# Magnetic and non Magnetic Characterization of Intracellular Biogenic Crystals Synthesized by Freshwater Magnetotactic Bacteria

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## ABSTRACT

Intracellular biogenic crystals synthesized by freshwater magnetotactic bacteria (MTBs) from La Fe Dam and Calima Lake – Colombia were separated from water/sediment samples using a magnetic field of 6 Gauss. Bacterial concentrates were observed by transmission electron microscopy (TEM) and analyzed with ray X microdiffraction (microXRD), Fourier transformed infrared spectroscopy (FTIR) and magnetic measurements. Magnetosomes were measured and analyzed with EDX/TEM (Energy dispersive X – ray analysis). Results showed magnetite crystals with cuboctahedral, hexagonal prismatic and bullet morphology; most crystals were classified in the single domain magnetic state, with an average length smaller than 100 nm. Samples with MTBs were saturated in a field close to 100 mT, showing ferrimagnetic behavior. These results open the path to others studies with MTBs in Colombia that could lead to find future applications for magnetic biominerals in nanobiotechnology and medicine.

***Keywords***: magnetotactic bacteria, biogenic magnetite, magnetosome, single domain, nanotechnology.

## 1 INTRODUCTION

Understanding the magnetic and non-magnetic properties of magnetite ($Fe_3O_4$) or greigite ($Fe_3S_4$) crystals produced by magnetotactic bacteria (MTBs) is important in fields of geoscience, biomineralization and nanoparticle magnetism [1]. However, the measurement of MTBs magnetic properties at the present state of the art do not allow generalizations due to difficulties in obtaining sufficient quantities of bacteria from natural environment and the difficulty for cultivation.

MTBs are a diverse group of motile, aquatic bacteria including vibrioid, coccoid, rod, spirilla and multicellular forms which orientate and migrate along the geomagnetic field lines [1,2,3,4]. Magnetosomes, crystals surrounded by a cellular membrane, are characterized by a size between 30 and 120 nm, chemical purity and a morphology specific of species [5.6]. Magnetic biominerals can be used in medical applications such as biosensing, cell separation, agent contrast, site specific chemotherapy and nanotechnology [7,8,9,10].

## 2 METHODOLOGY

### 2.1. Sampling and magnetic separation

In order to isolate MTBs, samples were collected from the water/sediment interface at depths between 160 - 310 cm in La Fe dam, Antioquia (6°6'57" north 6°6'00" north, 75º30'30" west 75º29'36" west) and Calima lake, Valle del Cauca (-76°38'32,78'' west, -76°31'33,16'' East, 3°57'55,7'' North y 3°50'54,24'' South), Colombia.

Samples used in all preparations were magnetically separated, using a magnetic field of ~6 Gauss, generated by a solenoid, according to literature [11,12].

### 2.2. Non magnetic techniques

***2.2.1 Transmission Electron Microscopy (TEM):*** Drops of water with MTBs magnetically separated were put onto Formvar-covered electron microscopy Cu grids. Samples were dried at room temperature. They were observed and analyzed in a Phylips Tecnai $G^2$ transmission electron microscope equipped with an accessory for energy-dispersive X-ray (EDX), operating at 200 kV. At the microscope MTBs, with intracellular magnetosomes, were studied to characterize their morphology. Measurements of length and width of crystals were made using the software Imagin Pro-Plus 6.2, for 350 crystals.

***2.2.2 X – Ray Microdiffraction (microXRD):*** MTBs magnetically separated were deposited in an eppendorf tube with a Neodymium magnet of 12.8 Gauss. Bacteria, washed with filtered lake o dam water, were concentrated onto a cellulose membrane with 0.22 µm porous size. Analysis was carried out in a Pananalytcal Diffractometer X'pert Pro MPD, using a sensor Pixcel A medipix[2], and Cu kα radiation. XRD spectral data were recorded from 5 and 80 2θ, 45kV and 40mA.

***2.2.3 Fourier Transform Infrared Spectroscopy (FTIR):*** MTBs magnetically separated, were deposited in an eppendorf of 1.5 ml. The water was extracted and the MTBs were dried and mixed with spectroscopy quality KBr. Vibrational spectra were recorded using Spectrum

One Perkin Elmer spectrophotometer. Transmittance spectral data were recorded from 4000 - 450 $cm^{-1}$.

### 2.3. Magnetic Measurements

Magnetic measurements at 80, 150 and 300 K were carried out. 20 µl of MTBs obtained by magnetic separation were encapsulated in paraffin. Hysteresis loop and magnetization saturation measurements were taken at a magnetic field of 10000 Oe (1 T), using a Quantum Design Vibrating Sample Magnetometer (VSM).

## 3 RESULTS AND DISCUSSION

Two different morphological types of live MTBs were observed in La Fe dam, they included coccoid and rod-shaped bacteria and only coccoid shape MTBs in Calima Lake. All of them aligned parallel to the direction of magnetic field and reversed their direction of motility by 180° when the field direction was reversed. MTBs collected were North seeking (NS).

### 3.1. Non magnetic techniques

#### *3.1.1. TEM study of magnetosomes.*

*Morphology of the biogenic crystals:* Magnetosomes observed in MTBs from La Fe dam showed an anisotropic morphology: magnetococci showed elongated hexagonal prismatic crystals (Fig. 1a-1c), while rod MTBs showed bullet – shaped crystals (Fig. 1d-1f). In Calima Lake, coccis with cubo-octahedral magnetosomes (Fig. 1g) and coccis with elongated hexagonal prismatic crystals (Fig. 1h-1i) were observed.

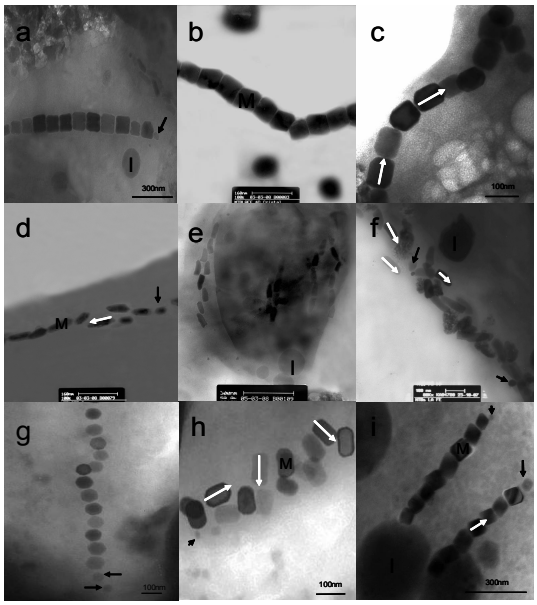


**Figure 1.** TEM imagen of MTB crystals. a – c) Elongated hexagonal prismatic crystals of coccis from La Fe dam. d – f) Bullet shaped magnetosomes of rod type MTBs La Fe. g) Coccis cube-octahedral magnetosome from Calima Lake. h – i) Coccoid elongated hexagonal prismatic magnetosomes from Calima lake. Grouped crystals (h) and crystals in chain (i). Black arrows show immature crystals. White arrows show the length of crystals. I (inclusion), M (magnetosome).

It has been hypothesize, that the biological hexagonal elongation observed in crystals of MTBs permits particles to achieve larger volumes and increase magnetic dipole moments without crossing into the multidomain state, allowing the cells to make fewer magnetosomes and to have the same magnetic orientation energy [13].

The length of magnetosome chains was different among cells taken at the same place of sampling, with an average of 1.09 and 3.8 µ for cocoid and rod MTBs from La Fe respectively, and 0.7 µ for cocoid MTBs with magnetosomes forming chain in Calima.

Elemental energy-dispersive X-ray analysis spectra of magnetosomes showed crystals composed of Fe and O but no sulfur in all MTBs observed (Fig. 2a, 2b). Spectra showed small quantities of P, S, K, Ca and Mg which possibly belong to bacterial inclusions near to magnetosomes, cytoplasm and cellular membrane. A sulphur inclusion is confirmed by EDX (fig. 2c) and in agreement with reports by other authors [14,15].

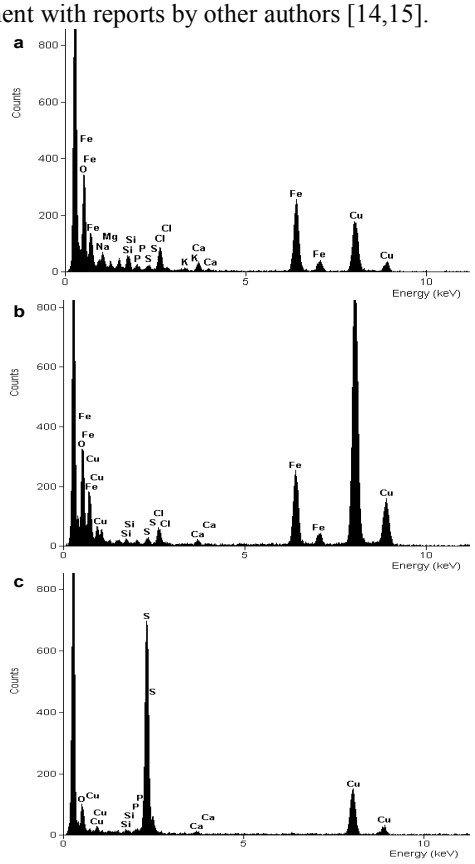


**Figure 2.** EDX spectra of magnetosomes. a) Spectrum of elongated hexagonal prismatic crystals from La Fe dam. b) Spectra of elongated hexagonal prismatic crystals from Calima Lake. c) Spectra of a inclusion belonging to an MTB from Calima Lake.


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*Number of magnetosomes per cell:* The number of magnetosomes per cell observed was 16 ± 3 and 90 ± 34 for coccoid and rod MTBs respectively from La Fe dam, and 15 ± 4 magnetosomes for magnetococcis from Calima Lake. This result shows a minimal difference between magnetococcis from the two places under study. The trend shown by these results is that the number of magnetic particles per cell depends not only on the growth conditions or nutrient availability, but also on the MTB strain, confirming that every strain execute a strict control on the synthesis and growth of crystal [4,6,16].

*Size of magnetosomes:* MTB magnetosomes from La Fe and Calima showed a wide crystal size distribution. In general, crystals are longer than wider, only 8.23% were wider than longer and 0.66% had a square shape. The crystal length was defined according with the direction of the chain extension throughout the cell. The shape factor analyses showed that crystals belonging to the coccis from La Fe are wider than those from Calima and that the rods have longest crystals. Mean values of the aspect ratio were 0.41, 0.84 and 0.7 for rods, coccis crystals from La Fe and coccis from Calima, respectively. These results are comparable with literature data where long magnetosomes of 35 to 140 nm are reported [6,17,18,19].

***3.1.2 X – ray Microdiffraction***

MicroXRD of a MTB concentrate showed two magnetite principal peaks at 33.5 and 43.35 2θ confirming that the intracellular crystals synthesized by the MTBs were magnetite and not greigite. Elemental Sulfur peaks were also seen at 19.979, 23.073 and 29.206 2θ corresponding to the sulfur inclusions inside the cell. Other minor peaks reported in the literature for magnetite were seen in 8.5, 14, 16.5, 18.26, 36.08, 43.35, 53.47, 58.43, 62.60, 71.28 and 74.34 2θ [1,20].

***3.1.3 Fourier transforms infrared spectroscopy (FTIR)***

Results of FTIR are relate to those obtained by microXRD and EDX/TEM, which shows presence of Fe compounds, showing that the magnetic minerals synthesized by MTBs in study could be magnetite. In this way, FTIR analyses showed Fe – O bands, located around of 632, 585 and 570 $cm^{-1}$, observed by other authors [21,22].

By FTIR were observed broad bands between 3414 and 1635 $cm^{-1}$ which are attributed to stretching vibrations N - H and the curved mode of $NH_2$ of free groups $NH_2$ respectively. These bands in our samples can belong to bonds presents in bacterial proteins, wall and cellular membrane and also to water vibration [21,22].

Also were observed bands in 2125 $cm^{-1}$. Magnetite nanocrystals covered by lipoproteins could have absorption bands in 2125 $cm^{-1}$, typical band of organic material due to stretching vibrations of C – H bonds; band observed on 886 $cm^{-1}$ is due to vibrating bend of $NH_2$ group [22] and this observed around 1090 $cm^{-1}$ apparently belongs to Si-O-Si bonds, present in sediment deposited during the concentration of MTBs [21,22].

**3.2. Magnetic Measurements**

Hysteresis loops (Magnetization (M) Vs. Magnetic field (H)) for MTB showed that samples were saturated in a field of 100 mT (1 KOe), indicating presence of ferrimagnetic minerals like maghemite or magnetite, at the three temperatures.

Comparing the values of Ms for biogenic and synthetic magnetite with similar particle sizes, temperature and magnetic field, it was observed that the synthetic magnetite has a larger saturation value than biogenic magnetite due to the presence of non-magnetic lipid membranes with 3 to 5 nm thick in the outer layer of magnetosomes [20].

Ms for MTBs from La Fe dam and Calima lake at 300 K were 6.8 x $10^{-5}$ y 3.75 x $10^{-5}$ emu respectively, suggesting that the relative quantity of cells on the samples was approximately 1.8:1, estimating a cells number of 3.7 x $10^{7}$ y 2.1 x $10^{7}$, for La Fe and Calima (assuming a magnetic moment of an individual MTB as 1.8 x $10^{-12}$ emu) [1, 23].

Hysteresis parameters are properties that depend mainly on grain size because they are influenced by the magnetic domain state of the sample, which is too a function of the grain size. Thus, reduced values of Mr/Ms (table 1) and the magnetosomes size are indicating a mixture of SD and SPM particles on MTB concentrates from La Fe y Calima, similar to literature data [24].

Using an Ms value of 0.0924 emu for pure magnetite [20], the magnetite content by mass of cell is 0.073% (MTBs from La Fe) and 0.04% (MTBs from Calima). The magnetite content of cells of these two places is significantly lower than observed in other strains of MTBs (*M. magnetotácticum* and strain MV-1), which have magnetite contents ranging from 0.5 to 2%, however our results are comparable with values obtained for *Desulfovibrio magneticus* strains RS1-AF and RS1-BS which reported values of 0.02% and 0.04% [19].

| | Temp. (K) | Ms ($emu10^{-5}$) | Mr ($emu10^{-5}$) | Hc (mT) | Hcr (mT) | Mr /Ms | Hcr /Hc |
|---|---|---|---|---|---|---|---|
| **Calima Lake** | 300 | 3,75 | 1,10 | 17,4 | 41,3 | 0,29 | 2,37 |
| | 150 | 3,72 | 1,19 | 25,5 | 51,3 | 0,32 | 2,01 |
| | 80 | 3,74 | 1,22 | 36,1 | 78,6 | 0,33 | 2,17 |
| **La Fe Dam** | 300 | 6,80 | 1,65 | 25,8 | 55,0 | 0,24 | 2,13 |
| | 150 | 6,50 | 1,75 | 31,3 | 68,2 | 0,27 | 2,16 |
| | 80 | 6,80 | 1,90 | 34,4 | 80,0 | 0,28 | 2,32 |

**Table 1.** Values of coercitivity (Hc), coercitivity remanence (Hcr), saturation remanence (Mr), saturation magnetization (Ms) and the ratio Mr/Ms at 80, 150 and 300 K for MTBs from La Fe and Calima.

Using the Day diagram, where the ratio Mr/Ms is plotted against the ratio Hcr/Hc, to provide information about a particle behaving as SD, MD (multiple domain) or PSD

(pseudo-single domain) [18,25], the MTB crystals from La Fe and Calima could be classified as PSD (Fig. 3), due to the presence of biogenic non-magnetic components which hide the magnetic properties of bacterial magnetite, reducing the efficiency of the technique to detect biogenic magnetite [24].

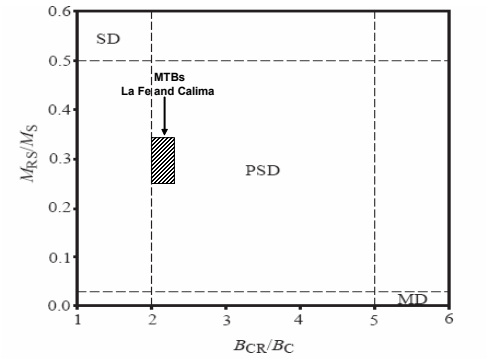


**Figure 3.** Day diagram. The black box shows the location of the crystals of MTBs from La Fe dam and Calima Lake.

A problem seen with the magnetic measurements was that biogenic magnetite is found on low levels and is necessary to capture a large number of MTBs in order to reach a proper signal to noise ratio for the instrument. To quantify the amount of sample used for the measurement is imprecise because the number of crystals in a cell varies depending on cell morphology. There is variability on the magnetosome morphology, which interferes with the results of the measurement, therefore, the availability of crystals in chain alignment or grouped affects the magnetic cell moment, interfering in the total magnetic moment, showing different values of magnetization between samples from La Fe dam and Calima Lake.

Given that the samples had millions of dehydrated cells, cellular agglomeration and shrinkage of cell membrane and cytoplasm could substantially increase the interactions between magnetosomes and chains of magnetosomes.

## 4. CONCLUSIONS

Results obtained by EDX/TEM, microXRD, FTIR and magnetic measurements determined that the magnetosomes synthesized by MTBs from La Fe dam and Calima lake correspond to magnetite.

Differences resulting from shape, size and magnetization of magnetosomes between MTB concentrates studied can be the result of the crystalochemical control exercised by each microorganism in the synthesis process, without environmental influence. However, it is necessary to do more research to confirm this hypothesis.

Biogenic crystals of magnetococcis from two sites in consideration show few morphological differences; results of size, shape and EDX analysis of magnetosomes do not show significant differences between the two types of coccis found. However, these small differences can be signs of having two different bacterial species. In order to confirm this hypothesis it will be necessary do phylogenetic analysis of magnetotococcis.

Results presented indicate that magnetosomes of MTBs from La Fe dam and Calima lake consist of SD magnetite evidenced by the relatively high Hc (~ 25 to 36 mT) and Hcr (~ 35 to 80 mT), the low saturation magnetization field (<100 mT) and the crystal size ranging from 30 and 120 nm and few SPM crystals (<30 nm).

It is important to notice that is possible to use techniques applied mainly to inorganic materials characterization to study biological samples, connecting areas such as physics, material engineering, geology and biology.

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