Biogenic ferroxides derived from *Leptothrix* bacteria for applications in electronics, biomedicine and biotechnology

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**Abstract.** The present work is focused on studying by-products derived from the metabolism of bacteria of the *Leptothrix* genus, which are among the first described microorganisms associated with the iron cycle in nature. The products of their metabolism are nanostructured biogenic iron oxides in the form of precipitating powders and sheath structures. The sheath structures can be considered as an organic matrix in which inorganic crystallites are discretely dispersed. We used X-ray diffraction, magnetic measurements, light microscopy and scanning electron microscopy to characterize biogenic products formed in a silicon iron glucose peptone medium under laboratory conditions. The studies showed a lack of significant differences between the naturally obtained and the artificially synthesized biogenic sheaths, i.e., an adequate laboratory technological process had been developed. From the point of view of nanoelectronics application, these biogenic by-products are unique because they are biocompatible, have specific electromagnetic properties and are potential candidates for various applications in biomedicine and electronics.

1. **Introduction**

The bacteria from the *Leptothrix* genus are typical \(\beta\)-proteobacteria capable of oxidizing \(\text{Fe}^{2+}\) and \(\text{Mn}^{2+}\) at neutral pH and are commonly found in aquatic environments \([1, 2]\). They grow as chains of cells into filaments 0.4 – 7 \(\mu\)m in width \([1]\).

This work is focused on studying by-products derived from the metabolism of *Leptothrix* bacteria. These bacteria do not pose a danger to the human body. Products of their metabolism are nanostructured biogenic iron hydroxides in the form of yellow/brown mats, which include also their sheath structures. As a result of the oxidation of iron cations (\(\text{Fe}^{2+} \rightarrow \text{Fe}^{3+}\)), different iron containing compounds are produced (oxyhydroxides, such as lepidocrocite, \(\gamma\)-FeOOH, etc.). The biotic or abiotic processes can continue in the walls of the sheath structures until ferroxide structures, such as magnetite (\(\text{Fe}_3\text{O}_4\)), maghemite (\(\gamma\)-Fe\(_2\)O\(_3\)), are formed. The review of the literature shows that there exist unclear points regarding the causes of oxidation of iron ions and their contribution to the metabolism of these environmentally important bacteria. The results of the research related to the formation of sheath structures are quite contradictory, as are the factors that influence their formation \([1]\). The sheath structures can be considered as an organic matrix in which inorganic crystallites are discretely dispersed. From the point of view of nanoelectronics application, these biogenic by-
products are unique because they are biocompatible, have specific electromagnetic properties and can be potential candidates for various applications in biomedicine, electronics [2], as pigments, catalysts, adsorbents, carriers [3-8] and as precursors for synthesis of the hybrid battery–super capacitor system „bioFe$_2$O$_3$+AC)/LiBF$_4$/AC“ [6]. Magnetized sheath structures could be oriented in a magnetic field higher than the saturation magnetization of the impregnated sheath structures; together with their anisotropic properties; this makes them suitable for creating biochips for use in electronics. These bioproducts open possibilities for application in biomedicine, for example radio frequency protection of pacemakers or other implants in a living organism.

The interest in materials produced by Leptothrix bacteria has grown in recent years [7, 9-12]. We present a study on iron bio-ferroxides in the sheathed structures of Leptothrix sp. during laboratory cultivation in a selective medium (SIGP medium [2, 10]). Such thorough investigations could have a major impact on applications concerning the industrial synthesis of Fe(III)-bearing compounds and on the possibility for these techniques to be replaced by environmentally-friendly biosynthesis procedures [13].

2. Materials and Methods

A pure culture from Leptothrix genus was used isolated from a natural stream in Vitosha Mountain located at an altitude of 1783 m (42°35’15”N/23°14’55”E) [10]. For the formation of sheath structures, laboratory cultivation was performed in a silicon iron glucose peptone (SIGP) medium [10]. The isolate was inoculated into the medium with iron cuttings and glass plates on the bottom serving for attachment of the bacteria and cultivated under dynamic conditions (70 rpm) at 20 °C.

Studying the morphology of the by-products formed in an SIGP medium under laboratory conditions was performed by light microscopy and scanning electron microscopy (SEM) (JEOL JSN-5510, JEOL, Japan). The structural characterization of the biogenic iron oxides was carried out by X-ray diffraction using a Bruker D8 diffractometer in the Bragg–Brentano reflection geometry with Cu Kα radiation ($\lambda = 1.5418$ Å). The magnetic measurements were measured by a physical properties measurement system (PPMS, Quantum Design).

3. Results and Discussion

The cultivation of Leptothrix sp. in an SIGP medium resulted in an increase in the fluffy ochrous deposits in the flasks. Light micrograph (figure 1a) and SEM (figure 1b) images revealed the approximate size of the sheath structures formed in a seven-day period of cultivation – around 10 μm with an average diameter of up to 1 μm (figures 1 and 2). Their diameters and lengths varied, probably depending on their maturity [2]. Spring [11] reported that the sheaths structures of Leptothrix sp. are brittle and easily broken into shorter fragments. Therefore, measurements of the length may not be reliable for these structures.

![Figure 1. Light a) and SEM b) images of sheath structures formed in an SIGP medium.](image-url)
The sheath structures obtained under laboratory conditions were compared with those taken from the streams of Vitosha Mountain using light microscopy and SEM (figure 2). Such structures are found in the natural environment samples; most of the sheaths are empty – they do not contain bacteria and their structure fully corresponds to the descriptions given by other authors [2, 12].

![Figure 2. Light a) and SEM b) images of sheath structures grown in a natural environment.](image)

The studies showed a lack of significant differences between the naturally and laboratory obtained biogenic sheaths, i.e., an adequate laboratory technological process had been developed and used for the production of such a useful material.

The results about the dynamics of the ferrous ions concentration during cultivation of the bacteria in an SIGP medium measured by titration with potassium permanganate (0.1 N KMnO₄) are given in figure 3. The rate of chemical oxidation of the ferrous ions is very low in the control sample tested.

The XRD data from our previous investigation of the same sample showed a single-phase composition of the iron-containing particles on the sheaths structures obtained (lepidocrocite – γ-FeO(OH)), as well as a uniform size of the iron oxihydroxide particles – 8 nm [10]. The spectrum confirmed the presence of iron oxide compounds in the walls of the sheath structures (the spectrum was obtained from a coating of biogenic sheath structures on a glass substrate and shows also the background lines of the substrate). Lepidocrocite is antiferromagnetic and in practice the sheath structures have no magnetic properties at room temperature in the earth's magnetic field.

The SEM/EDX analysis in our previous investigation [10] revealed that S, O, C, K, Ca, Na, Si, Fe, and P are the inorganic elements of cultured mature sheath structures. These results agree with those of other studies, which reported that the major inorganic component of the sheath structures is in the form of iron oxides that bind other inorganics, such as Si, P, and often Ca and S [14, 15].

![Figure 3. Dynamics of oxidation of the ferrous ions during cultivation in an SIGP medium.](image)

![Figure 4. Magnetic hysteresis curve M(H) at 300 K of an SIGP sample.](image)
The magnetic measurements performed by a physical property measurement system (PPMS) of the SIGP sample showed a weak magnetic behavior \( (M \approx 1.6 \text{ emu/g at 10 kOe}) \) (figure 4).

The by-product obtained from the SIGP medium exhibited the properties of a soft magnetic material \( (H_c \approx 20 \text{ Oe}, M_{rem} \approx 50 \text{ memu/g}) \) with an indication of the presence of nanoscale particles at room temperature, which we associate with structural changes in the surface of the discretely dispersed lepidocrocite particles in the organic matrix of the sheath structures.

Such magnetic behavior is expected to arise from a relatively small quantity of low-magnetic-response material dispersed in a non-magnetic matrix.

The results of the magnetic measurements at room temperature of a powder sample from the SIGP medium with a high content of biogenic sheath structures showed nearly paramagnetic properties. Such sheath structures of biogenic origin can be enriched with nanoscale magnetite by a simple technological procedure (co-precipitation at room temperature), which can enhance the magnetic response of the material, while keeping the magnetic structure intact. Sheathed structures enriched with such magnetite nanoparticles can be capable of adsorbing various substances that endanger the environment, i.e., dyes, Fe\(^{2+}\) ions, heavy metals [16, 17].

4. Conclusion

The insoluble hydroxides formed by *Leptothrix* bacteria after oxidation of the ferrous iron are deposited on the bacterial surface, and specific sheath structures are formed after laboratory cultivation in an SIGP medium. The iron-containing part of the sheaths is single-phase lepidocrocite exhibiting a weak magnetic behavior at room temperature. These sheath structures, as well as the hydroxide, are of great interest for application in various nanotechnologies, biomedicine and bioengineering as ferrofluids, pigments, adsorbents, or as a contrast agent for magnetic resonance imaging, detoxification of biological fluids and many others.

Acknowledgements

This work was supported by the Bulgarian National Science Fund of the Ministry of Education and Science under project T02-17/2014.

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