INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES
IJPAES
Volume-4, Issue-2, April-June-2014
ISSN 2231-4490
Coden : IJPAES www.ijpaes.com
Received: 20th Feb-2014 *Revised: 20th Mar-2014* *Accepted: 25th Mar-2014*
Research article

THE ISOLATION AND CHARACTERIZATION OF MAGNETOTACTIC BACTERIA FROM IRON ORE SOIL FOR SYNTHESIS OF MAGNETIC NANOPARTICLES AS POTENTIAL USE IN MAGNETIC HYPERTHERMIA

V.A Karande, R.N.Patil, A.P Tiwari, R.K.Satvekar, A.V.Raut, S.J.Ghosh and S.H.Pawar*.

Center for Interdisciplinary Research, D. Y. Patil University, Kolhapur - 416 006, [MS] India.

ABSTRACT: Magnetotatic bacteria have been isolated from iron cap belt of Cuddegali Voril Soddo iron ore mine. Here magnetic measurement and non magnetic analysis helps to detect biogenic magnetite in soil sample. The cultivation of magnetotactic bacteria was done in modified enrichment medium and incubated at room temperature (25–30^0 C). A short rod of magnetotactic bacteria contained two or more magnetosomes revealed under TEM, having the shape of rounded and irregular. The SEM was used for morphological study of MTB. Energy spectrum analysis with SEM showed that iron oxides were main component of magnetic particles. This study reports the isolation and characterization of magnetotactic bacteria isolated from the mining area of Goa region, India. It also points towards the ability of modified media to support growth of magnetotactic isolates. The result depicts that there is a direct correlation of soil magnetic susceptibility with biogenic magnetite, geology and soil process. The natural way for the synthesis of nanoparticles by microbes, which is potent eco- friendly, green synthesis leads to the development of advance research in nanotechnology. The result gives the direction for future work, that is it can be used as potential heating agent in magnetic fluid hyperthermia (MFH).

Keywords: Magnetotatic bacteria, Bacterial magnetite, Magnetosomes, Biomineralization, iron ore soil.

INTRODUCTION

Magnetotactic bacteria are morphologically, metabolically, and phylogenetically diverse prokaryotes [1]. They are known to synthesize intracellular ferromagnetic magnetite and or gregite magnetosomes and have significant roles in global iron cycling in aquatic systems, as well as rock magnetism [2]. Bacterial magnetite contributes to the magnetic signal of the sediments and is widely distributed mainly in natural habitats [3] such as fresh water lake [4], marine region [5], pond ecosystem [2], iron ore soil [6] and estuarine region [7]. In spite of their wide distribution and abundance in aquatic environments, most MTB are intractable, and so far only a few of them are isolated in pure culture [8]. Enrichment of ferromagnetic minerals and magnetite is observed in top layer of soil therefore all biogenic and abiogenic process facilitate to produce magnetite [9] (Yongxin et al., 2005). Soil minerals play a vital role in soil's fertility since mineral surfaces serve as potential sites for nutrient storage. However, according to reported research magnetotactic bacteria and magnetosome can contribute magnetite in soil [10,11]. Even though Goa is an India's smallest state and it accounts for just 0.11% of India's geographical area, then also it is India's leading producers of iron and manganese [12]. Therefore to detect magnetotactic bacteria in the iron ore soil and further quantify its contribution to the magnetic signal, we conducted one of the primary soil magnetic measurement (VSM) as well as complimentary non-magnetic analyses (TEM, FTIR). Our result demonstrates magnetism in soil contributed by bacterial magnetite and its growth of desired community is supported by modified medium. In the present study soil was analyzed for the presence of MTB and effective method was developed to isolate magnetotactic bacteria from iron ore soil. The culture based approach was used for the study. Pure isolate obtained were characterized on the basis of morphology, biochemical tests, physiology. They were screened for their metal accumulation ability which further extends to production of magnetic nanoparticles for hyperthermia application.

MATERIALS METHODS

Soil Sample collection: The collection of soil sample was done from Cuddegali Voril Soddo Iron Ore Mine Showing Geographical distribution Latitude 15^0 19' 30", Longitude 74^0 09' 45" having lease area 91.09 Ha, located at village Santona District South goa, Goa. Three different random samples were taken through soil profile. (Table 1).

Table-1: The environmental characteristics of soil samples collected from Cuddegal iron ore mine Goa region India.

Sample Name	Level	Source of Sample	Characteristic of sample
T1	Top Horizon	Surface soil	Dark Brown Surface soil
M1	Meadow Horizon	Intermediate soil	Dark Brown Surface soil
B1	Bottom Horizon	Bottom soil	Black primary iron ore

Magnetic Measurements of Soil

Magnetic measurements was carried out at room temperature using Molspin Vibrating Sample Magnetometer (VSM) which was conducted on 3 bulk soil samples from three level of iron cap belt (T1, M1, B1). For the measurement required up to 0.5 g in weight sample in a maximum applied field of 1 T [13].

Enrichment and Cultivation of MTB

The collected soil (1gm) was inoculated in 100 ml of enrichment medium (flask) incubated at room temperature in dark for 7 days [6]. The modified enrichment medium was used for isolation and cultivation of desired community. It contained 300 ml of soil extract, 0.1 ml of peptone, 5 ml of 0.01M ferric quinate, 0.1gm of yeast extract, 0.05 gm of sodium chloride, 0.05 gm of sodium thioglycolate. About 500 ml of soil extract was filtered through 0.45 micrometer filter membrane and pH was adjusted to 7.2-7.4. After pH adjustment, the medium was autoclaved at 121^0 c for 20 min. Initially, 2 ml enriched samples were distributed in each 20 ml of enrichment medium in capped bottles and incubated at 30^0C for 48 hrs with applied magnetic field (1 T).The soil sample profile characteristics were shown Cuddegali Voril Soddo Iron Ore Mine (Table no.1). The comprehensive study of MTB involves a series of processes including sample collection, Pre-enrichment, Isolation, Cultivation and purification that was achieve systematically.

Transmission electron microscopy studies

For TEM, cells were centrifuged at 1000 rpm for 10 min and supernatant was discarded. The pellet was suspended in 0.1 M phosphate buffer pH (7.4) and centrifuged with addition of little fixative into buffer (3:7). The pellet was fixed in a mixture of 2% par formaldehyde and 2.5 % Glutaraldehyde in buffer for 2 -3 hr at 4^0 for 10 min and buffer was added. This processed pellet was used for grid preparation and further proceeds to TEM (TECNAI 200 Kv TEM (Fei, Electron Optics) [15].

FTIR analysis

A 20-30 µl of isolated magnetosomes were suspended in distilled water and dried on a zinc disc. The FTIR spectrum of Magnetosomes was recorded on alpha ATR Bruker (Eco ATR) o.ver the wave number range of 500 – 4000 cm^{-1} under ambient condition [16].

Induction heating study of Samples

The extracted magnetosomes suspension was used to perform primary induction heating experiments. The change in temperature rise of extracted magnetosomes was measured by an induction heating unit (Easy Heat 8310, Ambrell; UK) with a 6 cm diameter (4 turns) heating coil by applying an AC magnetic field of 335.2- 502.0 Oe at a fixed frequency of 265 kHz. (Concentration 1 mg/mL).

RESULTS

Magnetic properties

Measurements of the magnetization using vibrating sample magnetometry (VSM) concerning applied magnetic field were carried out at room temperature with results for coercivity (Hc), saturation magnetization (Ms), and remanence (Mr) obtained from hysteresis loops presented in (Figure 1). By comparing the measured saturation magnetic moment with theoretical saturation magnetization the content of magnetic iron oxide of the sample was estimated [17].The Hysteresis loops are characterized by the pot bellied shape. However the standard hysteresis parameter differs significantly. Sample M1 shows typical hysteresis loop and has Ms, Mr and Hc are 96.85, 6.15 & 44.44 respectively in comparison with sample T1 and B1. Additionally the figure represents the ratio of saturation remanence to saturation magnetization (Mr/Ms) and coercivity remanence to coercivity (Hcr/Hc) is 0.063 and 40.91 respectively. Therefore hysteresis parameters indicate that the biogenic magnetite is present in soil.

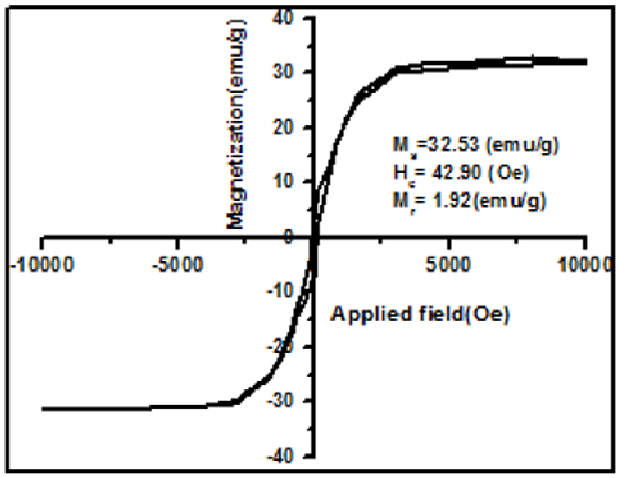
Enrichment and Cultivation of MTB

Pre enrichment of the soil sample is done by using simple flask and magnet to get desired community. MTB was successfully enriched from the middle horizon of iron ore belt using modified cultivation method. To obtain a pure culture of MTB the mixed culture was repeatedly subjected to racetrack purification and re inoculation in modified cultivation medium. Modified culture medium was analyzed for the growth of isolate culture.

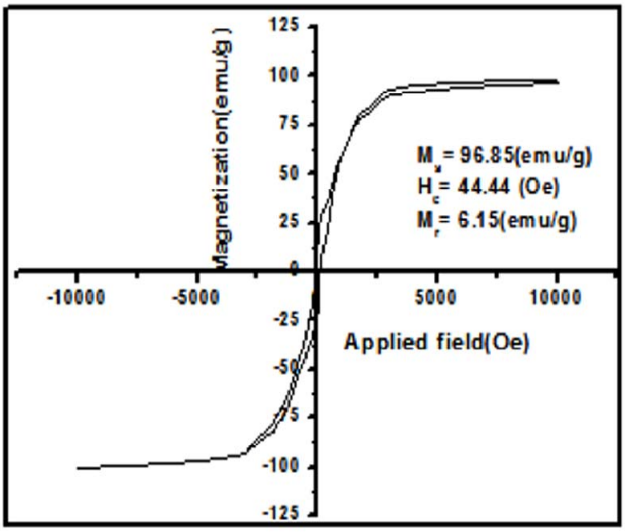
Electron Microscopic Studies of Isolate and Elemental analysis

Electron microscopic observation shows that the enriched cell samples contain rod cells (Figure 3). The width of isolated MTB is relatively uniform with mean 0.8 - 2 X 0.6 - 1 µm in size. The TEM was performed on the samples to investigate morphology and size changes in series of the formation of magnetosomes. Larger magnetosomes have a bullet shape with long axis, parallel to the magnetosome chain and smaller magnetosomes of spherical and irregular shape are dispersed uniformly into the cell. This is unique feature shown by soil MTB. In order to obtain information on the surface nature of magnetosome, the FTIR spectroscopy method was used to characterize the surface functional groups of magnetosome membrane [18]. The FTIR spectrum of the magnetosome is presented in Fig. 4. The FTIR spectrum at 3297.30 cm−1 was indicative of the existence of \OH and \NH groups of the magnetosome. The peaks observed at 2389.14 cm−1 can be assigned to the\CN group of the magnetosome. The peaks at 1640.20 cm−1 (mainly C_O stretching) can be attributed to amide I and amide II bands of protein peptide bonds. In primary induction heating study from the temperature kinetic curve, it is observed that magnetosomes reach the hyperthermia temperature within a short time span for applied field of 400,500 and 600 Oe. Heat generation by the magnetosomes was due to the, hysteresis losses, or from both of relaxation loss mechanisms.

Sample T1



Sample M 1



Sample B 1

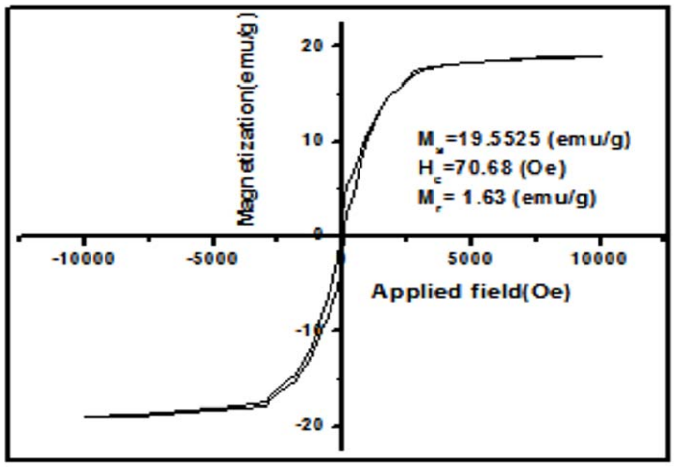


Figure1. Typical hysteresis loops of soil samples T1, M1and B1. The maximum applied field is 1T. Ms, Hc, Mr refer to saturation magnetization, coercivity and magnetic remanence respectively.

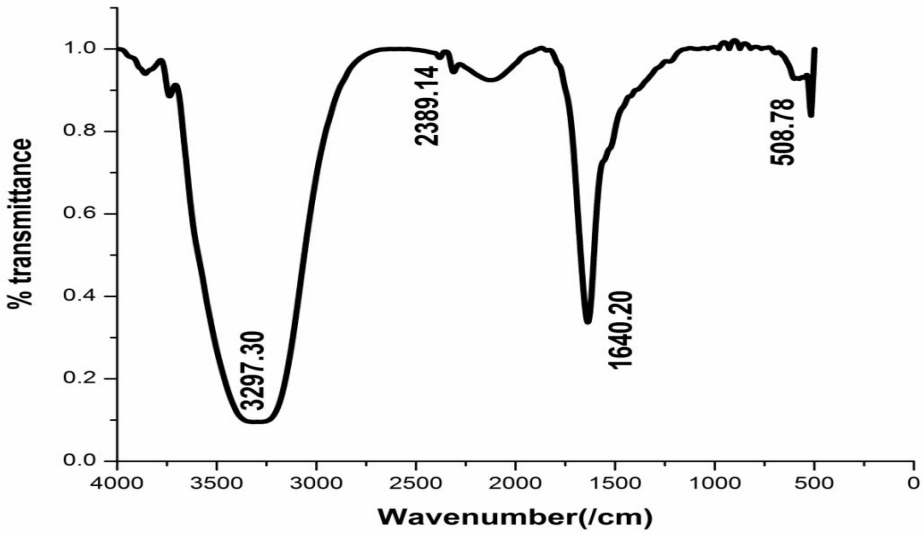


Figure 2. FTIR spectra of magnetosomes formed by isolate obtained from iron ore soil.

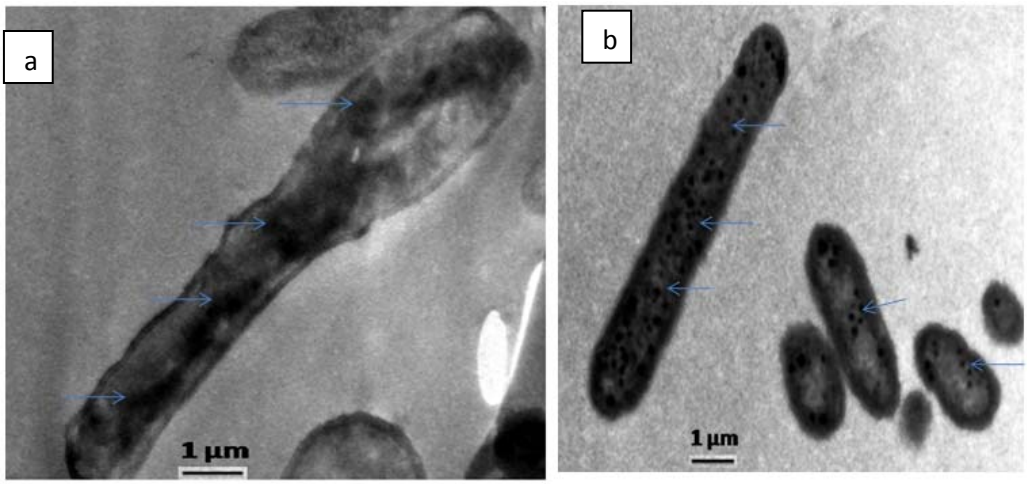


Figure 3. TEM images of (a) immature magnetosome, (b) Mature magnetosomes (blue arrow indicate the presence of magnetosomes)

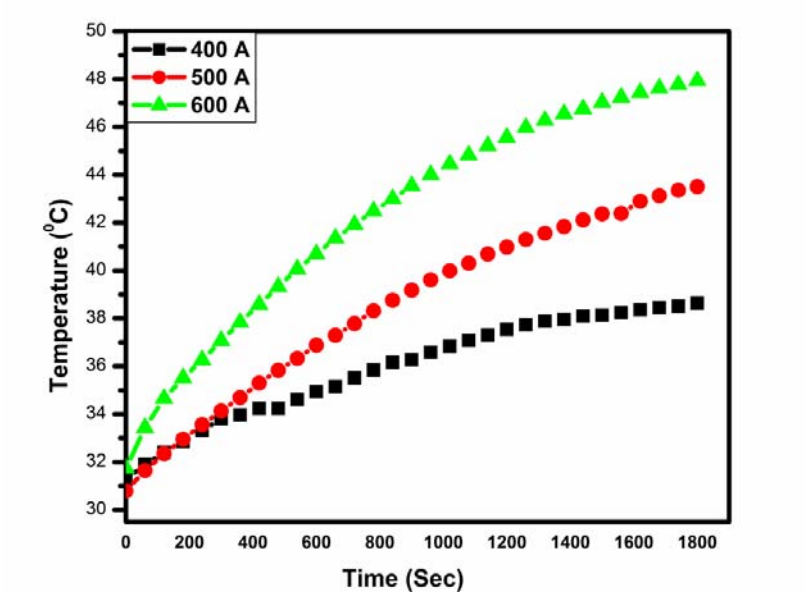


Figure 4. In Induction heating study (temperature curve) indicate the rate of temperature change of a solution containing extracted magnetosomes (Fe_3O_4) under the influence of different magnetic field conditions from 400, 500 and 600, at frequency 265 kHz.

DISCUSSION

Here we report the study on magnetic and non magnetic parameters of the ore environment and isolated MTB culture from soil. This information can serve as a guide to elucidate, potential mineral donors that contributes to the formation of magnetosomes and other intracellular bodies. Hence MTB were screened for their presence in different fractions of the soil environment. The magnetic measurement was carried out by VSM. The middle layer soil sample show greater hysteresis value than the top and bottom layers. The magnetic property varies with depth due to occurrence of MTB that influences the magnetism and chemical environment leading to dissolution of biogenic magnetite. Bioconsortium of MTB affects biogenic magnetism in topmost to bottom layer. However the correlation between magnetic properties and geochemical parameters defines the interaction of biogenic and abiogenic process which leads to formation of biogenic magnetite [19]. Therefore there is high possibility to have live MTB and biogenic magnetite in M1 sample. Hence M1 sample was chosen for further isolation studies. Therefore Pre enrichment of soil sample and series of process successfully isolate MTB. The pair arrangement of cells is attributed to the natural tendency of MTB. Here we report first time the variation in magnetic measurement and non magnetic analysis on purified sample containing solely MTBs. The TEM observation shows that magnetosomes dispersed vary well in cell. The main component Fe_3O_4 (Iron oxide) content of magnetosomes was determined by FTIR [19]. From the temperature kinetic curve, it is observed that Sample M2 reach the hyperthermia temperature within a short time span, it is observed that for M2 at the fields 400 Oe are not sufficient to reach hyperthermia threshold temperature while for applied field at 500 and 600 Oe, hyperthermia temperature is reached. The values of sufficiently high temperature observed for M2 was 48^0c under applied at 600 Oe. The essential changes in magnetic properties and NPs behavior provide major platform to perform magnetic hyperthermia in cancer treatment. [20] Further details Induction heating study theses magnetosomes is in progress and will be presented in future publication.

CONCLUSIONS

The present study is the first reporting on magnetic properties of soil samples and subsequent isolation of MTB from Cuddegali iron ore soil at Goa region .It has been found that the iron ore soil has high magnetism as compare to other rock . However the biogenic magnetite is significantly contributed by the magnetotactic bacteria through controlled biomineralization. The bioconsortium of magnetotactic bacteria increases with decrease in the oxygen concentration and is high just below the topmost layer under microaerophilic condition. The stronger interaction between the magnetosome moments attributes to magnetic properties of MTB. Iron oxide (Fe_3O_4) is the chief constituent of magnetosomes that is synthesized under anaerobic conditions with a series of anabolic and catabolic reactions performed by MTB. Immature magnetosomes and mature magnetosomes differs in their magnetic properties that reflect its motility and population density.

The live magnetotactic bacterium is a key magnetic mineral in Cuddegali iron ore mine. Biogeochemical and physical parameters of iron soil is affected by concentration of magnetotactic bacteria. Magnetosome formation and its deposition dominates biomagnetism in iron ore sediment. Here peculiar conditions in soil favors the synthesis of Fe hydrated phase overlying the surface of crystals of immature magnetosomes. The extracted magnetosomes can be used as an effective heat mediator hence can be of potential use in biomedical application and as a heat mediator for magnetic Hyperthermia. Moreover, increasing demand of magnetosomes and the study of isolation of MTB provides great milestone in the Interdiscipinary field of research.

ACKNOWLEGMENT

The authors wish to thank for financial assistance of the Department of Science and Technology (DST), New Delhi. We are grateful to Indian Institute of Geomagnetism (IIG Mumbai) for kind assistance to magnetic measurements. We are also thankful to All India Institute of Medical Sciences (AIIMS), New Delhi for TEM images and we also like to acknowledge Mr A.B. Mali from fomento Resources Goa, for soil sampling.

REFERENCES

[1] Amann, R.., Peplies, J., Schuler, D., 2007. Diversity and taxonomy of magnetotactic bacteria. In: Schuler D (ed) Magnetoreception and magnetosomes in bacteria. Microbiology monographs. Springer-Verlag, Berlin Herdelberg **(**3):25–36.

[2] Blackmore, R., 1982.Magnetotactic bacteria. Ann. Rev (36) Microbiol. 217-38.

[3] Currie, R., 1994. Ferromagnetic Materials. Academic Press London.

[4] Fassbinder, J., Stanjek, H., Vali, H., 1990.Occurrence of magnetic bacteria in soil. Nature. **(**343) 161-163.

[5] Flies, M., Jonkers, B., Bosselmann, K., Michael, E., Bottcher, D., Christine, B., 2005. Fresh water Diversity and vertical distribution of magnetotactic bacteria along chemical gradients in freshwater microcosms.FEMS Microbiology Ecology (52) 185–195.

[6] Geological Survey of India. 2006. Government of India. Bangalore Geology and mineral resources of The States of India.

[7] Hergt, R., Hiergeist, R., Zeisberger, M., Schuler, D., Heyen, U., Hilger, I., Kaiser, W., 2005.Magnetic properties of bacterial magnetosomes as potential diagnostic and therapeutic tools. Journal of Magnetism and magnetic material **(**293) 80-86.

[8] Kundu, S., Kulkarni, G., 2010. .Enhancement of magnetotactic bacterial yield in a modified MSGM medium without alteration of magnetosomes properties. Indian J. Expt. Microbiol. **(**48) 518-523.

[9] Lei, Y., Yue, X., Zhang, S., Chen, P., Xu, Z., Li, Y., Li, H., 2012. Biocompatibility evaluation of magnetosomes formed by *Acidithiobacillus ferrooxidans*. Materials Science and Engineering C **(**32) 1802-1832.

[10] Li, W., Yu, L., Zhou, P., Zhu, M., 2007. A Magnetospirillum strain WM-1 from a freshwater sediment with intracellular magnetosomes. World J Microbiol Biotechnol. **(**23) 1489–1492.

[11] Liu, Y., Gao, M., Dai, S., Peng, K., Jia, R., 2006. Characterization of magnetotactic bacteria and their magnetosomes isolated from Tieshan iron ore in Hubei Province of China. Materials Science and Engineering C **(**26) 597– 601.

[12] Meldrum, F., Mann, S., Heywood, B., Frankel, R., Bazylinski, D., 1993 .Electron microscopy study of magnetosomes in a cultured coccoid magnetotactic bacteria. Proc. R. Soc. Lond. B (251) 231-236.

[13] Moskowitz, B., Frankel, R., Bazylinski, D., 1993. Rock magnetic criteria for the detection of biogenic magnetite. Earth Planet. Sci. Lett. (120) 283– 300.

[14] Pan, Y., Petersen, N., Winklhofer, M., Alfonso, F., Liu, D., Thomas, T., Hanzlik M.; Zhu R. 2005. Rock magnetic properties uncultured magnetotactic bacteria. Earth and Planetary Science Letters (237) 311– 325.

[15] Pana Y.; Petersen N.; Davila AF.; Zhang L.; Winklhofer M.; Liua Q.; Hanzlikd M.,; Zhu R. 2005.The detection of bacterial magnetite in recent sediments of Lake Chiemsee (southern Germany). Earth and Planetary Science Letters. **(**232) 109–123.

[16] Salazar, A., Morales, A., Marquez, M., 2011**.** Characterization of natural microcosms of estuarine magnetotactic bacteria. Dyna, ano 78 Medellin, Agosto **(**168) 72-80.

[17] Simmons, S., Sievert, S., Frankel, R., Bazylinski, D., Edwards, K., 2004.Marine Magnetotactic Bacteria in a Seasonally Stratified Coastal Salt Pond. Appl. Environ. Microbiol. **(**70) (10), 62-30.

[18] Staniland, S., Ward, B., Harrison, A., 2009.Biosynthesis of magnetite by microbes. Applications in Earth, Energy and Environmental Sciences, Neutron Scattering Applications and Techniques. L. Liang et al. (Ed.), Springer science 595-618.

[19] Xiang, L., Wei, J., Jianbo, S., Guili, W., Feng, G., and Ying, L., 2007. Purified and sterilized magnetosomes from *Magnetospirillum gryphiswaldense* MSR-1 were not toxic to mouse fibroblasts in vitro. Letters in Applied Microbiology. (45) 75–81.

[20] Sophie, L, Silvio, D., Urs O. Hafeli., Morteza, M., 2011. Magnetic fluid hyperthermia: Focus on superparamagnetic iron oxide nanoparticles. J. Adv in Colloid and Interface Sci (166)**,** 8-23.