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Microbial synthesis of iron-based nanomaterials—A review

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Abstract. **Nanoparticles are the materials having dimensions of the order of 100 nm or less. They exhibit a high surface/volume ratio leading to different properties far different from those of the bulk materials. The development of uniform nanoparticles has been intensively pursued because of their technological and fundamental scientific importance. A number of chemical methods are available and are extensively used, but these are often energy intensive and employ toxic chemicals. An alternative approach for the synthesis of uniform nanoparticles is the biological route that occurs at ambient temperature, pressure and at neutral pH. The main aim of this review is to enlist and compare various methods of synthesis of iron-based nanoparticles with emphasis on the biological method. Biologically induced and controlled mineralization mechanisms are the two modes through which the micro-organisms synthesize iron oxide nanoparticles. In biologically induced mineralization (BIM) mode, the environmental factors like pH, pO_2, pCO_2, redox potential, temperature etc govern the synthesis of iron oxide nanoparticles. In contrast, biologically controlled mineralization (BCM) process initiates the micro-organism itself to control the synthesis. BIM can be observed in the Fe(III) reducing bacterial species of *Shewanella*, *Geobacter*, *Thermoanaerobacter*, and sulphate reducing bacterial species of *Archaeoglobus fulgidus*, *Desulfuromonas acetoxidans*, whereas BCM mode can be observed in the magnetotactic bacteria (MTB) like *Magnetospirillum magnetotacticum*, *M. gryphiswaldense* and sulphate-reducing magnetic bacteria (*Desulfovibrio magneticus*). Magnetite crystals formed by Fe(III)-reducing bacteria are epicellular, poorly crystalline, irregular in shapes, having a size range of 10–50 nm super-paramagnetic particles, with a saturation magnetization value ranging from 75–77 emu/g and are not aligned in chains. Magnetite crystals produced by MTB have uniform species-specific morphologies and sizes, which are mostly unknown from inorganic systems. The unusual characteristics of magnetosome particles have attracted a great interdisciplinary interest and inspired numerous ideas for their biotechnological applications. The nanoparticles synthesized through biological method are uniform with size ranging from 5 to 100 nm, which can potentially be used for various applications.**

Keywords. **Nanoparticles; biosynthesis; microbes; iron reducing bacteria; sulphate reducing bacteria; magnetotactic bacteria.**

1. Introduction

Nanoparticles are considered to be the building blocks for nanotechnology and are referred to as the particles having one or more dimensions of the order of 100 nm or less (Huber 2005). Nanostructured materials have attracted considerable attention in recent years because they exhibit useful and unusual properties compared to conventional polycrystalline materials. They offer better built, long lasting, cleaner, safer, and smarter products for use in home, communications, medicine, transportation, agriculture, and industry in general. A key understanding of nanotechnology is that it offers not just better products, but a vastly improved manufacturing process. It covers fields from biology to material science, physics to chemistry, and can include development in a variety of specialities. The physical and chemical properties of metal nanoparticles are mainly determined by its size, shape, composition, crystallinity and structure (Addadi and Weiner 1992; Bazylinski *et al* 2007). Control over these parameters is crucial for a successful utilization of the size-dependent properties that are unique to nanoparticles like assembly of monolayer-protected nanoparticles into crystalline arrays of one-, two- or three-dimensions. These nanocrystalline particles have a high surface/volume ratio leading to magnetic properties different from those of bulk materials (Bazylinski *et al* 2007). The controlled synthesis of magnetic nanoparticles is of high scientific and technological interest. In particular, magnetite (Fe_3O_4) is a common ferritic material having a cubic inverse spinel structure. The compound exhibits unique electric and magnetic properties based on the transfer of electrons between Fe^{2+} and Fe^{3+} in the octahedral sites. Interest in the magnetite has centred on applications such as multiterabit magnetic storage devices, ferrofluids, sensors, spintronics, separation processes, MRI contrast enhancement agents, biomedical fields and especially in environmental remediation (Bharde *et al* 2005; Huang and Ehrman 2007). To synthesize such particles, several methods are used. The nanoparticles formed using

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Table 1. Physical and chemical methods for synthesis of iron nanoparticles.

Phase of synthesis	Synthesis method	Nanostructured material synthesized	Demerits	References
Vapour				
a) Chemical vapour condensation	Precursor molecules in short residence in heated tube start to decompose and gas stream expands rapidly to mitigate particle growth. Finally, nanoparticles condense on a cooled substrate, scrapped and collected	Metals (Fe, Cu, Co), metal oxides (MgO,TiO_2), carbides, nitrides (Fe/N, ε-Fe_3N), and composites	• Capital intensive • Low production rates • Difficult to control particle size • Difficult to scale up	Tavakoli *et al* (2007)
b) Laser pyrolysis	Laser heats a gaseous mixture of iron precursor and a flowing mixture of gas producing small, narrow size and no-aggregated nanoparticles	Maghemite nanoparticle of size 2–7 nm	• Low production rate • High energy consumption • Highly uneconomical	Kalyanaraman *et al* (1998)
c) Spray pyrolysis	Solution of ferric salts and a reducing agent in organic solvent is sprayed into a series of reactors, where the aerosol solutes condense and resulting dried residue consists of nanoparticles	Maghemite nanoparticle of size 5–60 nm	• High energy consumption • Highly uneconomical	Kalyanaraman *et al* (1998)
d) Inert gas condensation	Iron metal foil produces metal vapour when heated in a ceramic crucible in a chamber filled with an inert gas. This vapour cools and gives nanoparticles	Iron nanoparticle of size 5–40 nm	• Expensive method due to high energy consumption • Inability to control particle size	
Liquid				
a) Microemulsion	W/O microemulsion solutions are nanosized water droplets dispersed in continuous oil phase and stabilized by surfactant molecules, offering a microenvironment for formation of nanoparticles	γ-Fe_2O_3 nanoparticle of size 5–40 nm	• Expensive surfactant • Low production yield • Use of a large amount of liquids	Huber (2005); Capek (2004)
b) Hydrothermal	High temperature–high pressure aqueous solutions, vapours, and/or fluids react with solid materials	Iron nanoparticle (4–16 nm)	Difficult to control process reproducibility	Lester *et al* (2006)
c) Sol-gel	It includes four steps: hydrolysis, polycondensation, drying, and thermal decomposition	γ-Fe_2O_3 nanoparticle of size 6–15 nm	High cost	Yoshimura and Somiya (1999)
d) Thermal decomposition	Decomposition of iron organic precursors in presence of organic solvents and surfactants at 100°C	Monodispersed nanoparticle (size 4–20 nm)	Mechanically and kinetically very complicated	Huber (2005)
e) Sonochemical decomposition	Acoustic cavitation is used to provide localized heating, resulting in decomposition of iron precursor of stabilizer and formation of nanoparticles	Iron nanoparticle (3–8 nm)	Inability to control particle size	Huber (2005)
Solid				
Ball milling	A high mechanical energy will be applied on powders along with several heavy balls in a high speed rotar	Magnetite nanoparticle of size around 12 nm	• High energy requirement • Extensive milling time • Microstructure sensitivity • Numerous defects in product • Powder contamination by steel balls	Rawers *et al* (1999); Roh *et al* (2001); Pithawala *et al* (2000)

each method show specific properties. The objective of this review is to present the various methods of synthesis of metal nanoparticles of various sizes by different processes, to compare the biological method with other methods and touching upon its application.

2. Methods of synthesis of nanoparticles

Synthesis of nanoparticles to have a better control over the particle size, distribution, morphology, purity, quantity and quality by employing ecofriendly economical processes, has always been considered a challenge. Moreover, special attention is paid to monodispersed and stable particles formation. Different metals, metal oxides, sulfides, polymers, core-shell and composite nanoparticles can be prepared using a number of synthesis techniques. Currently a number of chemical and physical methods (table 1) are being used for iron based nanoparticle synthesis. The summary in table 1 encompasses the various physical and chemical methods used with their respective importance.

3. Biosynthesis of iron oxide nanoparticles

There are various modes of biosynthesis of iron oxide nanoparticles. Biogenic processes are important in the formation of iron oxide nanoparticles generally known as biomineralization. There are two modes of biomineralization as shown below.

3.1 *Biologically induced biomineralization (BIM)*

BIM mode allows extracellular synthesis of the magnetite crystals in the culture solution as a by-product and magnetite formation primarily depends on the environmental parameter, like pH, pO_2, pCO_2, redox potential, temperature. The microbial cell induces release of metabolites into the surrounding solution as a result of their metabolic activity. These metabolites in turn react with specific ions or compounds, either in solution or already adsorbed to the cell surface resulting in the mineral particle formation. Generally anaerobic bacteria undergo BIM process of biomineralization. Minerals produced by BIM are poorly crystalline with a broad size distribution and lack of specific, consistent crystal morphologies (Bazylinski *et al* 2007). BIM can be observed in the following microbes: Fe(III) reducing bacteria (*Shewanella* sps., *Geobacter* sps., *Thermoanaerobacter ethanolicus* etc) (Roh *et al* 2006a, b) and SRB (*Archaeoglobus fulgidus, Desulfuromonas acetoxidans).*

3.1a *Biosynthesis through Fe(III)-reducing bacteria (FRB)*: Dissimilatory iron-reducing bacteria such as *Geobacter metallireducens* and *Shewanella putrifaciens* are the most commonly studied microbes that produce crystals of magnetite as a by-product of their metabolism in the growth medium (Bazylinski *et al* 2007). Dissimilatory iron-reducing bacteria generally respire with oxidized Fe(III) compound in the form of Fe(III) oxyhydroxide under anaerobic condition and secrete poorly crystalline Fe(II) into surrounding environment. The Fe(II) so formed then adsorbs onto excess ferric hydroxide grain where it is transformed into magnetite (Yeary *et al* 2005; Bazylinski *et al* 2007). Magnetite formation is favoured by high pH:

$$CH_3COO^- + 8\,Fe\,(OH)_3 \rightarrow 8\,Fe^{2+} + 2HCO_{3^-} + 15OH^- + 5H_2O, \quad (1)$$

$$2OH^- + Fe^{2+} + 2\,Fe\,(OH)_3 \rightarrow Fe_3O_4 + 4H_2O. \quad (2)$$

Fe(III) reduction typically results with an increase in pH, ionic strength of the pore water and the concentration of a variety of cations. The amount of magnetite produced by FRB depends on the level of inorganic phosphate and availability of Fe(III) in the culture medium (Bazylinski *et al* 2007).

In figure 1, the mechanism of iron reduction through an iron-reducing bacteria *Geobacter sulfurreducens* is shown. The *Geobacter sulfurreducens* derive electrons from NADH oxidation and the electrons are passed to the 89 KDa cytochrome, then to the periplasmic 9 KDa cytochrome from where the electron is transferred to Fe(III) via 41 KDa cytochrome (figure 1). Magnetite crystals formed by FRB are epicellular, poorly crystalline, irregular in shapes having a size range of 10–50 nm super paramagnetic particles, with a saturation magnetization value ranging from 75–77 emu/g and are not aligned in chains. FRB produces 5000 fold more magnetite per unit biomass than magnetotactic bacteria (Bazylinski *et al* 2007; Moon *et al* 2007). Besides *Geobacter* and *Shewanella* sps., another FRB *Thermoanaerobacter ethanolicus* strain TOR

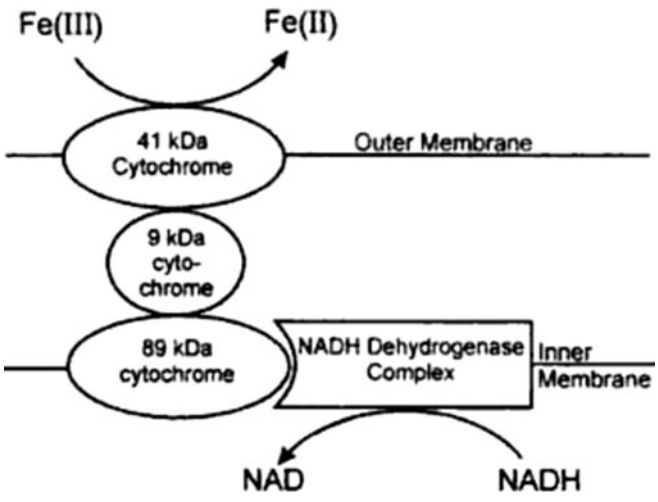

Figure 1. Mechanism of Fe(III) reduction in *Geobacter sulfurreducens* (Bazylinski *et al* 2007).

39 (Yeary *et al* 2005) and a *Gallionella ferruginea* also helps in magnetite formation. TOR strain 39 is an anaerobic, gram-negative, rod-shaped bacterium and ferments carbohydrates. It has a doubling time of 3 h with magnetite production rate of 2000 mg of Fe_3O_4 per litre of culture per day (Yeary *et al* 2005). TOR strain 39 produces single-domain tiny octahedral magnetic particles (<12 nm) (Mandal *et al* 2006). Metal substituted magnetite crystals can also be biosynthesized by the thermophilic iron reducing bacteria *Thermoanaerobacter ethanolicus* strain TOR 39 by using electrochemical process (Moon *et al* 2007).

3.1b *Biosynthesis through sulfate reducing bacteria (SRB)*: Some anaerobic sulfate-reducing bacteria produce particles of magnetic iron. The SRB respire with sulfate anaerobically releasing H_2S (dissimilatory sulfur reduction). Strains of *Desulfuromonas* are generally used for magnetite synthesis. They generally grow in defined basal anaerobic medium (Mandal *et al* 2006) at an optimal temperature of 30°C and pH of 6·5–8·5 (optimal 7·2–7·5). For SRB, ethanol, propanol, pyruvate, lactate, propionate, higher fatty acids and glutamate can serve as electron donor and carbon source. For growing SRB it generally takes at least four days.

Mechanism of Fe (III) reduction by the help of sulphate reducing bacteria (SRB): Magnetite can be formed at elevated temperatures from amorphous Fe(III) oxide in the presence of molecular hydrogen and sulfide produced enzymatically via microbial sulfate reduction. The following chemical reactions take place:

Sulfate reduction to sulfite by molecular hydrogen:

$$S + H^+ + 4H_2 \rightarrow HS^- + 4H_2O. \quad (3)$$

Fe(III) reduction by sulfide to yield Fe(II) and elemental sulfur:

$$2Fe(OH)_3 + HS^- + 5H^+ \rightarrow 2Fe^{2+} + S^0 + 6H_2O. \quad (4)$$

Magnetite formation from Fe(III) and Fe(II):

$$2Fe(OH)_3 + Fe^{2+} \rightarrow Fe_3O_4 + 2H^+ + 2H_2O. \quad (5)$$

Apart from this, *Actinobacter* species are also able to synthesize iron oxide nanoparticles through BIM mode of biomineralization. Similarly, other micro-organisms can also be used for synthesis of other nanoparticles (table 2), which can be used for a wide range of applications.

3.2 *Biologically controlled biomineralization (BCM)*

In BCM mode, the magnetite crystals are formed intracellularly and the crystal formation is under the strict control of the micro-organism. Initially, a specific site within the cytoplasm or the cell wall is sealed off from the external environment thereby creating geochemical environment

Table 2. Biosynthesis of metal nanoparticles by different microorganisms.

Microorganisms	Type of nanoparticle	Size	References
Bacteria			
Bacillus subtilis	Gold	5–25 nm	Yonghong *et al* (2006)
Pseudomonas aeruginosa	Gold	15–30 nm	Husseiny *et al* (2007)
Pseudomonas stutzeri	Silver	Up to 200 nm	Joerger *et al* (2000); Klaus *et al* (1999)
Desulfobacteriaceae	Zinc sulfide		
Thermoanaerobacter ethanolicus	Magnetite	35–65 nm	Yeary *et al* (2005)
Magnetospirillum magnetotacticum	Magnetite	50–100 nm	
Rhodococcus sp.(Actinomycete)	Gold	5–15 nm	Ahmad *et al* (2003a)
Yeast			
Candida glabrata	Cadmium sulfide	20 Å	Dameron *et al* (1989)
Torulopsis sp.	Lead sulfide		
Schizosaccharomyces pombe	Cadmium sulfide	1–1·5 nm	Kowshik *et al* (2002)
MKY3	Silver	2–5 nm	Kowshik *et al* (2003) Roh *et al* (2001)
Fungi			
Fusarium oxysporum and *Verticillium* sp.	Magnetite	20–50 nm	Bharde *et al* (2006)
Fusarium oxysporum	Gold	20–40 nm	Mukherjee *et al* (2002)
	Silver	5–15 nm	Ahmad *et al* (2003b)
Fusarium oxysporum	Cadmium sulfide	5–20 nm	Ahmad *et al* (2002)
Fusarium oxysporum	Zirconia	3–11 nm	Bansal *et al* (2004)
Colletotrichum sp.	Gold	20–40 nm	Shankar *et al* (2003)
Aspergillus fumigatus	Silver	5–25 nm	Bhainsa and D' Souza (2006)

independent from the outer one. The next step after organic matrix formation is the transfer of sequestered specific ions of choice to the isolated compartment where their concentration increases up to a supersaturation level. Nucleation is controlled by exposing ligands with distinct stereochemical and electrochemical properties tailored to interact with specific hydrated ions. The organic functional groups act as surrogate oxyanions and stimulate the first layer of the incipient nuclei (Mann 1993). The crystals then grow in a highly ordered manner having their orientation, morphology and size governed by the overall ultrastructure of the membrane bound compartment. Minerals produced by BCM are well-ordered crystalline with a relatively narrow size distribution and specific, consistent morphology. MTB (*Magnetospirillum magnetotacticum, M. gryphiswaldense*) and sulphur reducing magnetic bacteria (*Desulfovibrio magneticus*) synthesize iron nanoparticles through BCM mode.

3.2a *Biosynthesis through magnetotactic bacteria (MTB)*: MTB are gram negative, aquatic, motile and microaerophilic. MTB can be isolated from freshwater swamps and ponds (Bazylinski *et al* 2007), saltmarsh ponds (Sparks *et al* 1989), freshwater sediments (Posfai *et al* 2006), marine sediments (Bazylinski *et al* 2007), soils or the places having high iron content, neutral pH and are not well oxygenated. Highest number of MTB (10^5–10^6/ml) are generally found in chemically stratified water column or sediments where they occur predominantly in or below the micro-aerobic redox transition zone, between the aerobic zone of upper waters or sediments and the anaerobic regions of the habitat are found at the oxi–anoxic transition zone. They are the heterogeneous group of aquatic micro-organisms which share the ability to orient themselves along magnetic field lines, a phenomenon known as magnetotaxis (Blakemore 1975, 1982). Magnetic orientation is due to the presence of magnetosomes, which are intracellular membrane-bound crystals of magnetic iron mineral which consists of magnetite or greigite (Blakemore *et al* 1979; Bazylinski *et al* 2007). Diverse MTBs including cocci, spirilla, rods, vibrios, and multicellular generally form aggregates (Bazylinski *et al* 2007). Most MTBs are members of the α-proteobacteria while some MTBs from the α-subclass are closely related to nonmagnetic, nonsulfur purple bacteria (Schuler 1999). MTB synthesizes iron nanoparticles through BCM process of biomineralization (Bazylinski *et al* 2007). Biomineralization of iron oxide is a process with genetic control over the accumulation of iron that proceeds through the deposition of the magnetic crystal within a specific compartment as well as the assembly, alignment and intracellular organization of particle chains. Magnetite crystals produced by MTB have uniform species-specific morphologies and sizes, which are mostly unknown from inorganic systems. The unusual characteristics of magnetosome particles have attracted a great interdisciplinary interest and inspired numerous ideas for their biotechnological application.

Magnetosomes are the specialized organelles synthesized by magnetotactic bacteria for geomagnetic navigation in their aquatic habitats. The magnetosome membrane (MM) structure in Magnetospirillum strains (*M. magnetotacticum* or *M. gryphiswaldense*) consists of a bilayer of about 3–4 nm containing phospholipids and proteins (Frankel *et al* 1983). All magnetotactic bacteria synthesize in ferrimagnetic crystals of either magnetite (Fe_3O_4) or the iron sulfide-greigite (Fe_3S_4) (Frankel *et al* 1983; Bazylinski *et al* 1993). The size, morphology, and chemical composition of magnetite crystals are subject to a species-specific genetic control. Different MTBs display a considerable diversity with respect to magnetosome morphologies (figure 2) which are mostly unknown from magnetite particles formed by chemical synthesis. The size and arrangement of magnetosomes are important in determining the magnetic properties of the bacterium. The magnetosomes of size range 35–120 nm show permanent magnetism and thus the magnetotaxis behaviour i.e. the passive orientation of magnetotactic bacteria along the earth's geomagnetic field lines (Blakemore 1975). While those above 120 nm do not show magnetotaxis behaviour as they are unable to show permanent magnetism. When the magnetosomes are arranged in chains with the help of magnetosome membrane, the magnetic interactions between the magnetosomes make each magnetosome moment along the chain axis parallel with each other. Due to this, the magnetic moment of the magnetosome chain is the sum of the magnetic moment of individual magnetosomes in the chain. In contrast, when magnetosomes are not aligned in chain, they are free to float in the cytoplasm and may form aggregates having a much smaller net dipole moment than the magnetosome chain with the same number of magnetosome crystals (Varadan *et al* 2008). It is observed that in natural environments, magnetotaxis enables the cells to locate and maintain an optimal position in water columns or in sediments with respect to their main metabolic needs: molecular oxygen and organic nutrients (Schuler 1999).

Mechanism of magnetite biomineralization in magnetospirillum species: Fe(III) is actively taken up by the cell possibly via a reductive step. Iron is then thought to be reoxidized to form a low density hydrous oxide which is dehydrated to form a high-density Fe(III) oxide (ferrihydrite). In the last step, one-third of the Fe(III) ions are reduced, and with further dehydration, magnetite is produced within the magnetosome vesicle (Schuler 1999). The magnetosome membrane contains specific proteins called as "Ferritin", which are thought to have crucial role in the accumulation of iron, nucleation of minerals and redox and pH control (Schuler 1999) (figure 3). Ferritin is a ubiquitous intracellular protein that stores iron and releases it in a controlled fashion. Ferritin is a globular protein complex consisting of 24 protein subunits and is the primary intracellular iron-storage protein in both prokaryotes and eukaryotes, keeping iron in a soluble and non-toxic form. Ferritin that is not combined with iron is called apoferritin (Theil 1987).

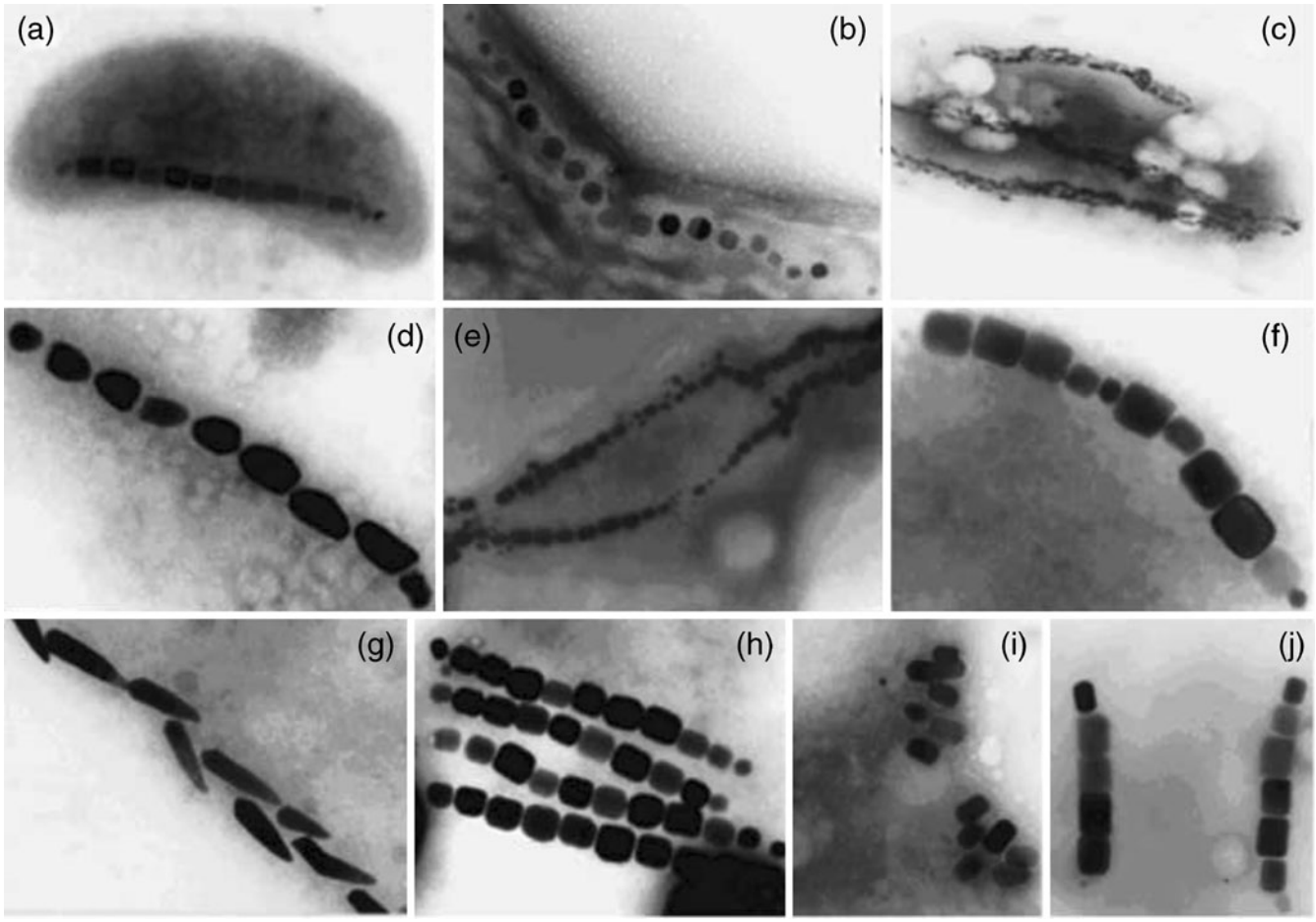

Figure 2. Diversity of magnetosome crystals and arrangements in various MTB (Characteristic crystal habits found in various MTB are elongated prisms (a, e, f, h, i, j), cubo-octahedral (b), and bullet-shaped morphologies (c, d, g). Crystals can be arranged in single or multiple chains) (Frankel *et al* 1983).

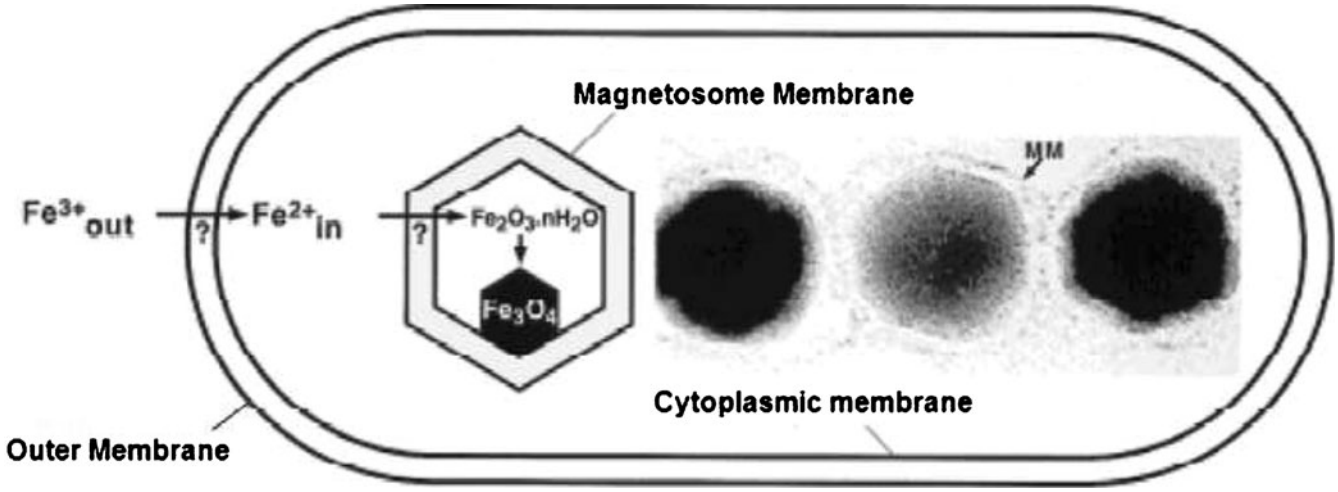

Figure 3. Model for magnetite biomineralization in *Magnetospirillum* species (Schuler 1999).

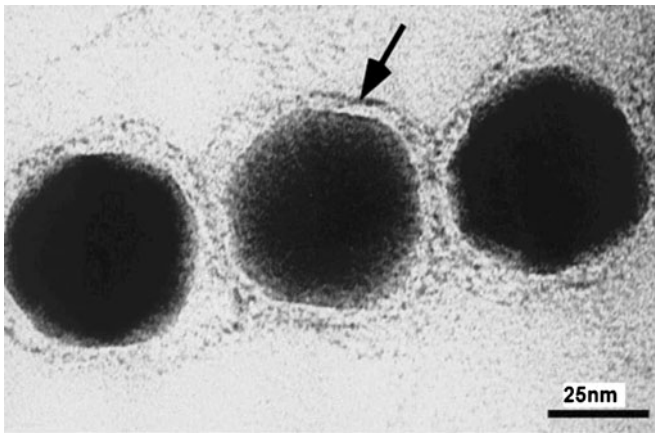

Figure 4. Magnetosome particles isolated from *M. gryphiswaldense* (Thomas-Keprta *et al* 2000). The magnetite crystals are typically 42 nm in diameter and are surrounded by magnetosome membrane (arrow).

Thomas-Keprta *et al* (2000, 2001) have identified six properties that they claimed as collectively unique for MTB-NPs which are: (i) unusually truncated hexa-octahedral morphology; (ii) few crystallographic defects; (iii) elongated habit; (iv) narrow size distribution restricted mainly to the single domain field; (v) high purity; and (vi) alignment in chains (figure 4).

4. Conclusions

(I) Current physicochemical methods (sol–gel technique, chemical vapour deposition, hydrothermal synthesis, precipitation method, micro-emulsion method) of oxide nanoparticles synthesis are hazardous, eco-unfriendly, cumbersome, costly and require high temperature, pH and/or pressure for synthesis.
(II) Biosynthesis of iron oxide nanoparticles with the help of Fe(III)-reducing bacteria (*Shewanella* sps.*, Geobacter* sps.*, Thermoanaerobacter ethanolicus* etc.), SRB (*Archaeoglobus fulgidus, Desulfuromonas acetoxidans)* and MTB (*Magnetospirillum magnetotacticum, M. gryphiswaldense),* are reliable eco-friendly and economic at ambient temperatures, pressures and neutral pH, that can be used in environmental remediation. For example, zerovalent iron nanoparticles can be used for removing chromium, nitrate, nitrite, organic contaminants etc from waste water.

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