Bacterial Magnetic Particles for Potential Applications in Nanoscale NDT&E

E. Siores, A. S. Lamb, and L. M. Swallow.
Centre for Materials Research and Innovation, The University of Bolton, Bolton, BL3 5AB, England
es1@bolton.ac.uk

Abstract - Current NDT techniques are capable of identifying sub millimetre defects in structural materials. Nanoscale NDT however, necessitates development of new techniques for identifying and sizing surface and more importantly subsurface imperfections. The reliability of micro and especially nano scale components heavily depends on such new developments. This paper presents a first attempt to explore the potential of magnetotactic bactreria for applications in NDT&E. Such bacteria having dimensions of a few microns can form single crystals of iron magnetite encapsulated in a magnetosome membrane. Crystals are structured in a long chain arrangement and polarised in the north / south direction, thus allowing the bacteria microorganisms to behave as magnets. Since their discovery in the 70s, different magnetotactic bacteria morphologies producing various magnetic minerals have been observed and a few have been successfully cultured. At present, emphasis is placed on understanding the mechanism of magnetic synthesis and associated crystalline structure behaviour, while a few applications are beginning to emerge. Such applications include magnetic storage devices, cell separation and magnetic resonance imaging (MRI).

Keywords: Magnetotactic bacteria, Non Destructive Testing, Magnetic Bacteria Inspection, Nanoscale.

1 Introduction

Magnetotactic bacteria were first described in the work of Bellini [1], whilst observing bog sediments microscopically, a certain group of bacteria oriented themselves in accordance with the Earth's North Pole and from this observation he named them "magnetosensitive bacteria." However it was with Blakemore [2] that the first peer reviewed report on magnetotactic bacteria was submitted. Many species of magnetotactic bacteria have been reported *Magnetospirillum magnetotacticum* was the first reported experimentally [2] since which time strains such as *Magnetotacticum gryphiswaldense*, *Magnetotacticum magneticum* [3], and *Magnetobacterium bavaricum* [4] [5] have been discovered. *Desulfovibrio magneticus* a magnetotactic sulphur reducing bacteria was investigated by [6]. In this work it was noted that the bacteria were capable of orientating in line with the Earth's Magnetic field from South to North, and the adjective "Magnetotactic" was first derived. The term Magnetotactic has no taxonomic significance and represents a heterogeneous group of fastidious prokaryotes [7]. The morphology of the bacteria was part of the work carried out by Freitas et.al. [8] who investigated envelope ultra-structure of uncultured naturally occurring magnetic cocci. They observed numerous membrane vesicles on the bacteria surface and also that the bacterial flagella were organized into bundles. They also reported that, "Capsules and S-layers are common structures in magnetotactic cocci from natural sediments" and may be involved in the inhibition of metal precipitation on the cell surface or indirectly influence magnetotaxis.

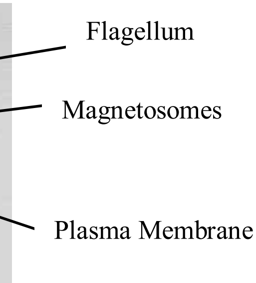

Figure 1 : The magnetotatic bacterium *Magnetospirillum magnetotacticum*. Image credit: R. Frankel

The bacteria are usually found in oxic-anoxic transition zones (OATZ), the zone between the oxygen rich and oxygen poor regions in water or sediment. The bacteria can survive in sediment regions where oxygen and nutrient are in poor supply.
The magnetotactic bacteria's (MTB) mode of operation relies on the capability of the bacteria to synthesize specific intracellular structures called magnetosomes [9] - [10]. Magnetosomes comprise nano-sized, membrane bound crystals of magnetic iron minerals (see Figure 1). The formation of magnetosomes (see Figure 2) is achieved by a biological mechanism that controls the accumulation of iron and the biomineralization of magnetic crystals with a characteristic size and morphology within membrane vesicles.

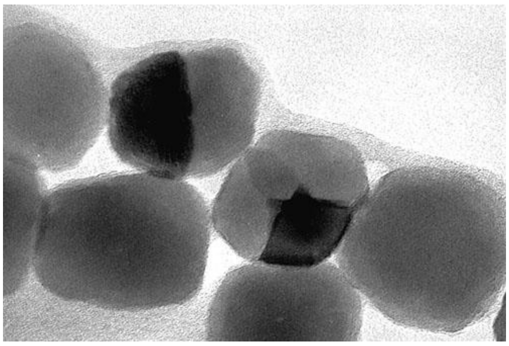

Figure 2 : Magnetospirillum S-1 magnetosomes (45 nm) (B. Devouard).

It has been reported [11] that several different morphologies of magnetotactic bacteria exist which further differ for number, layout and pattern of bacterial magnetic particles (BMP) that the cells contain. These morphological types include coccoid, rodid, vibrioid and spirillum, [12] - [14]. The magnetosomes are produced in chains, and the magnetic dipole of the BMP orientates the cell and thus overcomes the resistance caused by the supporting material [9]. They are distributed in various morphological types in MTB (see Figure 3). In the majority of MTB, magnetosomes are aligned in chains of various lengths along the cell axis. Dispersed aggregates clusters of magnetosomes occur in some MTB usually at one side of the bacterial cell usually the side of flagellar insertion. All magnetotactic bacteria contain magnetosomes, which are intercellular structures made up of magnetic iron crystals enveloped by a phospholipids membrane [15].

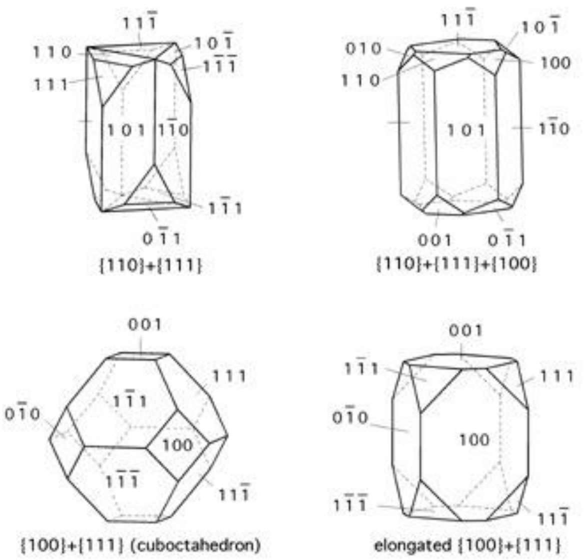

Figure 3 : Different Morphological Forms of Magnetosomes (taken from **www.calpoly.edu/~rfrankel/mtbphoto.html**)

Magnetotactic bacteria can thus be divided into two sub-categories according to the different cell productions: greigite (Fe_3 S_4) or magnetite (Fe_3O_4). The MTB that utilise greigite are usually strictly anaerobic.

The work of FreitaS ET.AL. [8] investigated envelope ultra-structure in magnetotactic cocci in response to magnetic fields. It was discovered that all bacteria investigated were gram negative and many of the bacteria possessed extensive capsular material and an S-layer formed by particles arranged with hexagonal symmetry. They observed no indication of metal precipitation on the bacterial surface. It was concluded that capsules and S-layers are common structures in magnetotactic cocci and that they be involved in inhibition of metal precipitation on the cell surface thus indirectly influencing magnetotaxis.

Work investigating the characteristics of magnetotactic bacteria and their magnetosomes has also been conducted by Liu et.al. [16]. In their work, transmission electron microscopy was employed to investigate the bacterial morphology. They also investigated energy spectrum analysis and discovered that iron oxides were in fact the main components of the magnetic particles.

The most abundant type of Magnetotactic bacteria are occurring in environmental samples, especially sediments is coccoid cells which possess two flagellar bundles on a somewhat flattened side. This "bilophotrichous" type of flagellation gave rise to the tentative genus *"Bilophococcus"* for these bacteria. In contrast to this, is one of the morphologically more conspicuous MTB, regularly observed in natural samples, but never isolated in pure culture, which are large rods containing copious amounts of hook-shaped magnetosomes (*Magnetobacterium bavaricum*).

Pulsed field-remanence measurements in two types of *Magnetobacterium bavaricum* were investigated by Hanzlik et.al. [5]. Iit was discovered that the different cell morphologies of the two types gave differing results. Wild type magnetic Vibrio shaped bacteria type was found to give square remanence curves with the reversal field H_{rev} reaching values of 825 Oersted (Oe). The high H_{rev} values were attributed to the elongated shape of the magnetosome, whereas in the work with rod shaped types it was found that the coercivity of remanence H_{cr} of cells always ranged between 600 and 700 Oe.

2 Experimental procedures and results

Magnetospirillum species is an extremely fastidious organism. *M. magnetotacticum* is described as an obligate anaerobe, which does not grow when the growth medium has free exchange with air [2], whilst *M.gryphiswaldense is aerobic* [17].

The bacterial sample was obtained from LGC (Teddington, UK). In the lab the following growth regime was undertaken. A 16 x 125mm tube of Magnetic Spirillum Growth Medium (ATCC 1653) was inoculated with the content of a thawed vile of sample. The tube was filled to create microaerophilic conditions thus facilitating the growth of the organism. The solution was then incubated at 28-30°C in a Townsen and Mercer Incubator (29±1°C) for seven days until growth was observed, a cloudy grey sediment was noted in the base of the tube. The contents were harvested via splitting the sample into two centrifuge tubes, and the centrifuge (Howe Sigma 215 Centrifuge) balanced as per good laboratory practice. The samples were then centrifuged at 7000rpm for 30 minutes. The supernatant liquid was discarded and the pellets of cells were re-suspended in a 125ml flask. For storage the bacterial pellet was stored in an Eppendorf tube under glycerol and then frozen till it was ready to be used.

2.1 Nanoscale magnetic bacteria inspection

Magnetic Particle Inspection (MPI) techniques for NDT&E have been around for years for the detection of defects in ferrous materials. Visualisation of the magnetic particles show magnetic field lines and any irregularity in field flow points to areas of weakness or flaws in the inspected medium.

The presence of a flaw in the material cause distortion to the magnetic flux, with the field leaking at the flaw site. The deformation of the magnetic field is not limited to the site of the flaw and extended a considerable distance causing a distortion site much larger than that of the defect.

Media used in MPI are usually micro-macro scale magnetic iron oxide particles held in a suspension of suitable liquids. The fast easy to adopt combination of magnetic flux leakage and visual testing makes MPI one of the most widely used NDT&E methods.

The proposed technique uses MPI with visual particles of nanoscale dimensions leading to the development of a system for the detection and evaluation of nano sized flaws. The resolution of MPI can be greatly increased if the visual media were reduced. Sufficiently sized media such as iron particles can limit the visual image of the flaw to the size of the particle. Nano/micro particles on the other hand can take the flaw detection resolution down to the nanometer scale giving greater accuracy to smaller flaws.

The synthesised BMP encapsulated in the cell wall were of consistent shape and size, in the diameter range of 50 – 100nm. Orientation of the BMP in chains along the magnetic field lines proved the existence of a magnetic dipole and through magnetotaxis [18] the bacteria were aligned along the geomagnetic filed lines such as the technique of the ferrous particles in MPI. As the MTB are the smallest organism to use the earth's geomagnetic field for navigation, visual resolution can be increased by reducing the resolution of flaw detection.

Another advantage with the use of nano/micro bacteria particles is reduction in contamination of the material under tests. The non pathogenic nature of MTB is not harmful for human contact thus being capable of taking the principle beyond laboratory curiosity. The nanoscale sized bacteria particles as well as increasing resolution are also less reciprocal to damaging mechanical systems. Misplaced particles of nanometer dimensions are of less harm to mechanical systems than their micrometer counter parts.

Under experimental laboratory conditions work was undertaken as described; a magnetic field was generated across a microscopy slide holding a 2ml suspension of MTB (see Figure 4). It was observed that the MTB formed chains from North to South along the lines of magnetic flux. A crack was created under controlled conditions and a 2ml solution of suspended MTB applied to the slide. The magnetic field was reapplied and it was observed that a forced alignment due to the crack was created. Aiding proof, the principle of using MTB as a possible nanoscale method for NDT&E.

Figure 4 : Placement of bar magnets alongside the Magnetospirillum sample

3 Conclusion

The work undertaken has shown that Magnetic bacteria can be used as a diagnostic tool in the detection of imperfections even at the nano scale. The experiment was carried out using a glass Microscope Slide specimens with simulated defect characteristics, but the same procedure can be adopted in the detection of flaws in other materials.

4 References

[1] S. Bellini, *Su di un particolare compartamento di batteri d'acqua dolce*, Instituto di Microbiologia dell Universita di Pavia, 1963

[2] R. Blakemore *Magnetotactic Bacteria*, Science, 190:377-379, 1975.

[3] U. Heyen and D. Schüler *Growth and magnetosome formation by microaerophilic Magnetospirillum strains in an oxygen-controlled fermenter*, Appl. Microbiol. Biotechnol., 61:536-544, 2003.

[4] C.B. Flies, J. Peplies and D. Schüler *Combined Approach for Characterization of Uncultivated Magnetotactic Bacteria from Various Aquatic Environments*, Appl. Environ. Microbiol., 71(5):2723-2731, 2005.

[5] M. Hanzlik, M. Winklhofer and N Petersen *Pulsed-field-remanence measurements on individual magnetotactic bacteria*, Journal of Magnetism and Magnetic Materials, 248(2) :258-267, 2002.

[6] R. Kawaguchi, J.G. Burgess, T. Sakaguchi, H. Takeyama, R.H. Thornhill, and T Matsunaga, *Phylogenic analysis of a novel sulphate-reducing bacteria magnetic bacterium, RS-1 Demonstrates its membership of the delta pro-bacterium*. FEMS Microbiol. Lett., 126:277-282, 1995.

[7] D.A. Bazylinski and R.B. Frankel, *Magnetosome formation in prokaryotes*, Nature Reviews/Microbiology, 2, Mar 2004.

[8] F. Freitas, C.N. Keim, B. Kachar, M. Farina and U. Lins, *Envelope ultra-structure of uncultured naturally occurring magnetotactic cocci*. FEMS Microbiol. Lett., 219(1):33-38, 2003.

[9] D.A. Bazylinski, *Structure and function of the bacterial magnetosome*, ASM News 6:337-343, 1995.

[10] D. Schüler, *Formation of magnetosomes in magnetotactic bacteria*, J. Mol. Microbiol. Biotechnol., 1:79-86, 1999.

[11] D. Schüler, *"The biomineralization of magnetosomes in Magnetospirillum gryphiswaldense"*, 2002 Int. Microbiology, 2002.

[12] D.A. Bazylinski, A.J. Garratt-Reed, R.B Frankel, *Electron microscopic studies of magnetosomes in magnetotactic bacteria*, Microscopy Research And Technique, 27(5) :389-401, Apr 1994.

[13] S. Spring and K.H. Schleifer, *Diversity of magnetotactic bacteria*, Systematic and Applied Microbiology, 18(2):147-153, 1995.

[14] D. Schüler and R.B. Frankel, *Bacterial magnetosomes: microbiology, biomineralization and biotechnological applications*, Applied Microbiology And Biotechnology, 52(4) :464-473, Oct 1999.

[15] Y.A. Gorby, T.J. Beveridge and R.P Blakemore, *characterization of the bacterial magnetosome membrane*, Journal of Bacteriology. 170:834-841, 1988.

[16] Y. Liu, M. Gao, S. Dai, K. Peng and R. Jia, *Characterization of magnetotactic bacteria and their magnetosomes isolated from Tieshan iron ore in Hubei Province of China*, Material Science and Engineering, 26(4):597-601, 2006.

[17] K.H. Scleifer, D. Schuler, S. Spring, Weizenegger, M.R. Amann, W. Ludwig and M. Kuhler, *The genus Magnetospirillum gen. Nov. description of Magnetospirillum gryphiswaldene sp. nov. and transfer of Aquasiprillum magnetotactium to Magnetospirillium magnetotactium comb. nov.* Systematic and Appl. Microbiol., 14(4):379-385, 1991.

[18] R.B. Frankel and D.A. Bazylinski, *Magnetotaxis and Magnetic Particles in Bacteria. Hyperfine Interactions*, 90(1):135-142, 1994.