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Orginal Research Article

Biosynthesis of iron oxide nanoparticles by cytoplasmic extracts of bacteria lactobacillus casei

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ABSTRACT

Nowadays, tend to use nanotechnology in various fields such as medical science and pharmacology have increased. Making nanoparticles can be done by different ways, but, due to the hazards and environmental pollution caused by them, green synthesis has attracted much attention. Green synthesis of biological resources such as plants, green algae, and microorganisms like bacteria and yeast are used for the production of nanoparticles. For the production of iron oxide nanoparticles this research, in line with the objectives of green synthesis, used the lactobacillus casei extract as a biological source. In this study, green synthesis of iron oxide nanoparticles were performed using lactobacillus casei extract as a biological source cytoplasmic extract of lactobacillus casei and iron sulfate solution 10^{-3} M [pH=5.6] were mixed in a V/V 10% volume ratio, and incubated for 3 weeks at 37 °C in the presence of 5% carbon dioxide. Synthesizing iron oxide nanoparticles was studied by electron microscope and x-ray microdiffraction. After three weeks of incubation, the color of iron sulfate and the extract solution was changed from colorless to black. According to XRD analysis, synthesis of iron oxide nano crystals was confirmed. The average synthesized nanoparticles diameters as determined by transmission electron microscopy (TEM) was found to be about 15 nm with a spherical shape. Production of iron oxide nanoparticles through green synthesis method using cytoplasmic extract of lactobacillus casei as a microorganism probiotic is biologically safe, of low cost, simple, efficient, and eco-friendly treatment that has attracted a lot of attention in medicine, pharmacology, and targeted drug delivery.

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Graphical Abstract

Introduction

Nanotechnology is a field of applied knowledge and technology that covers a wide range. In fact, it is exploiting the chemical, physical, and biological characteristics of the materials in sizes less than 100 nanometers in different industries and sciences [1, 2]. One of the main research areas in nanotechnology is the use of various metal nanoparticles. Today, nanoparticles are produced by different chemical methods that have disadvantages including instability of the solution, various particles sizes, low efficiency, and need to advanced equipments. Therefore, in order to produce nanoparticles, researchers use biological systems which have the least environmental hazards and are simple and eco-friendly. So, biological resources such as bacteria, green algae, fungus, plants, plant extracts and metabolites are applied for the synthesis of nanoparticles [3]. Although various biological ways to produce bio-metal nanoparticles have been known, using plants or other intermediates to prepare metal nanoparticles is expensive and has limitations. Therefore, easy biological providing of nanoparticles with controlled sizes and shapes is of great importance.

Meanwhile, using such a microorganism that can make nanoparticles outside the cell stably, environmentally safe, with good particle size distribution, and spending lower cost is of particular importance [4]. In this research in line with the objectives of green synthesis for the production of iron nanoparticles, lactobacillus casei extract as a probiotic bacteria and biological source was used.

Experimental

Materials and methods

Lactobacillus casei strain with ID [PTCC 1608] was bought from microbial bank of Scientific and Industrial Research Organization of Iran. Lyophilized pipe of bacteria Lactobacillus casei was broken in sterile condition and inoculated in 1 CC of sterile distilled water. After moving in a MRS Broth medium, it was incubated for 24 hours at 37 °C [5].

Preparing lactobacillus casei 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 5.1 mL phosphate buffered saline (Merk) and centrifuged at 3000 g for 10 min (Washing step with phosphate buffered saline was repeated three times). Afterwards, it was placed inside the nitrogen tank (Liquid nitrogen -196 °C) for 5 minutes and then it was also placed in steam bath for 15 minutes (37 °C). Finally, after centrifuging at 12,000 g for 30 minutes, the supernatant was collected as the cytoplasmic extract [6].

Nanoparticles synthesis by cytoplasmic extract of lactobacillus casei

For synthesizing iron oxide nanoparticles, aqueous iron sulfate solution [10^{-3} M] was prepared and mixed with the lactobacillus casei cytoplasmic extract in V/V% 10 volume ratio. After adjusting pH=6.5, the solution was incubated in the darkness for 3 weeks at 37 °C and in the presence of 5% carbon dioxide [5, 7]. 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 sulfate 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 poured into a sterile glass and placed in oven at 40 °C for 24 hours to be dried [8].

Result and discussion

The first sign of formation of iron oxide nanoparticles (Fe_3O_4) was incubated solution color-change after about three weeks. After reduction of iron ions by lactobacillus casei cytoplasmic extract, color of iron sulfate solution changed from colorless to black. Color intensity is due to active surface plasmon vibrations in the iron oxide nanoparticles (Figure 1).

XRD analysis of iron oxide nanoparticles (Fe_3O_4) in Figure 2 by examining the XRD graph and using Debye-Scherrer method, particles sizes were calculated 15 nm.

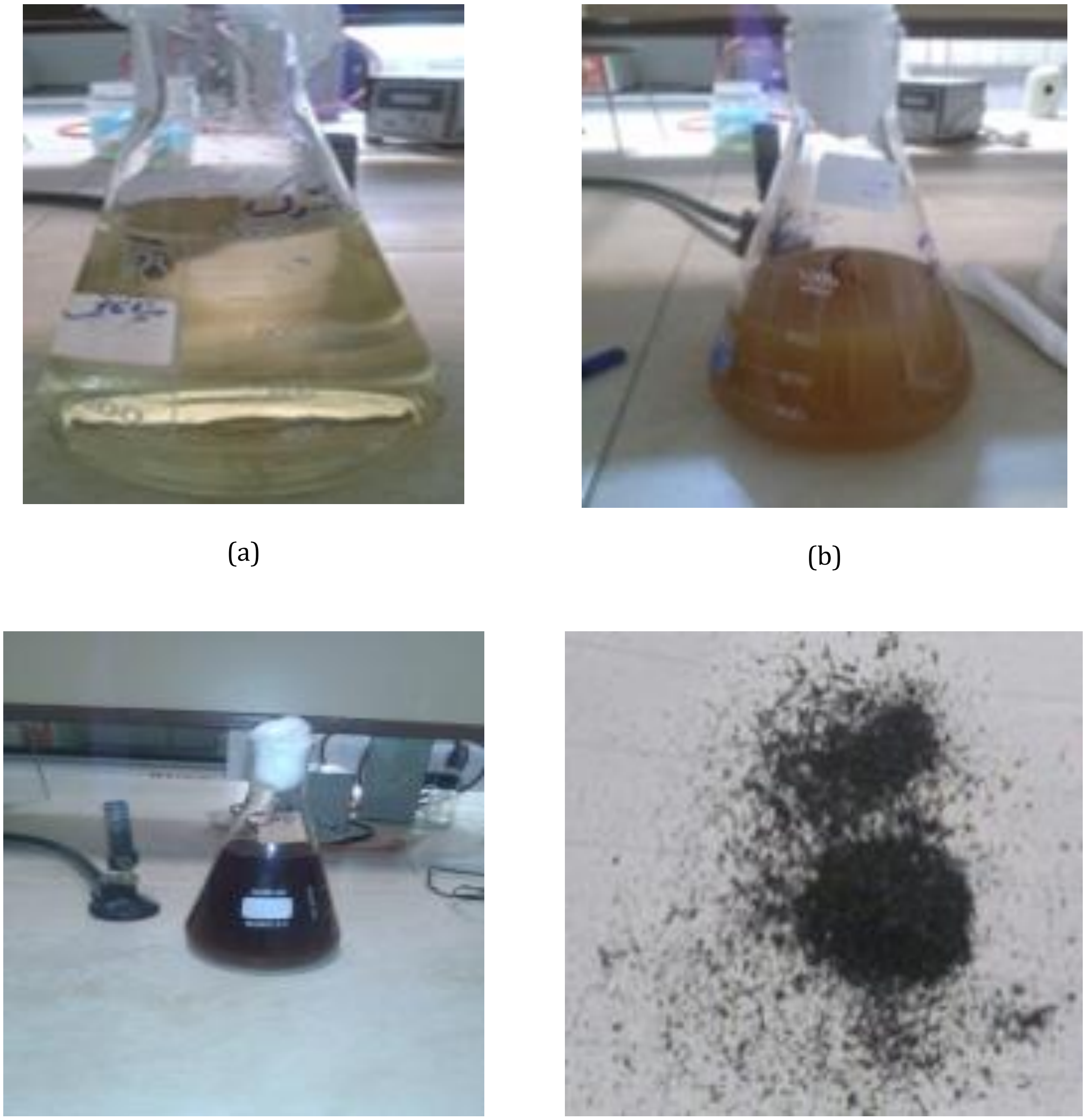
TEM sample images (Fe_3O_4) in Figure 3 indicated that the nanoparticles were spherical in shape with average diameters of 10-15 nm which agreed with sizes calculated using XRD diagram.

Green chemistry means production of new products using new methods that fit the three goals of the environment, economy, and stable society. In today's world, finding a suitable way for synthesis of nanoparticles used in nanotechnology is highly regarded.

Using nanoparticles in medicine, pharmacology, and biological sciences is increasing. In the biological production, microorganisms are capable of producing metal nanoparticles by reducing metal ions in the environment. Of course, variety of enzymes, reducing substances etc. are effective in the reduction process.

In this research, the first sign of the formation of iron oxide nanoparticles was incubated solution color-change after about three weeks. This color-change from colorless to black is due to a phenomenon called surface plasmon resonance. In fact, by absorbing visible light free electrons of nanoparticles are excited and move to a higher energy level. As the electron in excited state is unstable, by emitting a photon it returns to the ground state. Since the excitation of free electrons, when exposed to light, is in the form of resonance, the light that they emit is within the visible spectrum with a specific color. This phenomenon helps to realize the formation of particles microscopically [9]. The results of the study by *Omidi* et al. in 2010 showed that the first sign of the formation of silver nanoparticles was the color-change of silver nitrate from colorless to brown [7]. *Ashouri* et al. (1392) synthesized iron nanoparticles using green tea extract which was determined by formation of brown color [10]. Bacteria that do not tolerate high concentrations of metal ions can also be used as microorganisms suitable for the production of nanoparticles. Probiotic bacteria, particularly Lactobacillus casei*,* are able to produce metal nanoparticles among their cells [11].

Through the biological reduction of toxic metal ions of the microbes' growth environment into less toxic metallic elements (Using a specific enzyme such as NADH reductase) microorganisms cause the production of metal nanoparticles [12]. OF course, production of organic material by microorganisms and presence of polysaccharides in the cell may also cause the reduction of metal ions and production of metal nanoparticles [13]. At the study conducted by *Omide* and colleagues, silver nanoparticles were synthesized by lactobacillus fermantom supernatant which were 15 nm, spherical, stable, little, and impressionable [7]. *Selvarajan* and colleagues could synthesize ZnO nanoparticles using cytoplasmic extracts of lactobacillus plantarum that were reported to be moderately stable, spherical, hexagonal phase, with particles in size range within 7−19 nm in diameter [14]. Sherin's study results depicted that iron oxide nanoparticles synthesized by the bacteria streptomyces were spherical with a smooth surface and diameter of 39 nm [15].



(a) (b)

(c) (d)

Figure 1. a) Pure iron sulfate solution, b) the iron sulfate solution and ions by lactobacillus casei cytoplasmic extract, c) production of iron oxide nanoparticles through green synthesis method using iron sulfate solution and cytoplasmic extract of lactobacillus casei, d) biosynthesized iron oxide nanoparticles

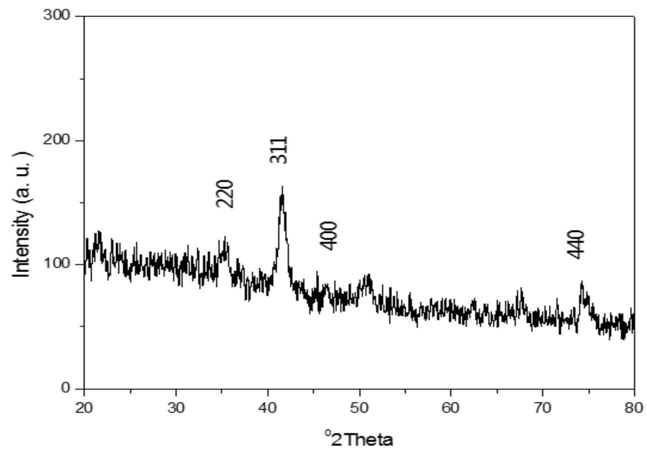


Figure 2. XRD curve of iron oxide nanoparticles (Fe_3O_4)

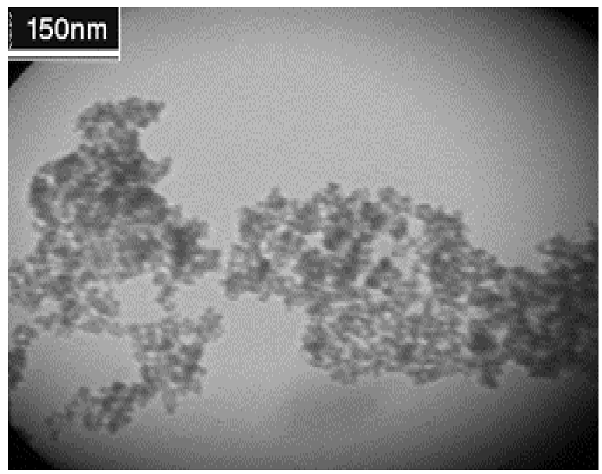


Figure 3. TEM sample image (Fe_3O_4)

Iron oxide nanoparticles produced through biological way rather than chemical generated particles are more valuable due to the absence of toxic organic residues on the surface, creating minimal waste and non-consumable materials in the production process, high production volume, and reproducibility. Although in green method than the chemical method more time is needed to produce iron oxide nanoparticles; green synthesis is suggested because of its immense benefits. For metabolic activities, doing vital processes, providing energy and structural metals, reducing environmental toxins, microorganisms sediment nano-sized metals into inside or outside the cell or into the cell wall. Using microorganisms for synthesizing nanoparticles has advantages: no need for stabilizing compounds to prevent mixing of nanoparticles and becoming macro particles, possibility of synthesis at low temperatures even at room temperature, no toxicity, ease of working with them, and lower costs. Moreover, microorganisms are able to synthesize bread and metal particles in sizes from 1 to 200 nm using cheap and renewable reducing agents such as laktatya acetate [16, 17].

Conclusion

Based on the results obtained in this study, we managed to synthesize iron oxide nanoparticles by lactobacillus casei bacteria extract. Synthesized iron oxide nanoparticles, in this way, were very small, spherical, with low impressionability and a size of 15 nm. It seems that presence of reducing compounds and certain enzymes in the cytoplasmic extract of lactobacillus casei causes the metal ion reduction and production of iron oxide nanoparticles. On the other hand, lactobacilluess are from the large family of probiotics which are safe with no pathogenic effects. According to what mentioned above, synthesis of iron oxide nanoparticles using cytoplasmic extracts of lactobacillus as a biological source, without using any chemical reducing and stabilizing agent, is an efficient, effective, eco friendly , and low cost method which can be used in medical purposes and drug delivery.

Disclosure statement

No potential conflict of interest was reported by the authors.

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