by analytical techniques like UV-Visible Spectrophotemetry, FT-IR Spectroscopy and SEM studies. The UV-Visible absorption spectrum showed an absorption maxima at 249 nm which is a characterstic of iron nanoparticles. The FT-IR spectrum of iron nanoparticles showed absorption peaks ranging between 3439.19 cm^{-1} to 542.02 cm^{-1} range. SEM images showed 155.7 nm of spherical shape. Iron nanoparticles showed anti-microbial activity against both gram –ve , gram +ve bacteria, but Iron nanoparticles showed more activity against the Bacillus sps than E.coli.*

I. INTRODUCTION

Nanotechnology is an innovative field which influences all aspects of human life. Nanoparticles in a variety of majors such as "nanomedicine", they are paid so much of attention in this field. Nanoparticles are widely classified into two types of organic and inorganic ones. While organic nanoparticles [1] consists of carbon nanoparticles, a number of inorganic nanoparticles contain magnetic nano particles (iron nanoparticles), the noble metal nanoparticles (like gold and silver) and semiconductors nanoparticles like (titanium oxide and zinc oxide). Since inorganic nanoparticles are recently utilized as catalysts, semiconductors, optical devices, biosensors, encapsulation of drugs and contrast agents. In addition, inorganic nanoparticles biomass nanoparticles i.e., of noble metal nanoparticles catering for better quality material properties with functional flexibility, there is an increasing interest in their formation.[2]

The majority of the chemical and physical techniques employed for the synthesis of the nanoparticles are very costly. Moreover, it contains the poisonous and dangerous chemicals responsible for the different biological hazards,[3] This matter increases the necessity of developing environmentally friendly procedure by means of biological methods. For the synthesis of metallic nanoparticles, living extracts have been utilized by the researchers. They followed easy process such as the procedures of reducing the metal ions. In doing so, they made use of biomass as a basis of extracellular or intracellular reductants. Nanoparticles possess significant and antimicrobial properties and thus it has immense efficacy in the management of various diseases.[4]

It was reported Bacteria and Fungi mediated synthesis of gold, silver and iron nano particles in several studies. But very less work was attempted for synthesis of yeast mediated iron nano particles, its characterization, assessment of antibacterial, anticancer properties.[5] Literature has reported investigations on biosynthesis of iron oxide magnetic nano particles using clinically isolated pathogens, by endophytic fungi and some other species. Present research paper discussed about promising approaches of yeast mediated iron oxide nano particles at laboratory conditions using yeast cells and by different chemical constituents.[6] The present work is extended to study optimization methods, characterization of synthesized iron nano particles by analytical techniques like UV-Visible spectroscopy, identification of functional groups by FT-IR and particle size determination by SEM. [7]

II. MATERIALS AND METHODS

2.1 Method of Preparation

Yeast cells were isolated in the microbiology laboratory by using yeast powder (Figure 1) and yeast obtained by the potato water as a source of yeast which were grown on the Potato dextrose agar media and incubated for 24hrs (Figure 2). After incubation, the yeast cells were transferred on to PDA slants under aseptic conditions. The yeast cells are inoculated into 250ml conical flask containing mixture of ferrous sulphate and ferric chloride solutions.[8] The constituents of the medium

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for preparing PDA slants was kept at pH=7.0 and incubated at 30^0Cfor at least three days. The slants preserved at 4^0C and subculture twice a week. (Figure 3)

Fig. 1. Stock culture of yeast by potato water


Fig. 2. Stock culture of yeast powder


Fig. 3. Sub cultured Yeast slants

2.2 Biosynthesis of Iron Nanoparticles

2.7801grams of Ferrous sulphate hepta hydrate was weighed and taken in a beaker containing 100ml of distilled water. (0.1M) .0.0162grams of anhydrous Ferric chloride was weighed and taken in another beaker containing 100ml of distilled water. (0.001M). [9] The Ferric chloride solution was transferred to the beaker containing Ferrous sulphate solution. Contents are stirred using sterile glass rod. The mixture was inoculated with freshly prepared cultures of yeast. It was incubated in rotary shaker at 30^0 C at 120 RPM for 2-3days. [10] The synthesis of iron nanoparticles was identified by appearance of brown color in the mixture. After filtration the biosynthesized nanoparticles were collected and air dried to get the dry powder of Iron nanoparticles. (Figure 4)


Fig. 4. Synthesized Iron oxide nanoparticles

2.3. Characterization of the Iron Oxide NPs

[11] Light Absorbance maxima of Iron nano particle was done using UV-Visible Spectrophotometer (Shimadju UV - 1800). The UV-Vis spectrophotometry analysis at different time intervals of the reaction provides key information on the success of generation of the NPs. Further, bond level characterization by Fourier-transform infrared (FTIR) spectroscopy of the Fe_2O_3-NPs was recorded on a 1000 FTIR instrument (Shimadu). [12] The size and shape of the as-synthesized Fe_2O_3-NPs were investigated using scanning electron microscopy (Jeol co.JP). The SEM analysis provides insight on the size and shape of the biosynthesized NPs.

2.4 Evaluation of Anti-microbial Activity

Agar well diffusion method [13] is the primary method to determine the anti-microbial activity of the nanomaterials. It is important to mention here that it is only suitable for the diffusive materials.[14] The agar well diffusion test is quantitative, easy to perform, and simple. The methodology includes the inoculation of the bacterial cells on nutrient agar petri dishes and after solidification and 6mm wells were made using the sterile borer and the test samples were poured into the well by under sterile conditions. In the first two wells, 30µL of Ofloxacin (100 mg/mL) for bacterial strains (*Bacillus subtilis,E.Coli*) and in the next well, 100µL of the prepared Fe_2O_3-NPs was inoculated. The plates were further incubated at 37˚C for 24 h in the incubator. The entire anti microbial study was done in triplicates.[15]

III. Results and Discussion

3.1 Identification and Characterization of Iron nanopaticles

Identification and assessment of Yeast mediated synthesis of Iron nano particles [16] were performed by Chemical and Analytical studies. Chemical tests were performed by solubility studies and synthesized Iron nano particles were characterized by UV-Visible spectroscopy, IR-spectroscopy and SEM analysis.[17]

3.2 Solubility Studies

Noted the solubility of iron nanoparticles in water, alcohol, acid and alkali by placing a small amount of sample in the test tube and adding few ml of solvent and warming if necessary. It showed a clear solubility in acid (0.1N HCl) and

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alkali (0.1N NaOH). It showed a poor solubility in alcohol. It showed insolubility in water. (Figure 5)

Fig. 5. Solubility tests

3.2. Characterization of Iron Oxide Nano Particles

3.2.1. UV-Vis Analysis. A UV spectrum of Iron oxide nanoparticles was recorded by scanning between 200-600nm [18]. From the spectrum, Iron nanoparticles showed maximum absorbance at 249nm. (Figure 6) The UV-Vis Analysis confirmed formation of Iron oxide nano particles.

3.2.2. FTIR Analysis. The FTIR analysis of the synthesized Iron oxide nano particles detected an outcome in the range of wavenumber 400-4000 cm^{-1} that ascertained the chemical bonds as well as functional groups. [19] The FTIR spectrum of the synthesized Fe_2O_3-NPs exhibited defined peaks at 542,559, 572, and 601 cm^{-1} C-H stretching. The appearance of two well-defined peaks at 517cm^{-1} and 621cm^{-1} was due to the vibrational intrinsic stretching of metal oxygen bond vibrations (here, it was Fe-O), which indicated that the developed NPs were iron oxide [20]. The presence of a slightly dwarf peak at 1022 cm^{-1} was due to Fe-O asymmetric stretching. The absorption peaks appeared at 3416 cm^{-1} and 3439 cm^{-1} indicating the bending vibration of absorbed water and surface hydroxyl (-OH) groups, which might be due to the use of NH_4OH in the synthesis of the Fe_2O_3NPs[21]. These findings were further corroborated from the previous report which confirms that the developed NPs were Iron oxide.(Figure 7)

Fig. 6. UV-Visible spectrum of Iron oxide nano particles

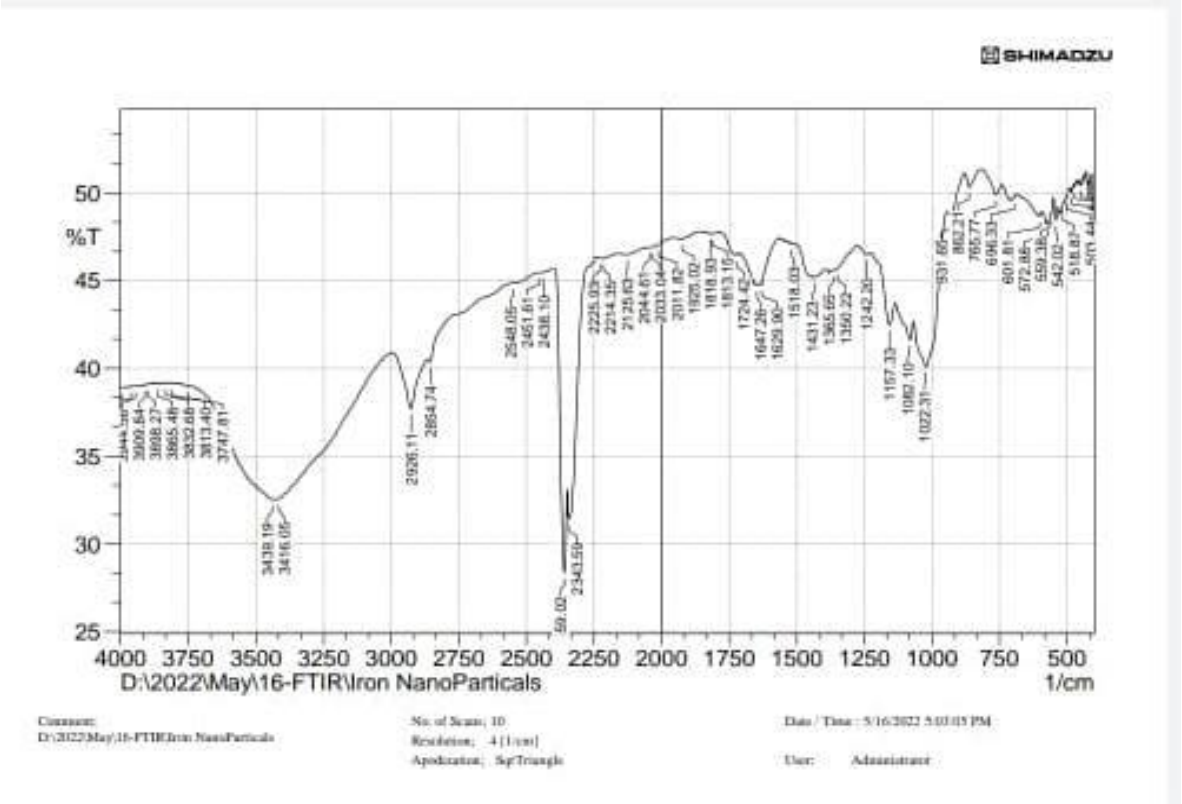

Fig. 7. FT-IR spectrum Iron oxide nano particles

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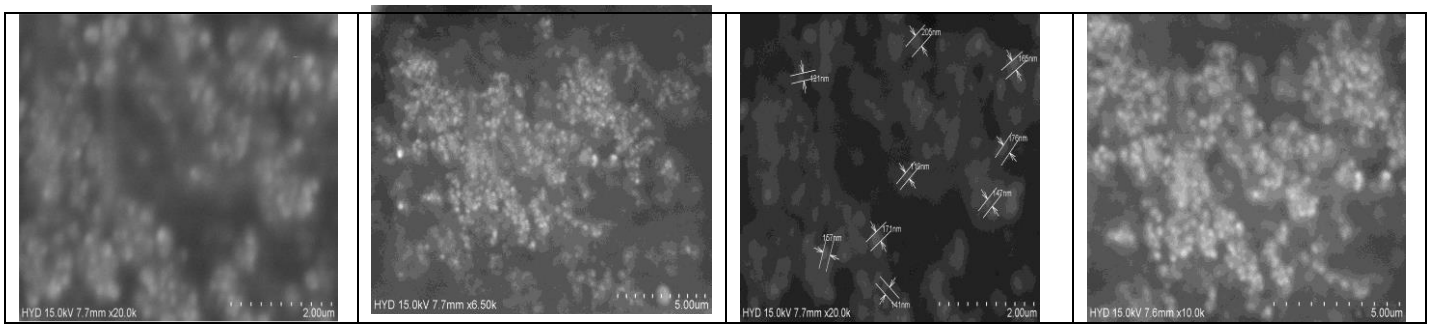

Fig. 8. SEM studies Iron oxide nano particles

Fig. 9. Anti microbial studies of Iron oxide nano particles (*Bacillus subtilis*)

Fig. 10. Anti microbial studies of Iron oxide nano particles *(E.coli*)

3.2.3. Morphological Characterization of Iron oxide nano particles by SEM Analysis. [22] The morphological characterization of the developed Fe_2O_3-NPs was performed by scanning electron microscopy. The SEM image demonstrates the formation of spherical shape Fe_2O_3-NPs, with an average size of ~70-100 nm. Mostly, the NPs were well dispersed, whereas some of these were slightly agglomerated.[23] The few large particles were found which could be due to the agglomeration of smaller particles around 150 nm, however, the majority of the particles in the SEM images were less than 100nm in size. (Figure)

3.4. Antimicrobial activity of the synthesized Iron oxide nano particles

[24] The antimicrobial activity was tested by agar well diffusion method. Compared to the standard Ofloxacin, Fe_2O_3-NPs have shown significant antimicrobial activity against Gram positive bacteria *B. subtilis*, with zone of inhibition (ZOI): 21 mm. (Figure 10) However, Fe_2O_3-NPs has showed less activity towards Gram negative bacteria *E. coli* with the zone of inhibition 18 mm.[25]

IV. Conclusion

The present study explored the ability of yeast in the synthesis of iron nanoparticles (Fe_2O_3-NPs). Bio-synthesis of iron nanoparticles employing yeast cells is an eco-friendly process for the synthesis of iron nanoparticles. Iron nanoparticles synthesized through the reduction of aqueous ferrous sulphate and ferric chloride in 1:2 ratio. The Fe_2O_3-NPs were characterized by UV-Visible spectroscopy, IR Spectroscopy and Scanning Electron Microscopy. In UV-Visible Spectroscopy the Fe_2O_3-NPs showed maximum absorbance at 249nm. The functional groups identified by IR Spectroscopy were 1^0amine(-NH), aldehyde stretching(-CHO), alkyne(C≡C), C-O stretching and C-H stretching. the average size of Fe_2O_3-NPs by Scanning Electron Microscopy was found to be 155.7nm but particles above this size were also found. Fe_2O_3-NPs showed anti-microbial activity against both Gram-positive and Gram-negative bacteria, but iron nanoparticles showed more anti-microbial activity to *B.subtilis* than *E. coli*. This eco-friendly bio method synthesis of Iron nanoparticle can potentially be applied in various products that directly come in contact with the human body, such as cosmetics, foods, and consumer goods, besides medical applications.

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