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Bio-synthesis of magnetite nanoparticles by bacteria

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Abstract: A promising avenue of research in materials science is to follow the strategies used by Mother Nature to fabricate ornate hierarchical structures as exemplified by organisms such as diatoms, sponges and magnetotactic bacteria. Some of the strategies used in the biological world to create functional inorganic materials may well have practical implications in the world of nanomaterials. The aim of our work is to examine the synthetic of magnetite nanoparticles under different conditions to show the influence in magnetic properties of magnetite nanoparticles. *Magnetospirillum* strain AMB-1 was used in this study in order to produce magnetite nanoparticles. Magnetite nanoparticles of average size~47 nm were obtained. The magnetic properties of magnetite nanoparticles under different incubation temperature were examined and a small influence in magnetic properties of magnetite nanoparticles was indicated.

Keywords: *Magnetospirillum,* Magnetite Nanoparticles, Temperatures, Magnetic Properties

1. Introduction

In recent years, nanotechnology research is emerging as cutting edge technology interdisciplinary with physics, chemistry, biology, material science and medicine. The prefix nano is derived from Greek word nanos meaning “dwarf” in Greek that refers to things of one billionth (10^{-9} m) in size. The primary concept of nanotechnology was presented by Richard Feynman in a lecture entitled “There's plenty of room at the bottom” at the American Institute of Technology in 1959. Nanoparticles are usually 0.1 to 1000 nm in each spatial dimension and are commonly synthesized using two strategies: top-down and bottom-up [1].

Microbes produce inorganic materials either intra- or extracellular often in nanoscale dimensions with exquisite morphology. Microbial resistance to most toxic heavy metals is due to their chemical detoxification as well as due to energy-dependent ion efflux from the cell by membrane proteins that function either as ATPase or as chemiosmotic cation or proton anti-transporters. Alteration in solubility also plays a role in microbial resistance [2, 3]. Therefore, microbial systems can detoxify the metal ions by either reduction and/or precipitation of soluble toxic inorganic ions to insoluble non-toxic metal nanoclusters. Microbial detoxification can be made either by extracellular biomineralization, precipitation or intracellular bioaccumulation. Extracellular production of metal nanoparticles has more commercial applications in various fields. Since the polydispersity is the major concern, it is important to optimize the conditions for monodispersity in a biological process [4].

Magnetite, $Fe^{3+}(Fe^{2+}, Fe^{3+})O_4$, is an “inverse” spinel and the unique electronic and magnetic properties of magnetite are directly associated with the extremely rapid exchange of electrons among the octahedrally-coordinated iron ions. Other divalent and trivalent metal ions readily substitute for the iron atoms in both site types. Magnetite formed naturally inevitably contains impurity cations, the most frequent ones being Ti, Al, Mg, and Mn. The effect of metal substitution in magnetite produces systematic variation in magnetic and physical properties: saturation magnetization, curie temperature change; coercivity; magneto crystalline anisotrophy, cell parameter, and electrical resistivity changes. There are many approaches to the synthesis of magnetic nanoparticles such as size reduction through ball milling, chemical precipitation, and microbial synthesis[5,6,7].

Magnetic nanoparticles are promising as therapeutic or diagnostic tools in medicine. In terms of diagnosis they can be used both for *in vitro* and *in vivo* applications for example: in immobilization and detection of biomolecules [8,9,10], cell separation [11], purification [12] and gene transfer [14],

and serve as a contrast agents in magnetic resonance imagining [13]. They can also be applied for drug delivery system in target therapy [9] and for hyperthermia treatment, due to the heat they produce in an alternating magnetic field [l4].

The aim of this study is evaluate the physical conditions at which magnetic bacteria can produce magnetite nanoparticles with the best characterizations.

2. Material and Methods

For the isolation of magnetosomes; approximately 100 ml cell culture of *Magnetospirillum* strain AMB-1 was suspended in 100 ml of 20 mM HEPES-4 mM EDTA, pH 7.4, and then split up (disrupted) by sonication. The unbroken cells and the cell debris were removed from the sample by centrifugation (30 min, 9000 rpm), then the cell extract was placed on magnet (NdFeB-magnets, 1h). The black magnetosomes sediment at the bottom of the tube, whereas the residual contaminating cellular material was retained in upper part tube and then decanted. To eliminate the electrostatically bound contamination, the magnetic particles were rinsed first with 50 ml of 10 mM HEPES-200 mMNaCl, pH 7.4, and subsequently with 100 ml of 10 mM HEPES, pH 7.4. The magnetosome suspension (black sediment) was centrifuged (18000 rpm, 30 min). After centrifugation, the cell extract was placed on the magnet for 30 minutes. The magnetic particles were sediment at the bottom of the tube, whereas residual contaminating cellular material was retained in upper part tube. The last step was repeated ten-times to obtain well purified magnetosomes. The previous procedure had been done under different incubation temperatures (30, 40,50,60,70 $^{\circ}$C)

Transmission electron microscopy images were taken for magnetosomes and the magnetite nanoparticles. The size of the magnetite nanoparticles was analyzed by Beckman Coulter Particle Size Analyzer. The degree of magnetism of the nanoparticles was evaluated using vibrating sample magnetometer (VSM-9600-IDSM-LDG-USA) and the saturation magnetism (B_r), Retentivity (B_r), and Coercivity (H_c)

3. Statistical Analysis

All results were represented as mean ± SD. In order to study the statistical significance of the results significance regarding to Pearson's coefficient and sample size had been performed and the $p \leq 0.05$ had been taken as the significance limit.

4. Results

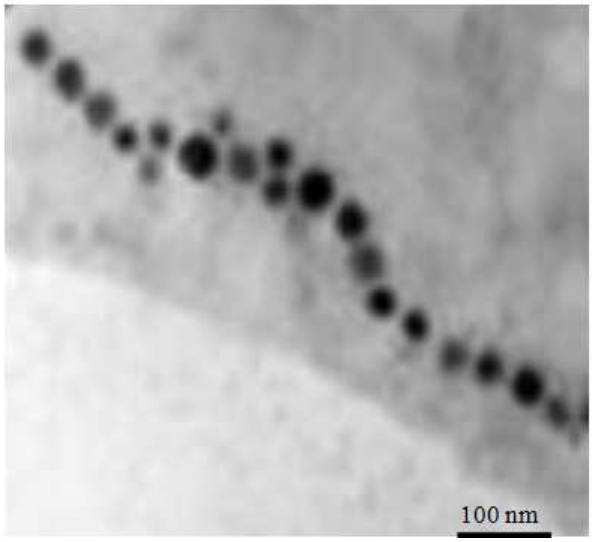
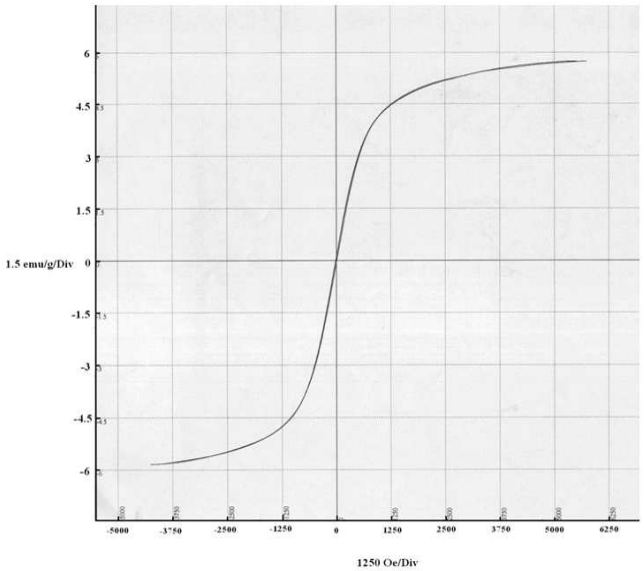
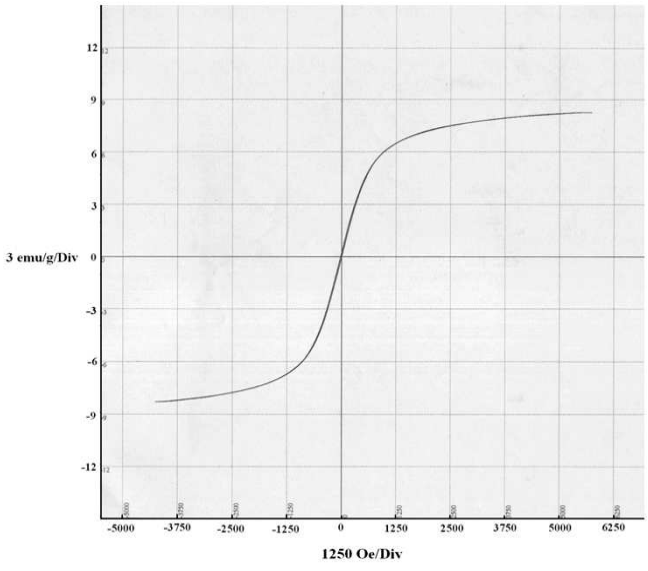


Fig. 1. *Nanoparticles TEM image.*



(a)



(b)

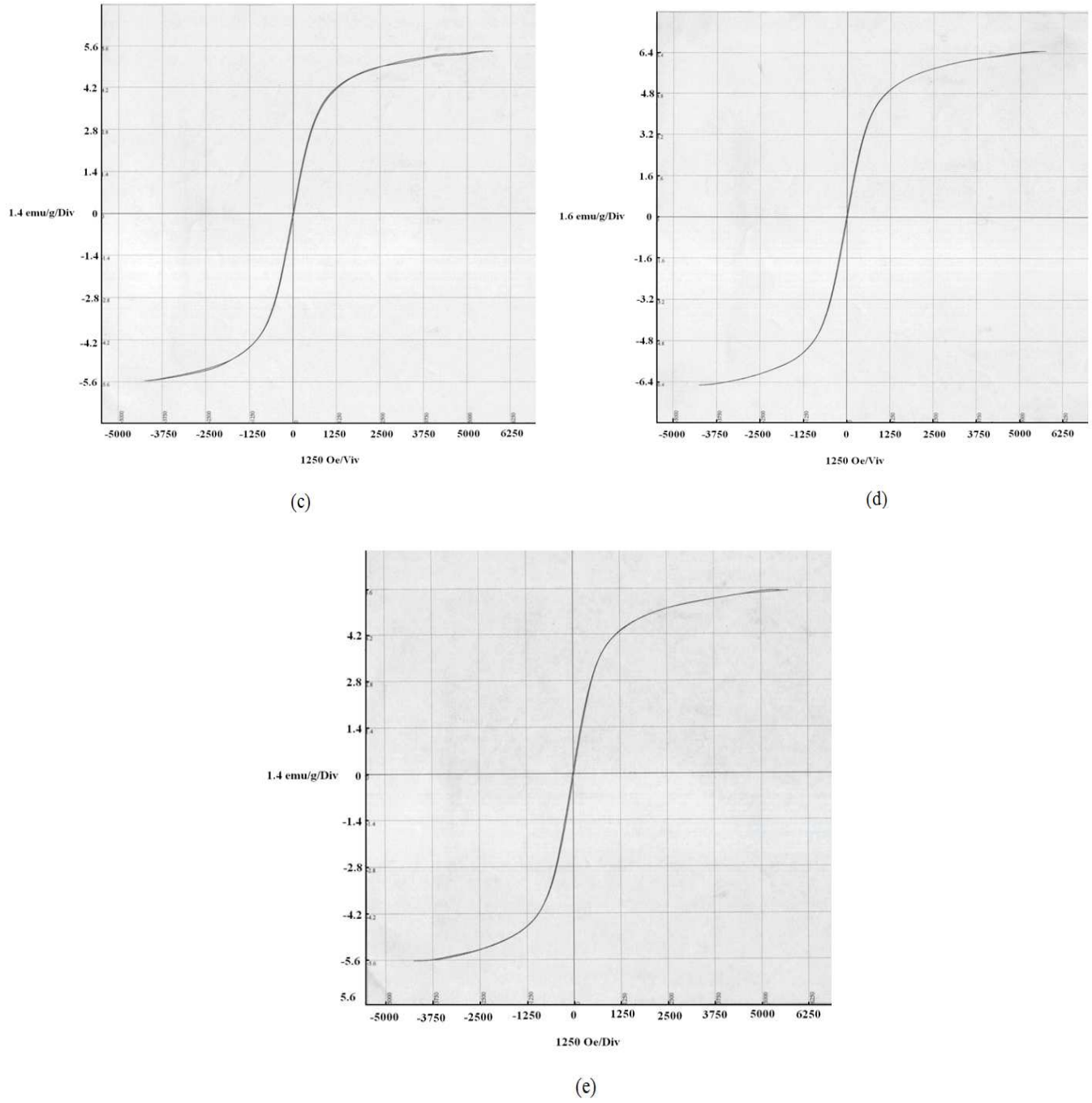


Fig. 2. *The hysteresis loop magnetite nanoparticles prepared at a) 30ºC, b) 40 ºC, c) 50 ºC, d) 60 ºC and e) 70 ºC.*

Typical electron micrograph of magnetosomes on surface obtained by TEM technique for prepared samples and is shown in Fig.1. For evaluation of different preparation conditions the size distributions of magnetosomes (from 100 particles) according to TEM photographs were prepared. The mean diameter of magnetosome prepared estimated from the size distribution of magnetosomes obtained by cultivation at different incubation temperatures was estimated as to be 45±2.5 nm, 49±2.5 nm, 46±1.5 nm, 48±4.5, nm 47±1.99 nm, respectively. They were the same size of magnetite nanoparticles obtained after separation from the bacteria. It was observed increased number of magnetosomes in part of higher and lower size of magnetosomes this causing distinct changed of size distribution and size of magnetosomes is more uniform. A small particles size was obtained at 30 ºC , but the maximum particles size was obtained at 40 ºC.

The magnetic properties including hysteresis loop, saturation magnetization and coercivity of magnetite nanoparticles were measured in this research. Fig.2 shows the hysteresis loops of paramagnetic magnetite nanoparticles. In which the internal area of hysteresis loop represents the capability of magnetic energy storage of magnetic materials,

which is an important parameter in electromagnetic absorption field. The hysteresis loop with great area brings on a large loss. The internal areas of hysteresis loops are great, which can be used as electromagnetic absorption materials. The figures represent the hystersis loops of magnetite nanoparticles prepared at different temperature range from 30 to 70 ^{o}C. In the first Fig.2(a) which represent magnetite nanoparticles prepared at 30 ^{o}C, in which the saturation magnetization was 5.89 emu/g, coercivity was 14.37 Oe and retentivity was 0.7615 emu/g. The second Fig.2(b) which represents magnetite nanoparticles prepared at 40 ^{o}C, in which the saturation magnetization was 5.404 emu/g, coercivity was 15.2 Oe and retentivity was 1.01 emu/g. The third Fig.2(c) which represents magnetite nanoparticles prepared at 50 ^{o}C, in which the saturation magnetization was 5.6 emu/g, coercivity was 13.91 Oe and retentivity was 0.6726 emu/g. The fourth Fig.2(d) which represents the magnetite nanoparticles prepared at 60 ^{o}C, in which the saturation magnetization was 5.626 emu/g, coercivity was 15.85 Oe and retentivity was 0.7133 emu/g. The fifth Fig.2(e) which represents the magnetite nanoparticles prepared at 70 ^{o}C, in which the saturation magnetization was 5.65 emu/g, coercivity were 16.49 Oe and retentivity were 0.71 emu/g.

The greatest size of the magnetite nanoparticles was obtained at 40 ^{o}C with average value of 48 nm Fig.3. There was no noticeable effect of variation of temperature on B_s values as indicated in Fig.4. A small variation due to the change in temperature was observed on the values of B_r and H_c as shown in Fig.5. and Fig.6.

The comparing means t-test for the obtaining data showed that the relation between temperatures and B_s , B_r , and H_C were significant $p<0.05$. In contrary the relations between temperature and particle size was insignificant $p>0.05$.

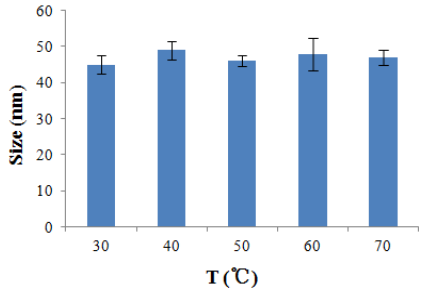


Fig. 3. *The variation in magnetite nanoparticles size with temperature.*

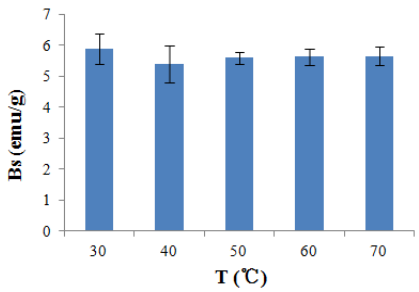


Fig. 4. *The variation in measured B_s of magnetite nanoparticles with temperature.*

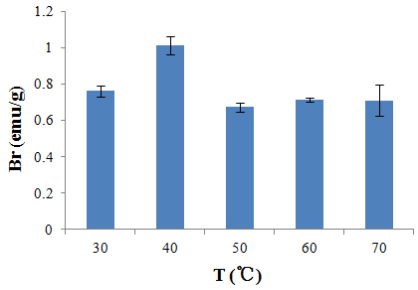


Fig. 5. *The variation in measured B_C of magnetite nanoparticles with temperature.*

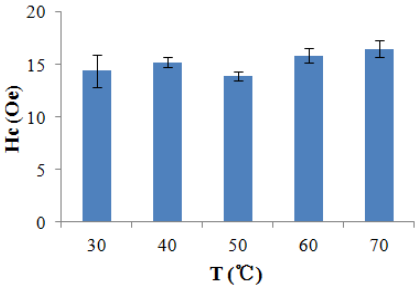


Fig. 6. *The variation in measured H_C of magnetite nanoparticles with temperature.*

5. Discussion

Biomagnetite production by magnetotactic bacteria and Fe(III)-reducing bacteria has been extensively studied[15]. In contrast, whether magnetite can be formed by Fe(II)-oxidizing bacteria remained still unclear. Here, we experimentally evidence that the nitrate reducing Fe(II)-oxidizing strain BoFeN1 can promote the formation of stable single domain magnetite. This strain can form a diversity of Fe-bearing minerals depending on culture conditions: lepidocrocite is obtained at neutral pH [16-17].

The possibility of using bacteria for the synthesis of oxide nanoparticles has also been explored. Most of the work in this direction has centered towards synthesis of magnetite nanoparticles, by taking inspiration from magnetotactic bacteria found in nature. For instance, laboratory-based studies on magnetite growth have focused mainly on the use of magnetotactic bacteria [18–19] and iron reducing bacteria, such as Geobacter metallireducens (a distant cousin of magnetotactic bacteria) [20]. In these studies, biosynthesis of magnetite was found to be extremely slow (often requiring 1 week) under strictly anaerobic conditions. It was however interesting to observe the ability of bacterium Actinobacter sp to synthesize magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) on incubation with suitable aqueous iron precursors under fully aerobic conditions [21]. These nanoparticles were formed extracellularly and showed excellent magnetic properties. The over expression of two inducible proteins was observed in Actinobacter-mediated synthesis of magnetite nanoparticles. When other aerobic bacteria (e.g. Bacillus sp., Aerobacter aero genes, and Micrococcus luteus) were investigated for magnetite synthesis under similar conditions, they did not result in synthesis of magnetite even after one

week of reaction. Kumar and co-workers also reported the extracellular synthesis of spinal-structured ferromagnetic Co_3O_4 nanoparticles using a marine cobalt-resistant bacteria strain, obtained from Arabian sea [22]. In agreement of the previous studies we were able to produce magnetite nanoparticle using *Magnetospirillum* strain AMB-1 and obtained average nanoparticles size of 47 nm.

The magnetic properties of these nanoparticles were determined by vibrating sample magnetometry. The hysteresis curve was obtained, the coercitivity was 1.54 Oersted. The low coercivity indicates that the particles are in super paramagnetic state due to their small particle sizes. The resulting saturation magnetization for small particles can be caused by the presence of super paramagnetic relaxation and/or non colinearity of the magnetic moments at the surface of the nanoparticles [23]. The magnetization saturation does not attain saturation at the highest magnetic field of 7 KOe. The fact that Mr/Ms values were below 0.5; where Mr is the remanent moment and Ms is the saturation moment; was explained from the effect of competition between interparticles interaction and intraparticles anisotropy on the spin relaxation process, which produces frustration [24,25]. The magnetic properties of Ni (Ni55 and Ni147) and Fe147 DENs were studied using SQUID magnetometry at temperatures ranging from 5 to 300 K with magnetic field (H) strength of 500 Oe. The effect of thermal energy on the magnetic properties of the DENs becomes apparent at 200 K for both sizes of Ni particles and at 6 K for Fe147. That is, at T>Tb no remanent magnetisation is observed, however, at T<Tb Ni55, Ni147 and Fe147DENs show hysteresis with magnetic saturation values (Ms) of 3.40 emu/gNi for Ni55, 3.95emu/gNi for Ni147 and 70.0 emu/gFe for Fe147. These values are significantly smaller than the bulk value of 55 emu/gNi and 220 emu/gFe at 300 K[26]. The M–H loop for the Fe55 DEN shows a hysteresis-free magnetism and complete saturation of the material was not observed over the range of magnetic fields studied. The absence of hysteresis and a blocking temperature indicate that the Fe55 DENs are super paramagnetic down to the lowest temperature used due their small particle size[27]. Our results showed that there are a small variation in magnetic properties of magnetite nanoparticles prepared using *Magnetospirillum* under different incubation temperatures. This indicted that and with agreement with the previous studies that it may be a slit effect of temperature on the magnetic properties of magnetite nanoparticles.

6. Conclusion

We concluded that synthesis of magnetite naoparticles using *Magnetospirillum* strain AMB-1 is a promising method in order to obtain nanoparticles with ideal size and magnetic properties suitable for biomedical applications. It is clear that the physical conditions under which bio-synthesis of magnetite naoparticles had been done, could have a small effect on their characterizations. Further study on the biocompatibility and toxicity of the bio-synthetic magnetite nanoparticles should be done to evaluate their suitability to be used in both medical and biological applications.

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