ISSN 0029-3865

CBPF-NF-025/85

A STUDY OF MAGNETIC PROPERTIES OF MAGNETOTACTIC BACTERIA

by

E. Wajnberg[+], L.H. de Souza[+], H.G.P. Lins de Barros*
and D.M.S. Esquivel*

*Centro Brasileiro de Pesquisas Físicas - CNPq/CBPF
Rua Dr. Xavier Sigaud, 150
22290 - Rio de Janeiro, RJ - Brasil

[+]Pontifícia Universidade Católica do Rio de Janeiro
Rúa Marquês de São Vicente, 225
22451 - Rio de Janeiro, RJ - Brasil

ABSTRACT

The average magnetic moment and its anisotropy are determined in natural samples of magnetotactic bacteria at 4.2K using a SQUID magnetometer. The results are in good agreement with estimates made from electron micrographs.

Key-words: Magnetism; Magnetotaxy; Magnetic microorganisms.

1 INTRODUCTION

Magnetotactic microorganisms, found in sediment from marine and fresh waters have recently been the subject of several studies which focused on different aspects of the magnetotaxis (1-9). These microorganisms show a peculiar behaviour: they orient and swim in the direction of a homogeneous magnetic field. This orientation is caused by the magnetic interaction between the microorganism and the magnetic field, and is sufficiently strong even in the geomagnetic field. It represents an important factor for the preservation of the species in their natural habitat (1,9,10). Fixed magnetotactic cells orient along the field lines and rotate when the field direction is reversed. Electron microscopy analysis has shown that all the observed magnetotactic microorganisms possess, inside their cytoplasm, geometrically regular, high-density regions, envolved by membranes (2,11,12). These organelles, the magnetossomas, are assumed to be the magnetic sensors responsable for the detection of the magnetic field (13). Mössbauer effect analyses of cells of the only available culture of a magnetotactic bacterium, (Aquaspirillum magnetotacticum), have identified those regions as composed of a high percentage of pure magnetite (Fe_3O_4). The Fe_3O_4 particles observed are in the single-domain size range. Morphological and structural studies show that they are single-crystals (14).

We present in this paper the results of a magnetic study on magnetotactic bacteria collected directly from its natural environment. The measurements were made at 4.2K using a superconducting quantum interference device (SQUID). We obtained the average magnetic moment of these bacteria in good agreement with the estimate which we have made from electron microscopy data. We show that the observed magnetic anisotropy has a period typical of a ferromagnetic material.

2 TECHNIQUES

2.1 Sample preparation

The samples were collected in a small freshwater river at a depth of approximately 50 cm. They showed a great number of magnetotactic bacteria which swim to the South magnetic pole. After standing in the laboratory for 2 or 3 weeks, the population of magnetotactic bacteria in these samples increases significantly even without any chemical enrichment. The samples have been magnetically concentrated using an appropriate vessel wich ends in a micropipet. This procedure permits us to obtain a very pure sample containing only magnetotactic bacteria. Their magnetotactic behavior has been checked by optical microscopy.The bacteria have been fixed in glutaraldehyde 2.5% in 0.1M phosphate buffer. Transmission electron microscopy of the sample assured us that we were observing exclusively magnetotactic bacteria (Fig. 1). We have counted the number of bacteria using an improved Neubauer chamber to evaluate their concentration.

2.2 Experimental procedure

We have used a very sensitive magnetometer based on a commercial SQUID, SHE model 130, in a first derivative configuration, coupled to a mixed electronic, for signal detection. The SQUID and the flux transformer are mounted in a home made liquid helium dewar in which measurements at 4.2K are taken (15). A steady field, H_0, is trapped by a lead screening. For the susceptibility measurement the samples have been oriented and frozen at liquid nitrogen temperature in the presence of a constant external magnetic field, H_{ext}. They were kept frozen until placed in the helium dewar. Figure 2 is a schematic design of the spherical sample holder and its accessories. It is made from an AV-8 CIBA araldite block. A cotton wire (dental wire) passing through the inox tube supports the sample holder. This mounting allows to change the orientation of the sample inside the dewar by rotating an external pulley supported by the same wire.

3 RESULTS

Figures 3a and 3b show typical signals for samples oriented and frozen in a magnetic field, H_{ext}, of about 6kG and a randomly oriented one, respectively. These signals were obtained in the absence of the magnetic field H_0 and show two contribuitions: from the bacteria and from the sample holder with glutaraldehyde solution. We have verified that the glutaraldeyde solution is diamagnetic while the sample holder is paramagnetic and both are isotropic.

The magnetization of the samples, M (in e.m.u.), can be obtained from the following expression:

$$M = \frac{\phi_0 \, \Delta V}{20 \times 10^{-3} . f . 4\pi . A_{eff}} \qquad (1)$$

where $\phi_0 = 2.07 \times 10^{-7}$ Gcm^{-2} is the quantum of the magnetic flux, ΔV is the peak-to-peak signal intensity (see figure 3) in Volts corrected for the contribuition of the blank, $f = 1.39 \times 10^{-2}$ is a calibration factor characteristic of the instrument used and $A_{eff} = 0.64$cm^2 is the samples effective area.

Since the interaction between the cell dipoles can be neglected, the bacteria in suspension behave, at rom temperature, to a good approximation as a paramagnetic liquid. The freezing of the sample to liquid nitrogen temperature in the presence of an external field is more rapid than the bacterial reorientation time. We have therefore assumed that the frozen samples have, approximately, the same average orientation as the liquid sample. M depends on the average orientation of the bacteria and, consequently, on the external field, H_{ext}. The average orientation of the bacteria is directly related to the Langevin function for paramagnetism, and we can write:

$$M = M\,(H_{ext}) = M_0 \, \langle \cos\theta \rangle \qquad (2)$$

where $M(H_{ext})$ and M_0 are the magnetization of a sample oriented in a field H_{ext} and of a fully oriented one, respectively; $< \cos \theta > = \mathcal{L}(m H_{ext}/kT)$ and $\mathcal{L}(x) = \coth x - 1/x$ is the Langevin function, m is the magnetic moment of a cell and $kT = 4.1 \times 10^{-14}$ ergs at 300K is the thermal energy. Eq (2) assumes, implicitly, that the fluid is composed of non-interacting permanent magnetic dipoles and $< \cos \theta >$ is their average orientation.

Using for M(0) the value of $5.5 \pm 0.5.10^{-15}$ e.m.u. measured in a desoriented sample, and for M_0 the value obtained in a sample frozen at 6kG, we obtain $< \cos \theta > \cong 10^{-2}$, corresponding to $\theta \cong 89^{o}$. This confirms our assumption that the limiting value of M_0 is~ $5.5 \pm 0.5 \times 10^{-13}$ e.m.u. Using this value and the sample concentration of $1.4 \pm 0.3 \times 10^{8}$ cells/cm^3 we obtain an average magnetic moment of a bacterium of $1.2 \pm 0.3 \times 10^{-12}$ e.m.u..

In anisotropy experiments the samples holder was held at a position, relative to the transformer coils, which gave the maximum signal and the sample was then rotated by intervals of about 0.48 rad. The dependence of the signal on the sample orientation is shown in fig. 4. This curve can be fitted by the expression:

$$M = M' + M'' \cos \beta \qquad (3)$$

This expression is associated with a ferromagnetic behavior, as confirmed by the remanent magnetization demonstrated by the existence of a signal with $H_0 = 0$. This result is in agreement with those obtained previously for the bacterial culture (4).

The magnetic moment can also be obtained from electron microscopy (EM). The E.M. of samples used in the present work shows, even after the freezing procedures, a great number of whole bacteria with a chain of geometrically regular high-density regions (Fig.1). These regions are analogous to those found in others magnetotactic microorganisms (2,5,8). We can estimate the magnetic moment of a bacterium if we assume

that these regions are composed of 80% magnetite (Fe_3O_4) (2,13). The total magnetic moment of a bacterium is thus equal to the product of the volume of each region by the number of regions and the magnetization per unit volume of magnetite (480 e.m.u/ cm^3). The average total magnetic moment obtained by this procedure is $1.3 \pm 0.4 \times 10^{-12}$ e.m.u..

CONCLUSIONS

Studies of cultured magnetotactic bacteria show that the conditions of growth alter significantly the magnetic properties of these organisms. These studies permit the use of sophisticated techniques and are of great interest to obtain information about the biomineralization process, but cannot provide knowledge about the adaptability and the importance of magnetic orientation in Nature. These informations must be obtained from samples collected directly from natural niches and without any chemical enrichment. The magnetotactic microorganisms collected directly in their habitat are present in low concentrations. This, together with high impurity concentration present in the natural samples, turns the study difficult. Efficient techniques have to be developped in order to obtain highly concentrated and pure samples.

Most of the measurements of average magnetic moment of magnetotactic bacteria grown in cultural media were performed using light scattering (16), birefringence (17) and electron microscopy (5,19). These results depend on the condition of growth and sample concentration. There exist only a few other determinations of the magnetic moment of natural samples without chemical enrichment made by the analysis of motion and by electron microscopy (8,9,18,20). These results show that the magnetic properties of these microorganisms depend on the conditions of the niche (local geomagnetic field, kind of water...). The size of microorganisms also determines their magnetic properties, however, the same species, collected in the same place, always possess a characteristic magnetic moment (8,9,20).

We present in this work what we believe to be the first direct determination of magnetic moment of magnetotactic bacteria as found in natural environment, using a technique other than the study of motion or the electron microscopy analyses.

We conclude that, at 4.2 K, a bacterial solution of concentration of about 10^8 cells/cm^3 behaves like a ferromagnetic material, suggesting that there is a magnetic interaction among the dipole moments. At room temperature this solution behaves like a paramagnetic fluid. It is possible that this fluid has the characteristic of a superparamagnetic fluid, since each crystal is a single domain-single crystal of magnetite.

The average magnetic moment measured leads to a $mB_0/kT \sim 3$ (magnetic to thermal energy ratio) for $B_0 \sim 0.25G$ (local geomagnetic field). This ratio was shown to be sufficient for an efficient magnetic orientation in Earth's field (9,20).

The value that we obtained for the average magnetic moment using the SQUID is in good agreement with the E.M. estimate. The magnetic concentration used here together with the use of SQUID provides a reliable method for determination of the magnetic moment of magnetotactic microorganisms collected directly form the environment. This determination is important to obtain more informations about the behavior of magnetotactic microorganisms in its natural habitat.

ACKNOWLEDGEMENTS

We wish to thank to M.Farina for the micrographs, G.Vieira for the sample preparation and Dr. George Bemski who critically read the manuscript.

FIGURE CAPTIONS

Fig. 1.2 - Micrograph of magnetotactic bacteria (transmission electron microscopy). Bar represents 1μm.

Fig.1.b - Crystal chain detail, Bar represents 2500 Å

Fig. 2. - Sample holder and accessories, a -spheric sample holder, b-cotton wire, c-inox tube and d-external pulley.

Fig.3 - Typical SQUID signal at 4.2K of bacterial solution Frozen to nitrogen liquid temperature, without steady field, H_o. Electronic scale: 0.4 V. a-oriented under a field of about 6kG b-completly desoriented.

Fig.4 - Anisotropy curve of the SQUID signal with H_o=10G for a sample oriented under a magnetic field of 300G. Electronic scale:0.4V.

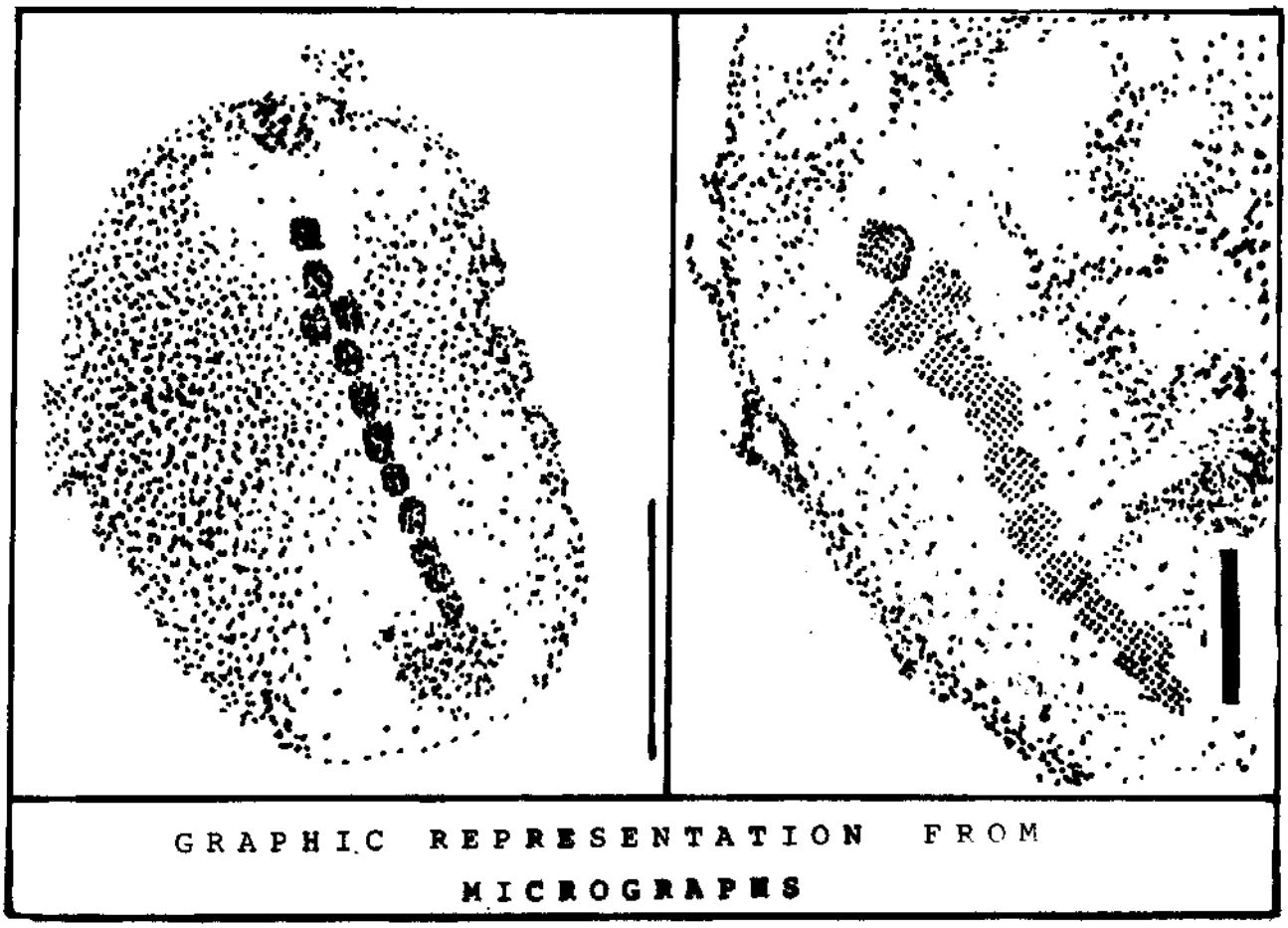

Figure 1a

Figure 1b

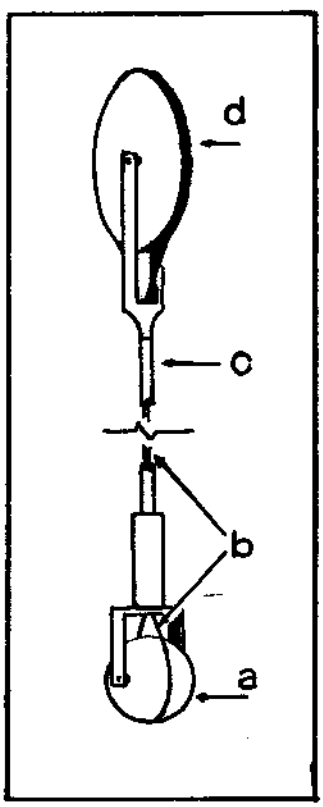

Figure 2

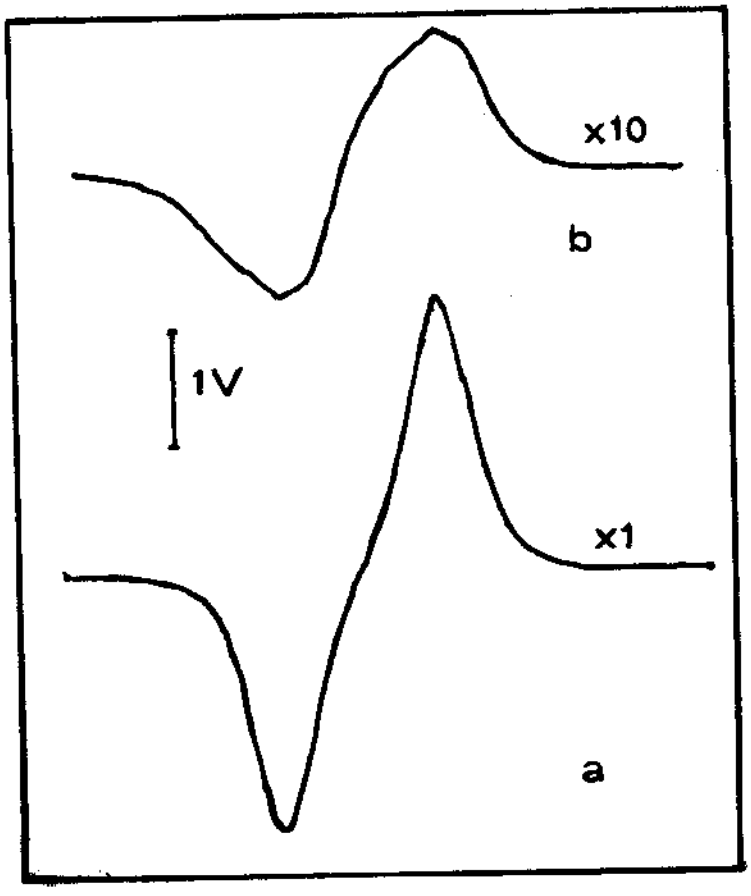

Figure 3

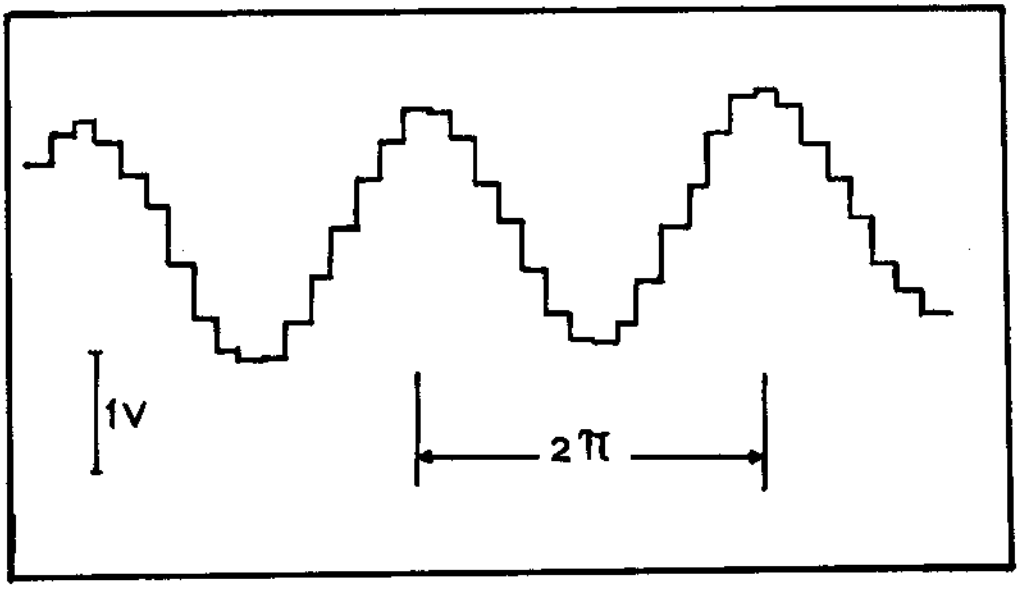

Figure 4

REFERENCES

1 - R.P. Blakemore, Science, 190, 377 (1975)

2 - K.M. Towe and T.T. Moench, Earth Plan. Sc. Lett., 52, 213 (1981).

3 - R.B. Frankel, R.P. Blakemore and R.S. Wolfe, Science, 203, 1355 (1979).

4 - C.R. Denham, R.P. Blakemore and R.B. Frankel, IEEE Trans. Magn. mag. 16, 1006 (1980).

5 - R.B. Frankel and R.P. Blakemore, J.Magn. and Mag. Mater, 15-18, 1562 (1980).

6 - R.B. Frankel, G.C. Papaefthymiou, R.P. Blakemore and W.O'Brien, Bioch. Biophys. Acta 763, 147 (1983).

7 - J.L. Kirschvink, "Biogenic Ferrimagnetism: A New Biomagnetism" in Biomagnetism, eds. S.J. Williamson, G.L. Romani, L.Kaufman and I.Modena. Plenum Press (1983).

8 - D.M.S. Esquivel, H.G.P. Lins de Barros, M.Farina. P.M.A. Aragão and J.Danon, Biol. Cell. 42 (2), 227. (1983)

9 - H.G.P.Lins de Barros and D.M.S.Esquivel, "Magnetotactic Microorganisms Found in Muds from Rio de Janeiro: A General View" in Magnetite Biomineralization and Magnetoreception in Organisms" eds. J.L. Kirschvink, D.S.Jones and B.J. Mac Fadden. Plenum Press (in press)

10 - R.B. Frankel, R.P. Blakemore, F.F.Torres de Araujo, D.M.S. Esquivel and J.Danon, Science, 212, 1269 (1981)

11 - D.L. Balkwill, D.Maratea and R.P. Blakemore, J.Bacteriol. 141(3), 1399 (1980)

12 - M.Farina, H.Lins de Barros, D.M.S.Esquivel and J.Danon, Biol. Cell. 48, 85 (1983).

13 - R.P.Blakemore, Ann.Rev. Microbiol. 36, 217 (1982)

14 - T.Matsuda, J.Endo, N.Osakabe and A.Tonomura, Nature, 302, 411 (1983).

15 - R.A. Nunes. Master Thesis."Medidas de susceptibilidades magnéticas de nitrosil-hemoglobina, utilizando um magnetômetro supercondutor" 61pp. PUC-RJ (1981).

16 - G.Rosenblatt, F.F.Torres de Araujo and R.B.Frankel, J.Appl. Phys.53 (3). 2727 (1982)

17 - G.Rosenblatt, F.F.Torres de Araujo and R.B.Frankel, Biophys. J., 40, 83 (1982).

18 - A.J.Kalmijn, IEEE Trans. Magn.Mag. 17, 1113 (1981)

19 - R.B. Frankel, Ann. Rev. Biophys.Bioeng., 13 85 (1984)

20 - D.M.S. Esquivel and H.G.P. Lins de Barros, Submited to J.Exp. Biol. (1985).