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Iron Oxide Nanoparticle Biosynthesis using Lactobacillus Fermentum Bacterial Cytoplasmic Extract

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AB ST R ACT

The manufacturing of nanoparticles has occupied much of the scientific and technological community's attention during the last decade. Nanoparticle synthesis may be accomplished in a number of ways, however these methods are inefficient and expensive. Consequently, biologic methods for producing nanoparticles are required. Nanoparticles are manufactured using a variety of biological structures, including plants, algae, and microbes including bacteria, string molds, and yeasts. The aim of this research is to employ green chemistry as a basis for the manufacture of iron oxide nanoparticles using cytoplasmic extracts of the probiotic microbe Lactobacillus Fermentum. We incubated the bacterial cytoplasmic extract of Lactobacillus Fermentum for three weeks at 37 °C with 5% carbon dioxide after preparing it using the freez-thow technique. We then added an equal volume ratio of iron sulfate solution (III) at a concentration of 10-3 M. The use of X-ray diffraction (XRD) and transmission electron microscopy (TEM) allowed for the investigation of nanoparticle production. As soon as iron sulfate nanoparticles begin to form, the solution's color will change to black. The XRD examination demonstrated that the cytoplasmic extract of Lactobacillus Fermentum could generate nano crystals of iron oxide. According to the results of the transmission electron microscopy (TEM) analysis, the typical size of the nanoparticles was 10-15 nm, and they had a spherical form. When it comes to producing iron oxide nanoparticles, there is an efficient biological process that is both cost-effective and ecologically benign. This method involves using Lactobacillus fermentum cytoplasmic extract.

Keywords: Iron oxide nanoparticles Lactobacillus Fermentum Biosynthesis Probiotic

Introduction

Various metal nanoparticles are the subject of much study in nanotechnology. Researchers are particularly interested in iron oxide nanoparticles with diameters less than 20 nm because of their unique combination of desirable magnetic characteristics and very low toxicity compared to other types of nanoparticles. There are several methods for producing nanoparticles, however they all have prohibitive costs, high energy requirements, and harmful environmental impacts. Thus, there is an ever-increasing need to manufacture nano-sized particles according to green chemistry principles. To do this, nanoparticles are produced using a wide range of biological structures, including plants, algae, bacteria, molds, yeasts, and filamentous molds. 1Due to their diverse metabolic profiles, high substrate specificity, rapid growth rate, and ability to withstand extreme environments, microorganisms are ideal candidates for use as biocatalysts in two-phase systems containing organic solvents and water, as well as the primary employees in environmentally friendly nanofactories that manufacture and assemble nano-sized Things that have metal in them are very important. 2-3 Small factories employing microorganisms in the microbial approach to nanomaterial production can synthesize metal nanoparticles (ranging in size from 1 to 100 nm) at room temperature or temperatures higher than thermophiles, using their inexpensive and renewable reducing agents, such as Laktatyaastat. Microbes may reduce metals in two ways: within cells and outside of them. Nanoparticle formation is regulated by the kind of microbes and enzymes that are present either within or outside the cell, which in turn dictates the reduction location of ions. 4 Consistent with green synthesis goals, this study synthesized iron oxide nanoparticles using a cytoplasmic extract of Lactobacillus fermentum.

1. Results and Discussion

After three weeks, the incubated solution's hue changed, which was the first indicator of iron oxide nanoparticle development (Fe3O4). The iron sulfate solution went from being colorless to black after the cytoplasmic extract of Lactobacillus fermentum reduced the iron ions.
XRD results of Fe3O4 nanoparticles are shown in Figure 1. According to the Debye-Scherrer technique and the XRD graph, the particles had a size of 15 nanometers. In agreement with the sizes determined using the XRD diagram, TEM images of the sample [Fe3o4] (Fig. 2) showed that the nanoparticles had a spherical shape and averaged 10-15 nm in diameter. In biological production, we see the reduction of enzymes, extracellular polysaccharides, and reducing materials, all thanks to various microorganisms that may reduce metal ions. To lessen the harmful effects of metal ions in the bacteria' growing environment, germs produce metal nanoparticles. To do this, they use an enzyme called NADH reductase or nitrate reductase to convert harmful metal ions into less harmful metallic components. Not to mention that certain polysaccharides are present and microorganisms inside the cells create organic materials, and culture media lead to the synthesis of metal nanoparticles.

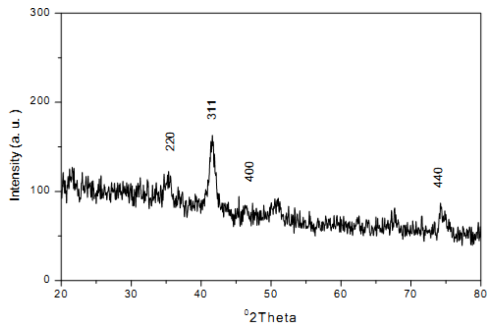

Figure 1. XRD spectrum of iron oxide nanoparticles [Fe_3O_4]: using Debye- Scherrer method, particles sizes were calculated 15-20 nm.

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.

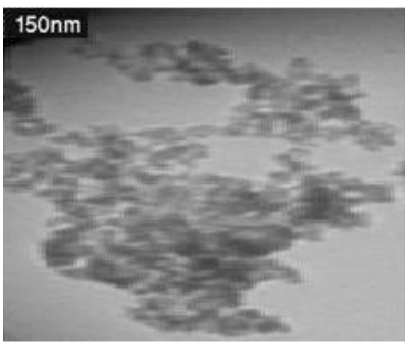

Fig 2. TEM image showed that the nanoparticles had average diameters of 10-15 nm.

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.

2. Conclusion

Due to its many advantages over chemical synthesis, including reduced cost, toxicity, and friendliness with the environment, green synthesis is often considered the superior method. Conversely, lactobacillus belongs to the vast family of probiotics, which means they are harmless and may be used for medicinal purposes without worrying about any harmful side effects.

3. Experimental

3.1. Materials and methods

3.1.1. 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.

3.1.2. 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]

3.1.3. 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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