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Biogenic oxides from neutrophilic iron bacteria and possibilities for application in the nanotechnology

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Abstract. The aim of this study is to obtain and characterize the ferric oxides/(oxy)hydroxides formed after cultivation of bacteria under laboratory conditions. The pure cultures of these bacteria isolated from natural habitats are identified by the methods of classical and molecular taxonomy as strains of the *Leptothrix* genus. Adler (AM) and Silicon iron glucose peptone (SIGP) media are the most appropriate ones for obtaining the iron oxides. The characterization of the oxides and sheaths is performed by different physical methods. The sheaths are formed in a SIGP medium. Light micrograph images and SEM revealed the average size and diameter of the sheaths. The XRD measurements showed the composition of the oxides obtained, as well as the average size of the iron particles (up to 30 nm). The TEM micrographs showed the shape of the biogenic nanoparticles, while the magnetic measurements demonstrated the superparamagnetic character of the magnetic part of the biomaterials. The new biogenic materials are promising for application in magneto electronic for building biosensors.

1. Introduction

The field of nanosized biogenic iron oxides is truly multidisciplinary. The objects of this investigation are the iron sub-products obtained by laboratory cultivation of neutrophilic iron bacteria. These bacteria are a large physiological group with different taxonomic status but united by their ability to oxidize Fe(II) at neutral pH [1] in the appropriate nutrient media. As a result of their metabolism, they form biogenic iron oxides/(oxy)hydroxides accumulated on the nanosized powder and organic sheaths. In the tubular structures, the iron oxide formations are magnetic single-domains discretely dispersed into an organic matrix. The superparamagnetic iron oxides domains with appropriate surface chemistry are promising materials for magneto electronic applications as biosensors. On the other hand, this structure absorbs light in the IR spectrum and can self-assemble in a magnetic field and replace the supra-molecular assembly of light sensitive polymers thus opening up possibilities of constructing molecular-level devices.

The bacteria-mediated formation of iron-containing tubular structures and their magnetic properties are currently poorly understood. It is also not clear why many of the strains do not form sheaths during cultivation under laboratory conditions [2]. This investigation is focused on the iron sub-products containing powder and sheaths obtained by two different types of cultivation, which are developed in the Department of General and Industrial Microbiology, Faculty of Biology, Sofia University [3]. The neutrophilic iron bacteria from the *Leptothrix* genus isolated from Vitosha Mountain (nearby Sofia, Bulgaria) are identified by the methods of classical [1] and molecular taxonomy.

2. Materials and methods

The cultivation of the bacteria of the *Leptothrix* genus is carried out in the following two different nutrient media:
(AM) - Adler’s medium – modification: Sodium lactate - 40.0 mg; Yeast extract - 1.0 g; Ascorbic acid - 0.1 g; MgSO₄·7H₂O - 0.2 g; K₂HPO₄ - 0.01 g; (NH₄)₂Fe(SO₄)·6H₂O - 0.01 g; dH₂O - 1000 ml; pH 7.0.

(SIGP) - Silicon iron glucose peptone: 1 g glucose, 1 g Bacto peptone (BD, France), 0.2 g Na₂SiO₃·9H₂O, 0.044 g CaCl₂·2H₂O, 0.041 g MgSO₄·7H₂O, 0.076 g Na₂HPO₄·12H₂O, 0.02 g KH₂PO₄·2H₂O, 2.838 g HEPES (N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid), and 0.05 mM FeSO₄ in 1000 ml of distilled water (pH 7.0).

As additional source of iron, iron cuttings are supplemented to both media. The cultivation is carried out at 20 °C and a pH of 7.0. The changes in the pH and the content of Fe(II) in the cultivation vessels are monitored periodically. During the whole period of cultivation, a microscopic observation is performed of the cultural liquid from all samples.

The analysis of the iron oxides/(oxy) hydroxides is performed by X-ray diffraction (XRD) which is used to determine the phase composition of the biogenic samples. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) are used to study the bacteria ability to produce sheaths in such an environment. The magnetic properties of the samples are measured by a physical properties measurement system (PPMS, Quantum Design). The real and imaginary parts of the differential magnetization MAC are measured from zero-field-cooled (ZFC) 10 K to 300 K in an AC 10-Oe magnetic field at a frequency of 1 kHz. Hysteresis loops at 300 K up to 10 kOe are also recorded of both types of samples.

All measurements are conducted on samples taken after 40 days of cultivation in order to determine the material type. The cultural liquid of the samples is filtered and dried.

3. Results and discussions
The bacteria formed the typical sheaths (figure 1a) during the cultivation on SIGP medium, while on AM medium the bacterial cells are observed only (figure 1b). The formation of the sheaths started after a seven-day cultivation period. Their average diameter is in the range of 500 – 1000 nm, with the length reaching approximately 7 μm. The structures disintegrated completely approximately after 90 days since cultivation.

![Figure 1](image_url)

Figure 1. SEM of sample from: a) a SIGP medium, ×5000; and b) an AM medium, ×5000.

The XRD spectra of the biogenic material obtained showed the type of the oxides/(oxy) hydroxides, their percentage ratio and the particle size [4]. The XRD data for the AM sample revealed three phases – lepidocrocite (γ-FeOOH), goethite (α-FeOOH) and magnetite (Fe₃O₄), with dimensions of the particles up to 30 nm. In the material collected from the SIGP-cultivated Leptothrix spp., the XDR spectra revealed a single-phase composition – lepidocrocite (γ-FeOOH) with an average diameter of the particles of 8 nm [5]. The tubular samples spectrum revealed the existence of an organic matrix.

As can be seen in figure 2 and 3, the magnetization of sample AM is one order of magnitude higher than that of SIGP. Such a result is to be expected because the SIGP sample consisted of 100 % antiferromagnetic (AF) lepidocrocite, while there is ~ 20 % of magnetite present in the AM sample. Nevertheless, the imaginary part of the differential magnetization corresponding to magnetic losses
increased rapidly at low temperatures and reached a sharp maximum at about 30 K for both samples. Moreover, the differential magnetization real part in the temperature range 10 – 50 K increased nonlinearly and also rapidly. Such an identical behavior of the differential magnetization could be due to a temperature transition in lepidocrocite. Under these measurement conditions, the peaks of the real and imaginary parts of the differential magnetization of magnetite are at about 215 K and 125 K, respectively [6]. At higher temperatures (above 50 K), both magnetization real parts became linear; that of AM increased slightly up to 300 K, while for SIGP, saturation could be seen up to 160 K, followed by a linear increase up to 300 K (figure 2). These differences at higher temperatures are due to the different compositions of the samples. The hysteresis loops at 300 K until 10 kOe seemed to be the same except for the strength of magnetic response.

![Figure 2](image.png)

**Figure 2.** Temperature dependence of the real and imaginary parts of differential magnetization MAC of the sample a) AM and b) SIGP at an AC magnetic field 1 kHz, 10 Oe. The circles show the real part, the asterisks, the imaginary part. The insets show the magnetization around the temperature transition.

![Figure 3](image.png)

**Figure 3.** The hysteresis curve $M(H)$ at 300 K up to 10 kOe for samples Adler (AM) and SIGP.

5. Conclusions
Two iron-containing biogenic compounds with different magnetic nature are compared. One of the samples consisted of 100 % AF lepidocrocite, while the other sample is a mixture of AF (lepidocrocite
and goethite) and ferrimagnetic (magnetite) components. The magnetic hysteresis loops at 300 K up to 10 kOe and the differential magnetization vs. temperature up to 50 K had the same behavior as AF lepidocrocite. The temperature dependence of the differential magnetization above 50 K of the two samples showed a different behavior. The magnetization of the magnetically mixed sample AM increased linearly and slightly until 300 K, while the magnetization of the AF SIGP sample saturated up to 160 K and increased linearly afterwards. Additional magnetic measurements are needed to understand fully the magnetic nature of these compounds.

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