

Synthesis of Sterile and Pyrogen Free Biogenic Magnetic Nanoparticles: Biotechnological Potential of Magnetotactic Bacteria for Production of Nanomaterials

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Abstract—Today, biogenic magnetite nanoparticles among magnetic nanoparticles have unique attracted attention because of their magnetic characteristics and potential applications in various fields such as therapeutic and diagnostic. A well known example of these biogenic nanoparticles is magnetosomes of magnetotactic bacteria. In this research, we used two different types of technique for the isolation and purification of magnetosome nanoparticles from the isolated magnetotactic bacterial cells, heat-alkaline treatment and sonication. Also we evaluated pyrogen content and sterility of synthesized the isolated individual magnetosome by the Limulus Amoebocyte Lysate test and direct impedimetric method respectively.

Keywords—Biogenic magnetic nanoparticles, Magnetosome, Magnetotactic bacteria, Nanobiotechnology

I. INTRODUCTION

A charming example for the biogenic nanoparticles is the biomineralization of magnetic nano-particles by magnetotactic bacteria (MTB). Biomineralization is a precision process that produces complex nano-structures consisting of organic and inorganic components of uniform size and highly ordered morphology that self-assemble into structures in a hierarchical manner [1].

Magnetotactic bacteria synthesize intracellular magnetic nano-particles (Magnetosomes), which are enveloped by a thin organic membrane. Because of the appearance of amino and carboxyl bases associated with the surface of the membrane, these bacterial magnetic nanoparticles could be used as the perfect carriers of antibodies, medical drugs and therapeutic radioisotopes. In addition, these nanoparticles can be easily dispersed in aqueous solutions because of their enclosing membrane. Thus they have potential for various biotechnological applications [1], [2], [3], [4]. Today, synthesis of nanoparticles of different chemical composition, controlled size is an important area of research in

nanotechnology. Magnetotactic bacteria synthesize nano-sized magnetite crystals that are highly consistent in size and morphology within bacterial species compared with artificial magnetite particles, which making them ideal nano-biotechnological materials [1], [4], [5]. MTB's particle sizes are typically 35–120 nm, which is within the single magnetic-domain size for magnetite and greigite [4], [6].

To date, although magnetic properties of magnetosomes have been evaluated [7] and it predicted that magnetosomes could be considered as good materials for the biomedical applications in various field [8], [9], [10], [11], [12], little has been done to a rapid and simple process for the purification and sterilization method for magnetosomes and a detection standard for purified magnetosomes.

In this study, we used a curve-shaped magnetotactic bacterium that has been isolated from Anzali lagoon in other research [13]. In this work we focused on various methods for isolation of biogenic magnetic nanoparticles from the magnetotactic bacterium and production of sterile and pyrogen free magnetic nanoparticles. The main purpose of this research was development a simple and comprehensive method for synthesis of sterile and pyrogen free MTB's magnetic nanoparticles with great potential in various medical and biotechnological fields.

II. MATERIALS AND METHODS

Chemicals were generally reagent grade from commercial sources.

A. Bacterial Strain and Growth Condition

A curve-shaped magnetotactic bacterium has been isolated recently from a water/mud microcosm collected from Anzali lagoon in Gilan province, Iran [13]. This strain was grown in a modified liquid medium (MLM) under a microaerobic headspace gas mixture of 2% oxygen and 98% nitrogen, which is optimal oxygen concentration for magnetosome formation [13]. The magnetotactic cells and their magnetic nanoparticles were observed by transmission electron microscopy (EM 208S transmission electron microscope (Philips) at 100 kV).

B. Preparation of the Bacterial Magnetic Nanoparticles

In this research, techniques for the isolation and purification of magnetosome particles from the magnetotactic bacterial

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cells are based two different types of processes. After bacterial cells harvesting by centrifugation (8000 rpm, 15 min, 4°C), solution of isolated magnetotactic bacteria in sterilized phosphate buffer solution (pH= 7) were divided in two separated parts. MTB of the first solution were precipitated and resuspended in 1N NaOH and boiled for 30 min to lyse the cells [13], [14]. The solution contained in the second part sonicated during 120 min at 30 W to extract the chains of magnetosomes from the whole bacteria [15]. The optical microscope was used to examine the effect of disruption.

Magnetosomes from the disrupted cells were accumulated within 60 min at the sides of the graduated cylinders nearest the magnet. The nonmagnetic fluid fraction was removed by aspiration. Lastly the bacterial magnetic nanoparticles attracted to the magnet were suspended in sterilized phosphate buffer solution (pH= 7).

C. Preparation of Sterile and Pyrogen Free Biogenic Magnetic Nanoparticles

Magnetosomes of the first solution were precipitated and treated in 1% sodium-dodecyl-sulfate (SDS) at 90°C for 1 hour to produce individual magnetosomes [15]. Finally, the individual magnetosomes were separated by a magnet and washed 5 times with PBS buffer and pyrogen content of them was assessed.

For sterilization test of the isolated individual magnetosome, them were dispersed in PBS buffer and sterilized with autoclave (121°C, 15 min).

D. Examination of Purified Magnetosomes

The purified bacterial magnetic nanoparticles were suspended in PBS [16] and put onto the carbon-coated copper grids. Samples were observed through transmission electron microscopy (EM 208S transmission electron microscope (Philips) at 100 kV).

The pyrogen content of the purified magnetosomes was assessed by the Limulus Amoebocyte Lysate (LAL) test. The vial contents were incubated at 37°C for 60 min for the presence of pyrogens according to the manufacturer's instructions.

Direct impedimetric method was used for the determination of sterility of purified magnetosomes. Impedance changes were monitored using the BacTrac 4300 (Bactrac, SYLAB, Austria). This method can produce results in about 24 h, instead of the 7-14 days needed by standard culture broth technique and can be easily realized in automatic form.

III. RESULT AND DISCUSSION

One of the key features of nanoparticles is their large surface area. As particles become smaller, their surface area to volume ratio increases (Fig. 1). For example, one gram of iron oxide (Fe_3O_4) would form a sphere of approximately 0.75 cm with a surface area of 1.77 cm^2 . The same gram of iron oxide, when composed of 20 nm nanoparticles, would have a surface area of approximately 60 m^2 [17].

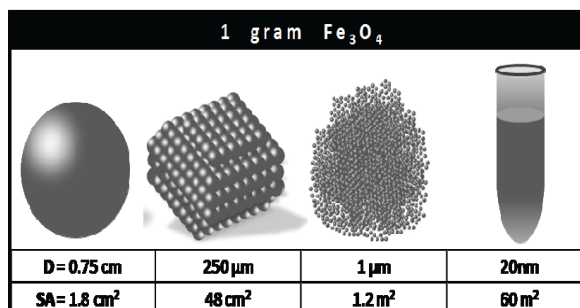


Fig. 1 Comparison of surface area and volume for different sized particles [17]

Magnetotactic bacteria synthesize more uniform magnetic nanoparticles in size and shape compared with artificial magnetite nanoparticles [5]. Nanoscale MTB's particles have many utilization for various biotechnological and medical applications. These biogenic nanoparticles are easily isolated and purified from broke MTB by magnetic separation using a magnet. A curve-shaped magnetotactic bacterium was isolated recently from a water/mud microcosm collected from Anzali lagoon. Transmission electron microscopy image of the isolated strain and its single chain of magnetosomes showed in fig. 2 [13].

In this study, we used two different types of techniques for the isolation and purification of magnetosome particles from the magnetotactic bacterial cells. Transmission electron microscopy images of the pure biogenic magnetic nanoparticles indicated that kind of processes of extraction and purification was important to protect the integrity of magnetosome's membrane (Fig. 3).

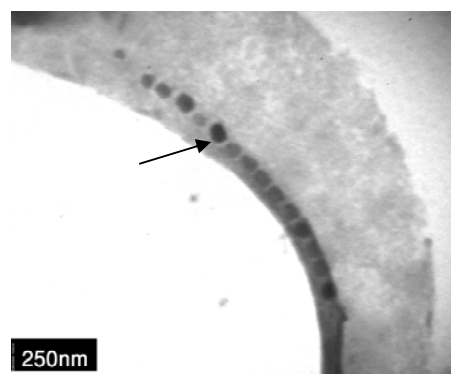


Fig. 2 TEM image of an unstained cell of the isolated strain showed a single chain of magnetosomes, arrow (\rightarrow) shows chain of magnetosomes inside the magnetotactic bacterium

According to fig. 3, it is concluded that the thermal extraction of the magnetosomes in the presence of NaOH can damage the biogenic membrane of the magnetosomes and it caused aggregation of magnetosomes. Fig. 4 shows a photograph from vials of the extracted bacterial magnetic nanoparticles mixed in phosphate buffer solution at pH 7 with used two different types of techniques for extraction.

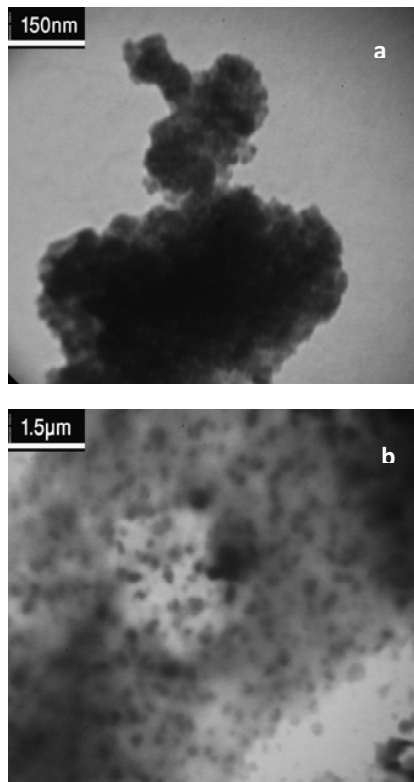


Fig. 3 TEM micrograph of magnetosomes extracted from the bacteria; (a) TEM micrograph of extracted magnetosomes by heat and NaOH treatments. (b) TEM micrograph of extracted magnetosomes by sonication method

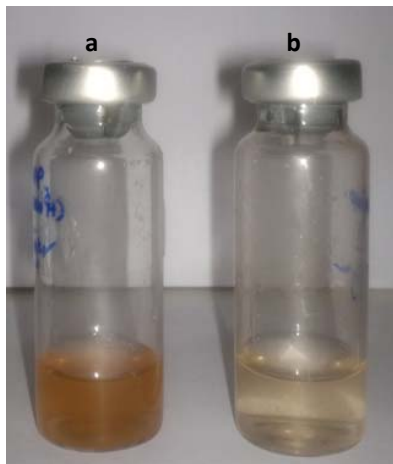


Fig. 4 The photograph shows vials of the extracted bacterial magnetic nanoparticles mixed in phosphate buffer solution at pH 7. (a) extracted magnetosomes by heat and NaOH treatments. (b) extracted magnetosomes by sonication method

Magnetosomes of the first solution were treated in 1% SDS at 90°C for 1 hour to produce pyrogen free individual bacterial magnetic nanoparticle solution. In 1970 the U.S. Food and Drug Administration (FDA) approved LAL for testing drugs, products and devices that come in contact with the blood [18].

Therefore, we used the LAL test for the detection and quantification of bacterial endotoxins in produced these nanoparticles solutions, in this study. The results demonstrated that these nanoparticles solutions are pyrogen free (Fig. 5).

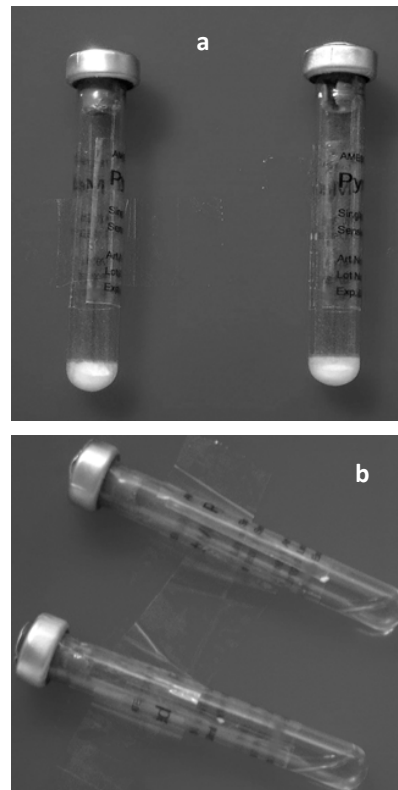


Fig. 5 (a) Vials of Limulus Amoebocyte Lysate test. (b) The used vials for detection of pyrogenic substances in the magnetic nanoparticles solutions

The isolated individual magnetosome were sterilized by autoclave. Contaminating microbes could not be detected in the sterilized magnetosomes with direct impedimetric method (Fig. 6).

BacEval M-Value

Results obtained with SY-LAB BacTrac

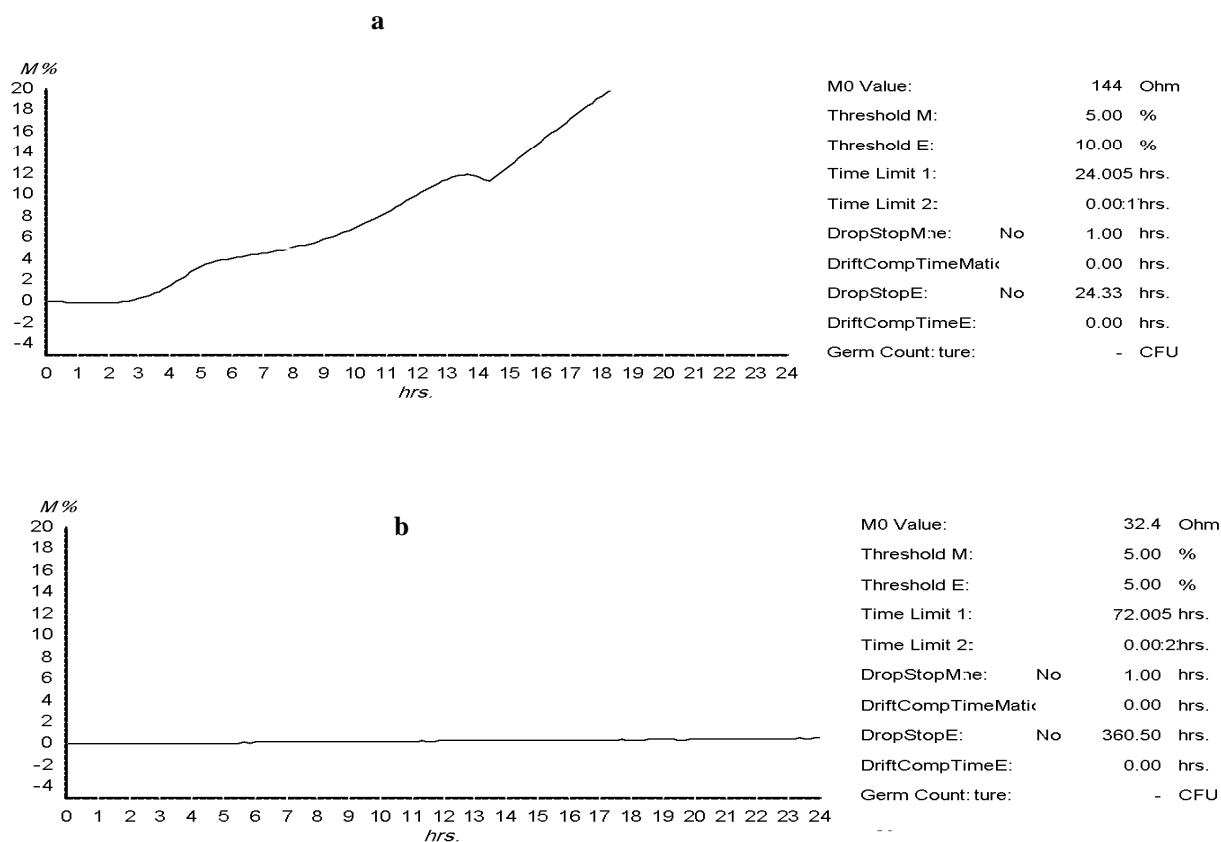


Fig. 6 Impedance growth curves. (a) Impedance growth curve of positive control. (b) Impedance growth curve of the magnetic nanoparticles solutions

IV. CONCLUSION

In this study, we only evaluated the biosynthesis of pyrogen free and sterilized biogenic magnetic nanoparticles by a magnetotactic bacterium. The results indicated that produced magnetosomes by these methods were pyrogen free and they can be candidates for medical application purpose. Further investigations of the safety and toxicity of magnetosomes are in examining in our laboratory.

REFERENCES

- [1] T. Matsunaga, T. Suzuki, M. Tanaka, and A. Arakaki, "Molecular analysis of magnetotactic bacteria and development of functional bacterial magnetic particles for nano-biotechnology," *Trends. Biotechnol.*, vol. 25, pp. 182-188, 2007.
- [2] L. Xiang, J. Wei, S. Jianbo, W. Guili, G. Feng, and L. Ying, "Purified and sterilized magnetosomes from *Magnetospirillum gryphiswaldense* MSR-1 were not toxic to mouse fibroblasts in vitro," *Lett. Appl. Microbiol.*, vol. 45, pp. 75-81, 2007.
- [3] D. Schüler, and R. B. Frankel, "Bacterial magnetosomes: microbiology, biomineralization and biotechnological applications," *Appl. Microbiol. Biotechnol.*, vol. 52, pp. 464-473, 1999.
- [4] D. A. Bazylinski, and R. B. Frankel, "Magnetosome Formation in Prokaryotes," *Nat. Rev. Microbiol.*, vol. 2, pp. 217-230, 2004.
- [5] T. Matsunaga, Y. Okamura, and T. Tanaka, "Biotechnological application of nano-scale engineered bacterial magnetic particles," *J. Mater. Chem.*, vol. 14, pp. 2099-2105, 2004.
- [6] C. Lang, and D. Schüler, "Biogenic nanoparticles: production, characterization, and application of bacterial magnetosomes," *J. Phys.: Condens. Matter.*, vol. 18, pp. 2815-2828, 2006.
- [7] H. Lei, S.Y. Li, Y. Yang, F-M. Zhao, J. Huang, and J. Chang, "Research on the structure and performance of bacterial magnetic nanoparticles," *J. Biomater. Appl.*, vol. 22, pp. 433-448, 2008.
- [8] M. Timko, A. Dzarova, J. Kovac, A. Skumiel, A. Jozefczak, T. Hornowski, H. Gojzewski, V. Zavisova, M. Koneracka, A. Sprincova, O. Strbak, P. Kopcansky, and N. Tomasovicova, "Magnetic properties and heating effect in bacterial magnetic nanoparticles," *J. Magn. Mater.*, vol. 321, pp. 1521-1524, 2009.
- [9] T. Matsunaga, and S. Kamiya, "Use of magnetic particles isolated from magnetotactic bacteria for enzyme immobilization," *Appl. Microbiol. Biotechnol.*, vol. 26, pp. 328-332, 1987.
- [10] T. Matsunaga, M. Kawasaki, X. Yu, N. Tsujimura, and N. Nakamura, "Chemiluminescence enzyme immunoassay using bacterial magnetic particles," *Anal. Chem.*, vol. 68, pp. 3551-3554, 1996.
- [11] T. Matsunaga, R. Sato, S. Kamiya, T. Tanaka, and H. Takeyama, "Chemiluminescence enzyme immunoassay using protein A-bacterial magnetite complex," *J. Magnet. Magnet. Mater.*, vol. 194, pp. 126-134, 1999.
- [12] J. Wei, Z. Dehua, L. Ying, T. Jiesheng, W. Zhenfang, and L. Jilun, "Submerged culture of *Magnetospirillum gryphiswaldense* under N₂-fixing condition and regulation of activity of nitrogenase fixation," *Chin. Sci. Bull.*, vol. 47, pp. 2095-2099, 2002.

- [13] P. Tajer-Mohammad-Ghazvini, R. Kasra-Kermanshahi, A. Nozad-Golikand, and M. Sadeghizadeh, "A green chemical technique for the synthesis of magnetic nanoparticles by magnetotactic bacteria," in press.
- [14] C. Yang, H. Takeyama, T. Tanaka, and T. Matsunaga " Effects of growth medium composition, iron sources and atmospheric oxygen concentrations on production of luciferase-bacterial magnetic particle complex by a recombinant *Magnetospirillum magneticum* AMB-1," *Enzyme. Microb. Technol.*, vol. 29, pp. 13-19, 2001.
- [15] E. Alphandéry, S. Faure, O. Seksek, F. Guyot, and I. Chebbi "Chains of magnetosomes extracted from AMB-1 magnetotactic bacteria for application in alternative magnetic field cancer therapy," *ACS Nano.*, vol. 5, pp. 6279-6296, 2011.
- [16] X. Zhijie, L. Bin, C. Jun, and H. H. Teng, "Design and application of the method for isolating magnetotactic bacteria," *Chin. J. Geochem.*, vol. 26, pp. 252-258, 2007.
- [17] Charles R. M. Glaus, "Development and analysis of radiolabeled magnetic nanoparticles for positron emission tomography and magnetic resonance imaging", *Georgia Institute of Technology*, Dissertation, December 2008.
- [18] [Http://en.wikipedia.org/wiki/Limulus_amebocyte_lystate](http://en.wikipedia.org/wiki/Limulus_amebocyte_lystate).