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Research Article

Microbial-Physical Synthesis of Fe and Fe_3O_4 Magnetic Nanoparticles Using *Aspergillus niger* YESM1 and Supercritical Condition of Ethanol

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Magnetic Fe and Fe_3O_4 (magnetite) nanoparticles are successfully synthesized using *Aspergillus niger* YESM 1 and supercritical condition of liquids. *Aspergillus niger* is used for decomposition of $FeSO_4$ and $FeCl_3$ to FeS and Fe_2O_3, respectively. The produced particles are exposed to supercritical condition of ethanol for 1 hour at 300°C and pressure of 850 psi. The phase structure and the morphology measurements yield pure iron and major Fe_3O_4 spherical nanoparticles with average size of 18 and 50 nm, respectively. The crystal size amounts to 9 nm for Fe and 8 nm for Fe_3O_4. The magnetic properties are measured to exhibit superparamagnetic- and ferromagnetic-like behaviors for Fe and Fe_3O_4 nanoparticles, respectively. The saturation magnetization amounts to 112 and 68 emu/g for Fe and Fe_3O_4, respectively. The obtained results open new route for using the biophysical method for large-scale production of highly magnetic nanoparticles to be used for biomedical applications.

1. Introduction

Recently, industrial demands for magnetic nanoparticles have increased as the number of potential applications for the material has grown. Due to their impact in many fields of applications such as drug delivery, magnetic resonance imaging, cell separation, antimicrobial activities, and hyperthermia treatment for cancer, soft magnetic nanoparticles have attracted the attention of scientists in the last few decades [1]. For that reason, many researches were performed regarding synthesis and optimization of these magnetic particles. However, it is hard to cheaply mass produce homogeneous magnetic nanoparticles between 5 and 50 nm. Successful commercialization of the technology hinges on factors of quality, cost, and availability. Many methods were developed for the synthesis of these particles that can be classified into chemical and physical methods. However, these methods are not suitable to produce biocompatible materials and the use of toxic chemical greatly limited their biomedical applications [2]. Therefore, the development of ecofriendly and nontoxic methods for nanoparticles synthesis is of utmost importance to expand their biomedical application [3]. These biological methods are manipulated by using bacteria, fungi, algae, actinomycetes, and plants [2]. One of the most well-known biological methods for production of magnetic nanoparticles is through magnetotactic bacteria. Magnetotactic bacteria are known to synthesize magnetite (Fe_3O_4) by a direct mechanism of mineralization. They produce intracellular magnetite (magnetosomes) of high purity and crystallinity, which shows consistent morphologies and narrow grain-size distribution that are within single domain size ranges [4]. Recently, the microbially mediated process became suitable for incorporating other metals into magnetite, including Co, Ni, Zn, Cr, Mn, and Pd and rare earths such as Nd, Gd, Er, Ho, and Tb. The work presented here focuses on the development of a low cost approach to produce Fe_3O_4 and Fe magnetic

nanoparticles having uniform size ranges within 5–100 nm. Fungi cultivation process can be a novel approach for large-scale production of nanometer-sized crystalline Fe_3O_4 and Fe nanoparticles. The biological agents secrete a large amount of enzymes, which are capable of hydrolyzing metals and thus bring about enzymatic reduction of metals ions [5]. In case of fungi, the enzyme nitrate reductase is found to be responsible for the synthesis of extracellular nanoparticles [6].

Herein, we are focusing on fungal synthesis of magnetite and iron magnetic nanoparticles rather than other microorganisms due to their ease in handling and low cost maintenance, as well as easy downstream process [7]. There are two possible mechanisms in production of nanoparticles by using fungi either extracellular or intracellular [8]. In extracellular method, microorganism usually secretes enzymes extracellularly to degrade particles [9]. Meanwhile, in the intracellular method, microorganism takes up particles inside its cells and begins to degrade them by intracellular enzymes [10]. However, in order to develop the magnetic behavior of the formed particles, another physical process is needed such as supercritical condition of fluid [11]. Such method helps in reduction of the formed particles by fungi to a stable magnetic phase after the heat treatment at the supercritical condition of fluid. The new approach of the combination of the biological and the physical processes is explained in the schematic diagram shown in Figure 1. Hence, in the work at hand, the combination of two methods (microbial and physical) is used for the synthesis of pure iron (Fe) and iron oxide (Fe_3O_4) magnetic nanoparticles.

2. Material and Methods

2.1. Microbial Process: Preparation of FeS and Fe_2O_3 Using Aspergillus niger. Production of Fe_3O_4 and Fe nanoparticles employing microbial processes in combination with physical process is demonstrated as shown in Figure 1. This approach presents high yield and good reproducibility, as well as low cost. *Aspergillus niger* YESM 1 isolated from Egypt [12] was cultivated in liquid Sabouraud Dextrose Media composed of 20 g/L dextrose and 10 g/L peptone and kept for 7 days in shacking incubator at 25°C. Then, the fungal homogenate is formed by taking a known weight (12.5 g) of the mycelia and suspending it in 50 mL of sterile water, and, by means of a cyclomixer, homogenized mycelia cells solution of concentration 250 mg/mL is ready. The second step is to add separately each of 10 mL of 2000 ppm $FeSO_4$ and $FeCl_3$ salts solution to 10 mL of the fungal homogenate solution and keeping them for 6 days in 2 separated flasks at room temperature. After 6 days of static incubation, the metal/fungus solutions were centrifuged at 6000 rpm for 30 min and the pellets were collected, washed with ethanol, centrifuged at 14000 rpm for 10 min, and dried at 65°C. The obtained pellets were characterized using X-ray diffractometer to identify a formation of FeS and Fe_2O_3 phase. Thereby, in the presence of the fungal solution, the $FeSO_4$ and $FeCl_3$ decomposed to form FeS and Fe_2O_3 particles, respectively.

2.2. Physical Process: Synthesis of Fe and Fe_3O_4 Nanoparticles at Supercritical Condition. Supercritical fluid (SCF) technology has also been proposed for manufacturing magnetic nanoparticles in a controlled regime. A SCF is a substance that exists as a single phase above its critical pressure and temperature [13, 14]. Some examples of SCFs being used in nanomaterial production are triethanol, acetone, carbon dioxide, diethyl ether, propane, nitrous oxide, and water. Employing the SCF method in combination with the microbial method for the synthesis of magnetic nanoparticles such as Fe_3O_4 and Fe nanoparticles allows the use of organic solvents to be avoided, thus representing a green science approach. Here, the formed FeS and Fe_2O_3 by the microbial method were injected separately to a stainless steel vessel in presence of liquid ethanol and heated up to 300°C for 1-hour reaction. Once the temperature inside the sealed vessel reached 300°C, the observed pressure was 850 psi and the ethanol becomes in the supercritical condition. The system was then left to cool down to room temperature and the magnetic particles were collected by permanent magnets.

2.3. Characterization of Nanoparticles. PANalytical X'Pert Pro MPD diffractometer under Cu Kα (λ = 1.5418 Å) was used for the phase structure measurement of the produced samples. Hitachi SU-70 scanning electron microscope (SEM) was used for investigating the morphology of the produced particles. Quantum design VersaLab Vibrating Sample Magnetometer (VSM) was used for magnetic properties measurements up to 3 T.

3. Results and Discussion

As mentioned before, during the microbial process, $FeSO_4$ decomposed to FeS which was trapped electrostatically inside *Aspergillus niger* mycelia cells and was kept intracellularly (Figure 2(a)). Briefly, the formed enzymes during the fungal cultivation process which are present in the cell wall bioreduced the metal ions and FeS nanoparticles are formed [9]. *Aspergillus* kept the formed FeS inside its cells and, during exposure to supercritical condition of liquid, FeS is reacted with the ethanol to form Fe particles and H_2S gas which acts as inert gas prevents Fe particles from further oxidation. In case of $FeCl_3$, no trapping has been caused by the fungus as shown in Figure 2(b), but we might assume that fungus secreted extracellular enzymes that led to decomposition of $FeCl_3$ to Fe_2O_3. The formed Fe_2O_3 was exposed to the supercritical condition reactor at 300° and 850 psi which was reacted with the supercritical ethanol to form a stable phase of iron oxide called magnetite (Fe_3O_4). Both the Fe and Fe_3O_4 magnetic nanoparticles were collected after cooling down the pressure reactor by permanent magnets.

The phase structure and the morphology of particles synthesized by *Aspergillus* have been characterized using XRD and SEM, respectively. Figure 3(a) displays the XRD pattern for the formed FeS particles by fungus before the physical process. The peaks are matching perfectly the standard reference peaks for FeS phase (blue bars). Figure 3(b) shows the XRD pattern for the formed Fe_2O_3 particles by the fungus before the physical process. The peaks are matching standard reference peaks of Fe_2O_3 phase structure.

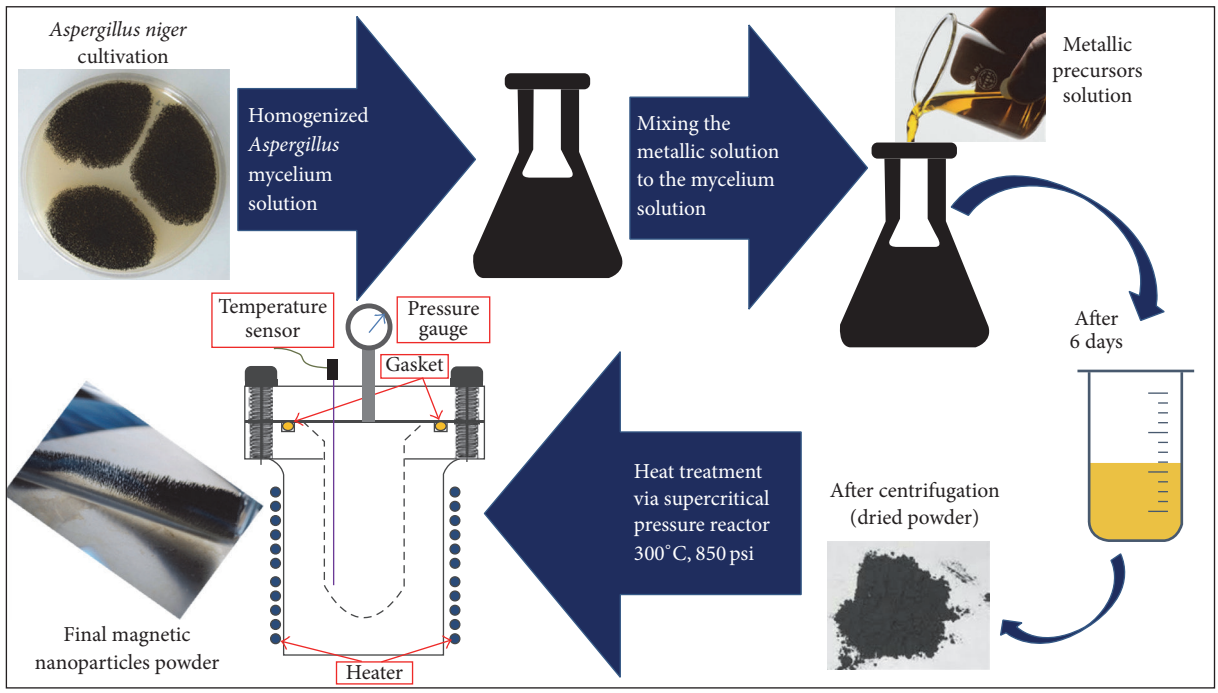
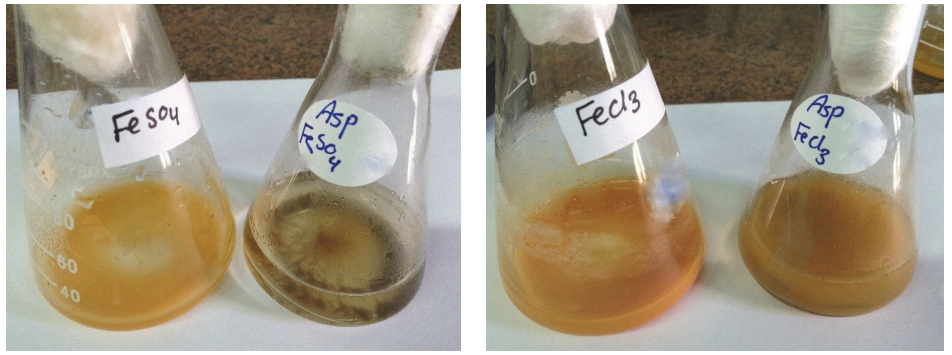


FIGURE 1: Schematic diagram of the biological-physical method for synthesis of magnetic nanoparticles.



(a) (b)

FIGURE 2: (a) *Aspergillus niger* liquid culture illustrating the trapping of $FeSO_4$ by the fungal cells, leaving the above solution clear. (b) *Aspergillus niger* in case of $FeCl_3$: no trapping happened and solution is turbid.

The morphology of the FeS nanoparticles was characterized by SEM as shown in Figures 4(a) and 4(b). The figures show *Aspergillus* cells trapping FeS particles; black arrows point to FeS particles, while the blue arrows point to *Aspergillus niger* cells. The presence of the particles was confirmed using energy dispersive X-ray (EDX) analysis unit which is attached to SEM. Figure 4(c) shows Fe_2O_3 particles formed outside fungus cells, which are pointed to by red arrows.

The phase structure and the morphology of the synthesized magnetic particles formed after heat treated at the supercritical condition of ethanol have been characterized using XRD and SEM respectively. Figure 5(a) displays the XRD pattern for pure Fe formed using supercritical conditions. The peaks are typical Fe cubic phase structure without presence of any related oxides peaks. The characteristic peaks for Fe nanoparticles appear at 43, 50, and 73°C and, from the full width of half maximum of the peaks, the crystal sizes were determined using Sheerer's equation [1] to be 9 nm.

Figure 5(b) shows the XRD pattern for the Fe_3O_4 particles formed after heat treatment of Fe_2O_3 particles at supercritical condition of ethanol. The synthesized sample using these precursors was characterized by XRD to be 90% of Fe_3O_4 and 10% of Fe_2O_3 phase structures. The characteristic peak for Fe_3O_4 nanoparticles appears at "30, 35, 37, 43, 47, 53, 57, 63, 66, 67, and 70°C" and, from the full width of half maximum of the peaks, the peaks reveal grain size of 8 nm for the formed Fe_3O_4 nanoparticles.

The morphology of the prepared nanoparticles was discovered by SEM, as shown in Figure 6. Figure 6(a) shows

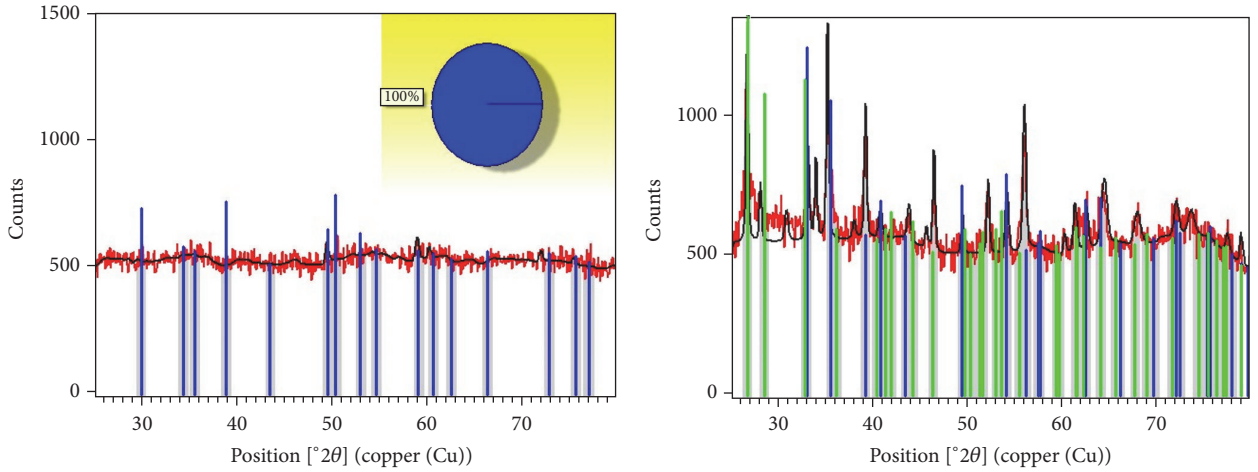


FIGURE 3: XRD analysis of nanoparticles formed using *Aspergillus niger* cells: (a) FeS and (b) Fe_2O_3 nanoparticles.

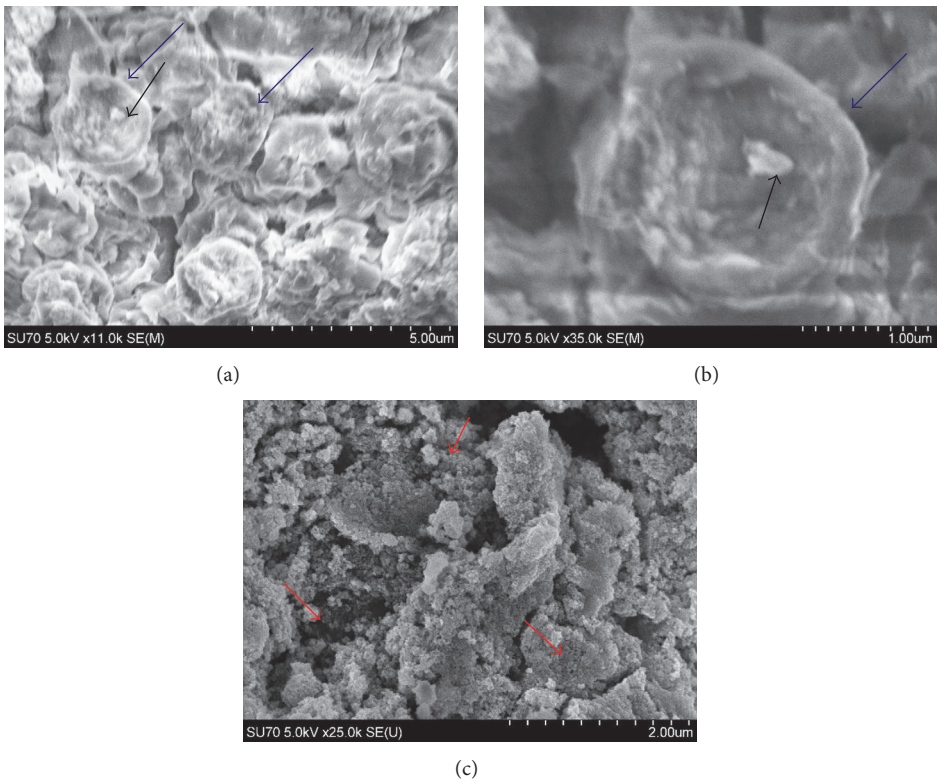
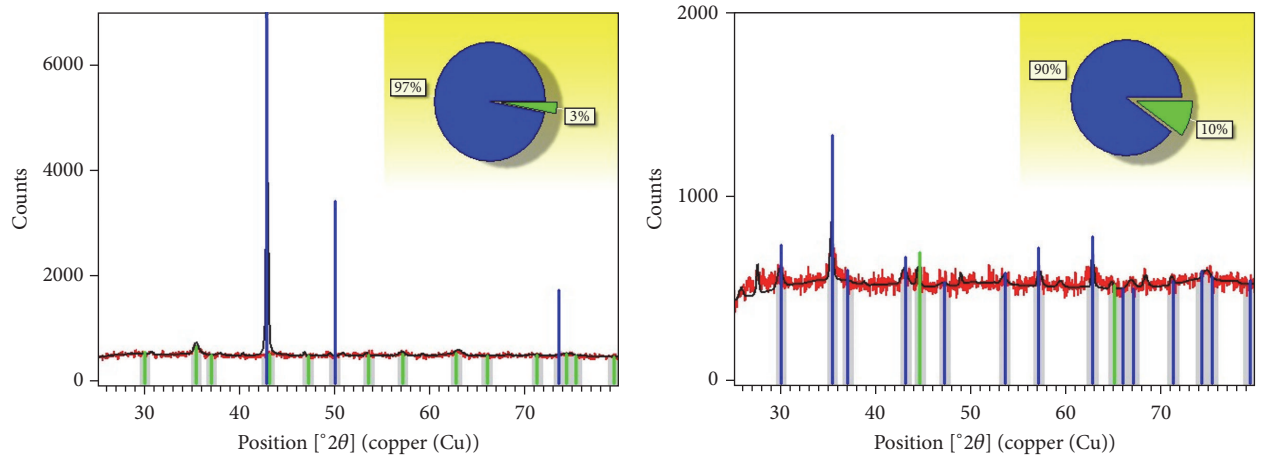


FIGURE 4: SEM micrographs of (a, b) FeS nanoparticles trapped by *Aspergillus niger* and (c) Fe_2O_3 nanoparticles formed by *Aspergillus niger* (c).

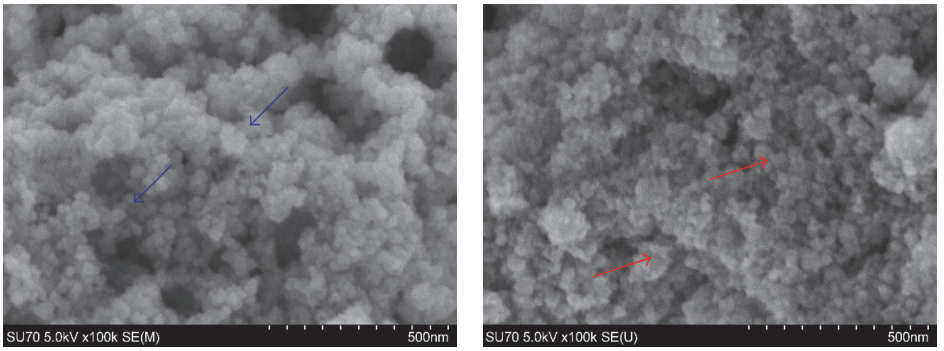
the formation of spherical Fe nanoparticles. Figure 6(b) shows Fe_3O_4 nanoparticles formed after using supercritical conditions of liquids. From SEM images, the size distribution of Fe and Fe_3O_4 nanoparticles yield average particle size of "18 ± 2" and "50 ± 1," respectively, as shown in Figure 7.

In order to investigate the magnetic properties of the samples, the VSM was used up to 3T at room temperature. Figure 8(a) shows the measured hysteresis loops of the formed Fe nanoparticles when exposed to external magnetic field. The Fe sample exhibits closed hysteresis loop that reveals superparamagnetic-like behavior with saturation magnetization Ms of 112 emu/g and with small remanence and coercivity. From the initial slope of $M \times H$ curve we can get information regarding the magnetic domain size of the formed particles [15] to be 7 nm for the synthesized Fe nanoparticles (Figure 8(b)).



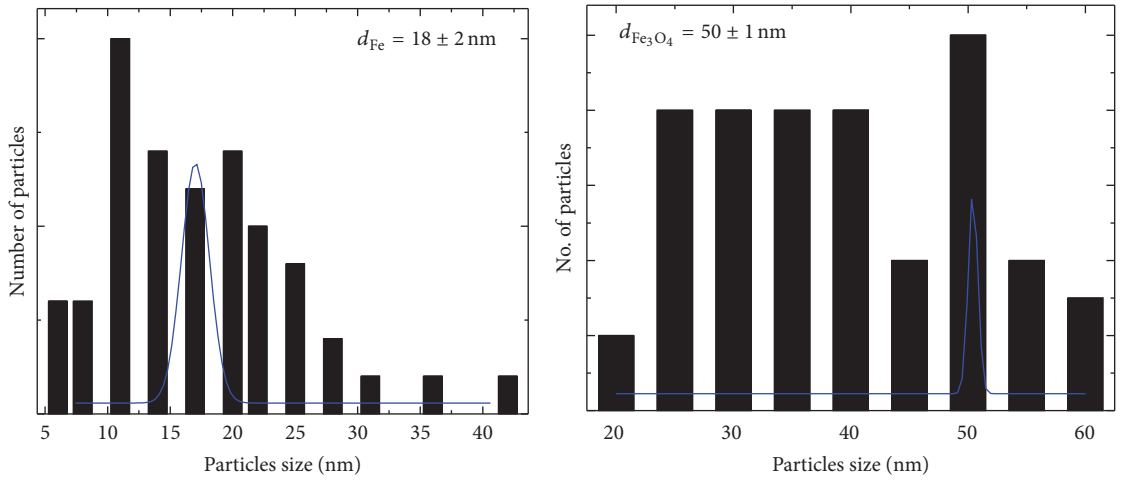
(a) Fe (b) Fe_3O_4

FIGURE 5: XRD analysis of nanoparticles formed at supercritical condition. (a and b) illustrate presence of Fe and Fe_3O_4 peaks, respectively.



(a) (b)

FIGURE 6: SEM micrographs of (a) Fe nanoparticles and (b). Fe_3O_4 nanoparticles.



(a) (b)

FIGURE 7: Size distribution of (a) Fe and (b) Fe_3O_4 nanoparticles extracted from SEM analysis. The blue line is Gaussian fitting.

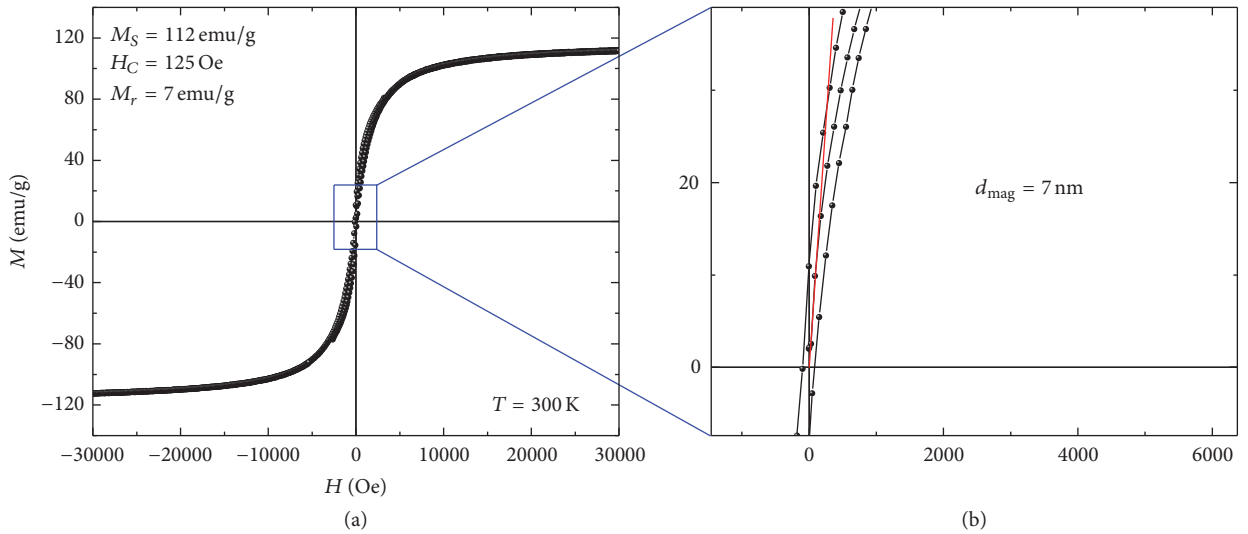


Figure 8: (a) Magnetization dependence on external magnetic field at room temperature for Fe nanoparticles. (b) Determination of magnetic domain size from the initial slope of $M \times H$ curve.

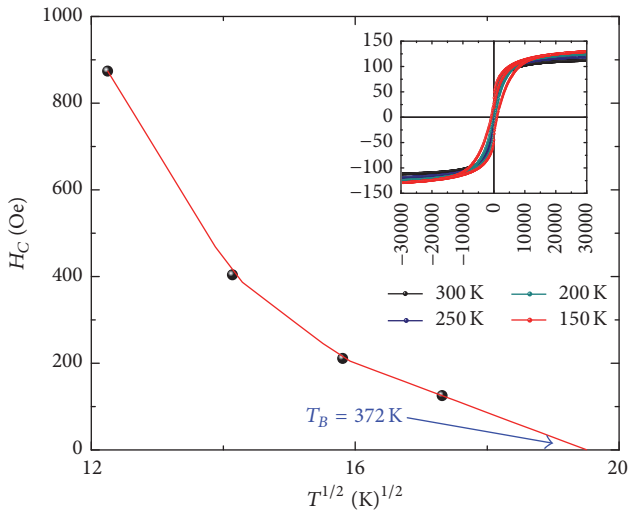


Figure 9: Coercivity dependence on temperature for Fe nanoparticles.

For all the previous investigations, the hysteresis loops were measured at different temperatures in order to determine the blocking temperature T_B which distinguishes between the ferromagnetic and the superparamagnetic behaviors (Figure 9).

The data from the hysteresis loops at different temperatures are listed in Figure 9 to exhibit decrease in the coercivity with temperature revealing T_B at $H_C = 0$ to be 372 K. Such value is suitable for using the synthesized nanoparticles for hyperthermia treatment for cancer [1]. On the other hand the Fe_3O_4 sample exhibits ferromagnetic-like behavior with magnetization M_s of 68 emu/g and observable values for H_C and M_r (Figure 10(a)). The magnetic domain size was calculated for the Fe_3O_4 nanoparticles to be 9 nm as shown in Figure 10(b).

The result provides better morphology and magnetic properties more than all the previous work reported by many researchers who used only the microbial method such as Bhargava et al. [16] and Lee et al. [4]. Such good results open route for many applications such as hyperthermia for cancer treatment and contrast agent for MRI and drug delivery.

4. Conclusion

Magnetic pure Fe and Fe_3O_4 nanoparticles were synthesized successfully using the fungus *Aspergillus niger* and supercritical condition methods. The XRD data showed exact peaks for the formed Fe and Fe_3O_4 nanoparticles. The SEM images reveal spherical nanoparticles for the formed particles which are suitable for heating mechanisms for hyperthermia treatment for cancer. The magnetic measurements yield good magnetic properties for the formed particles. The obtained values for magnetization and coercivity allow for the Fe nanoparticles to be used for medical application such as contrast agent for magnetic resonance imaging and hyperthermia treatment for cancer. The obtained magnetic nanoparticles with good yield using the microbial and the supercritical condition methods open new routes for large-scale synthesis of the magnetic nanoparticles with good properties for medical applications. Future work regarding the use of these particles for in vitro and in vivo biological experiments will be done.

Competing Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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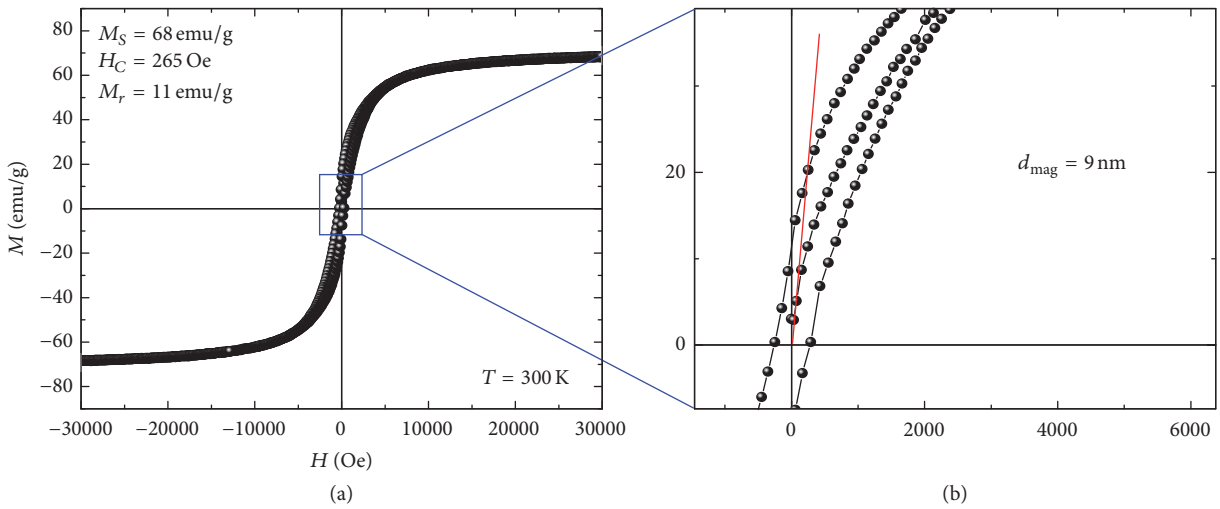

FIGURE 10: (a) Magnetization dependence on external magnetic field at room temperature for Fe_3O_4 nanoparticles. (b) Determination of magnetic domain size from the initial slope of $M \times H$ curve.

them to do the measurements. They also thank Microbiology Laboratory, Faculty of Science, Alexandria University, for participating in biological part.

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