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Biosynthesis of Iron Oxide Nanoparticles by Cytoplasmic Extract of Bacteria *Lactobacillus Fermentum*

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ABSTRACT

During the past decade, the attention of science and technology has focused on production of nanoparticles. There are various ways for synthesizing nanoparticles that are not cost effective due to power and material consumption. Therefore, production of nanoparticles through biologic ways is needed. For this purpose, different biological structures such as plants, algae, and microorganisms such as bacteria, string molds, and yeasts are used for nanoparticles production. This study is focused on biosynthesis of iron oxide nanoparticles by cytoplasmic extracts of bacteria *Lactobacillus Fermentum,* which is a probiotic microorganism, based on the method of green chemistry. After preparation of cytoplasmic extract of bacteria *Lactobacillus Fermentum* through freez-thow method, iron sulfate solution (III) with a concentration of 10^{-3} M was added in an equal volume ratio (V/V% 10) and incubated for 3 weeks at 37 °C in the presence of 5% carbon dioxide. Production of nanoparticles was investigated by X-ray Diffraction (XRD) and Transmission Electron Microscopy (TEM). Changing the color of solution to black is an indication of iron sulfate nanoparticles production. The formation of iron oxide nano crystals by *Lactobacillus Fermentum* cytoplasmic extract was shown by XRD analysis. The average nanoparticles sizes as determined by transmission electron microscopy (TEM) were found to be about 10-15 nm with a spherical shape. Using *Lactobacillus fermentum* cytoplasmic extract can be considered as an efficient biological method for the production of iron oxide nanoparticles .In addition to be environmentally friendly, this method is cost effective.

1. Introduction

One of the main areas of research in nanotechnology is the use of different metal nanoparticles. Iron oxide nanoparticles are often produced in sizes smaller than 20 nm which have attracted researchers' attention as compared to other nanoparticles due to features like suitable magnetic properties and very low toxicity. Making of nanoparticles can be done by different ways in terms of cost, energy consumption, and environmental pollution which are not affordable. Hence, the desire to produce nano-sized particles based on the principles of green chemistry is growing day by day. For this purpose a variety of biological structures such as plants, algae, and microorganisms such as bacteria, molds, yeasts, and filamentous molds are used to prepare nanoparticles.[1]

Microorganisms due to their unique characteristics such as living under harsh conditions and environmental stresses, High metabolic diversity, high substrate specificity, faster growth; and along with advantages such as possibility of using them under relatively mild conditions of temperature, pH, and pressure; their proper function in two-phase systems consisting of water and organic solvents as appropriate biocatalysts; and as main workers of eco-friendly nano factories for production and assembly of nano-sized Particles containing metal are of great importance.[2-3] Microorganisms in the microbial method for the production of nano materials are used in small factories which are able to synthesize metal nanoparticles using their cheap and renewable reducing agents such as *Laktatyaastat* in sizes from 1 to 100 nm at room temperature or temperatures above thermophiles. Metal reduction by microorganisms is possible to be done both intracellular and extracellular. Reduction position of ions and consequently nanoparticle production is determined based on the kind of microorganisms and enzymes involved in the reduction process placed within or outside of the cell.[4] Therefore, this research in line with the objectives of green synthesis used the *Lactobacillus fermentum* cytoplasmic extract for synthesizing iron oxide nanoparticles.

2. Results and Discussion

The first sign of formation of iron oxide nanoparticles [Fe_3O_4] was incubated solution color change after three weeks. After reduction of iron ions by *Lactobacillus fermentum* cytoplasmic extract, color of iron sulfate solution changed from colorless to black.

XRD analysis of iron oxide nanoparticles [Fe_3O_4] has been shown in **Fig** 1. By examining the XRD graph and using Debye-Scherrer method, particles sizes were calculated 15nm. TEM sample images [Fe_3o_4] (**Fig 2.**) indicated that the nanoparticles were spherical in shape with average diameters of 10-15 nm which agreed with sizes calculated using XRD diagram. Various microorganisms capable of reducing metal ions are effective in biological production where we witness reduction of enzymes, extracellular polysaccharides, and reducing materials. One reason for the production of metal nanoparticles by microorganisms is reducing the toxic effects of metal ions in the growth environment of microbes. They do this action through the biological reduction of toxic metal ions [using a specific enzyme NADH reductase or nitrate reductase] into less toxic metallic elements. In addition, the presence of some polysaccharides and

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organic materials produced by microorganisms inside the cells, and culture mediums cause metal nanoparticles production.

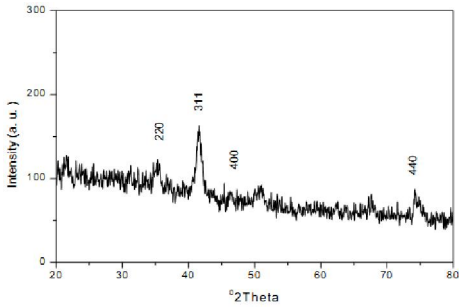

Figure 1. XRD spectrum of iron oxide nanoparticles [Fe_3o_4]: using Debye-Scherrer method, particles sizes were calculated 15-20 nm.

The biological materials, using functional groups, such as cysteine, histidine, aldehydes, and ketones reduce metal ions into metal nanoparticles.[7] Many studies have been done in this ground. At the study conducted by Omide and colleagues at 2014, silver nanoparticles were synthesized by *Lactobacillus Fermantom* supernatant which were reported to be very small, spherical, with high impressionability and average size of 15nm.[6] Prasad and Jaha in 2009 could synthesize zinc oxide nanoparticles by *Lactobacillus sprozhenz* .Results depicted that synthesized nanoparticles were hexagonal with a size of 5 to 15 nm.[8]

At the present study, iron oxide nanoparticles were successfully synthesized by *Lactobacillus Fermantom* cytoplasmic extract. In chemical methods, chemical materials were used to build and stabilize nanoparticles which are toxic and lead to produce byproducts that are incompatible with the environment. It also often leads to absorption of toxic substances on the surface of nanoparticles that may have deleterious effect on nanoparticle drugs. However, green synthesis is not only simple, low cost, and cost effective but also less toxic.[9]

So, synthesis of iron oxide nanoparticles using cytoplasmic extracts of *lactobacillus* fermentum, without using any chemical reducing and stabilizing agent, is an efficient, effective, and ecofriendly way for producing metal nanoparticles. It seems that presence of reduction-oxidation enzymes and other reducing compounds in the cytoplasmic extract of *Lactobacillus fermentm* causes the metal ion reduction and production of iron oxide nanoparticles.

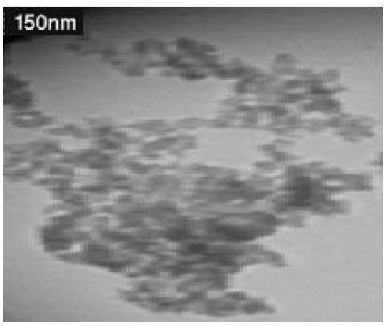

Fig 2. TEM image showed that the nanoparticles had average diameters of 10-15 nm.

The results also indicated that extracellular production of iron oxide nanoparticles is possible using *Lactobacillus fermentum.* Since in the extracellular production, metal ions on the surface of cells are trapped in which there is no need to extract the nanoparticles from inside the cells contrary to inside production. And this has led to the present method; even as compared to the intracellular production of nanoparticles which are also more affordable.[10] In green synthesis, more time is needed to produce iron oxide nanoparticles. It may be due to slow reactions done by enzymes placed in the biological compounds.

3. Conclusion

Green synthesis is recommended as the best replacement for chemical synthesis because of its immense benefits such as environmental compatibility, low cost and toxicity. On the other hand, *Lactobacilluess* are from the large family of probiotics, which are safe with no pathogenic effects that can be used for medical goals.

4. Experimental

4.1. Materials and methods

4.1.1. Purchase and cultivation of bacteria Lactobacillus fermentum

Lactobacillus fermentum strain with ID (PTCC 1638) was bought from microbial bank of Scientific and Industrial Research Organization of Iran. After moving in a MRS Broth medium was incubated for 24 hours at 37 ° C.

4.1.2. Preparing Lactobacillus fermentum cytoplasmic extract through freeze thaw method

After 24 incubation, the MRS Broth mediums containing the bacteria were centrifuged at 3000 g for 15 minutes. Then supernatant was discarded and the resulted sediment was added phosphate buffered saline and centrifuged at 3000 g for 10 min [washing step was repeated three times]. Afterwards, for 15 minutes was placed inside the nitrogen tank [liquid nitrogen -196 °C] and then in steam bath [Ben Murray] for 15 minutes [37 °C]. At the end after centrifuging at 12,000 g for 30 minutes, the supernatant was collected as the cytoplasmic extract.[5]

4.1.3. Nanoparticles synthesis by cytoplasmic extract of Lactobacillus fermentum

For synthesizing iron oxide nanoparticles, aqueous iron sulfate solution [10^{-3} M] was added to the *Lactobacillus fermentum* cytoplasmic extract in V/V% 10 volume ratio. After adjusting pH in 6/5 the solution was incubated in the darkness for 3 weeks at 37 °C and in the presence of 5% carbon dioxide.[6] After this period, extracellular accumulation of metal particles with ambient color change was observed. Changing color from colorless to black, indicated the production of iron oxide nanoparticles. After this time, the solution was poured into sterile Falcon tubes and centrifuged for 10 minutes at 2500 g. Then supernatant was discarded and the resulted sediment was washed twice with sterile deionized water and once with acetone. The resulted sediment was then placed in oven at 40 °C for 24 hours to be dried and was powdered using a porcelain mortar. The magnetic property of biosynthesized iron oxide nanoparticles was observed with a magnet.

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