Preparation and properties of ferrite derived from iron oxidizing bacteria

ZHANG WenQiang, CAI Jun* & ZHANG DeYuan

Bionic and Micro/Nano/Bio Manufacturing Technology Research Center, Beihang University, Beijing 100191, China

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The growth characteristics of *Sphaerotilus natans* and *Leptothrix discophora* SS-1 (ATCC43182) were first used to fabricate the ferrite and manganese ferrite. The scanning electron microscopy (SEM), energy dispersive X-ray spectrometry (EDS), X-ray diffraction (XRD), and vibrant sample magnetometer (VSM) were used to make clear their morphology microstructure, chemical composition, crystal structure, and magnetostatic characteristic. The result showed that the hollow ferrite fibers were prepared successfully, whose crystal structures were close to α -Fe₂O₃ and the average aspect ratio was over 50. Moreover, the soft magnetic manganese ferrite was fabricated with the saturation magnetization M_s at 25 emu/g and coercive force H_c at 796 A/m.

iron bacteria, ferrite, magnetic functional material, bio-growth forming

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Biological manufacturing is an emerging cross-disciplinary, which could achieve the efficient and environmentally friendly fabrication of micro/nano functional materials or devices [1,2]. Bio-growth forming is a new method to obtain the required functional materials or products with the direct use of the microbial growth characteristics [1]. For example, the microbe is used to pharmaceutical fermentation, or the magnetotactic bacteria is used to synthesize single domain Fe₃O₄ [3,4].

Currently, in the areas of materials, the study of magnetic materials represented by ferrite has become a hotspot, and it is widely used in the manufacture of new high-tech products, like electromagnetic absorbing materials, magnetic recording materials, magnetic fluids, catalytic materials, coating or paint [5,6]. It was shown that iron bacteria are widespread in various types of nature waters, which have the capacity of oxidizing and depositing ferrous ions and manganese ions in water efficiently. This microbe could be directly used to obtain composites with a variety of shapes such as fiber, tube and film [7–16] and has the potential to fabricate

the ferrite materials. Compared to traditional chemical synthesis [5], there may be a breakthrough in the aspects of physical control, particle reunion and industrialization.

In this paper, according to the enrichment and deposition mechanism of iron and manganese [7–12], the sediment composition, the microstructure characteristics [15] and other basic research of iron bacteria, the *Sphaerotilus natans* which were separated from activity mud of Xiamen sewage treatment plant and the *Leptothrix discophora* which was purchased from ATCC, were used to deposit the iron and manganese ions respectively. The Sediments were collected and heat treated at high temperature to obtain the ferrite. And then the product morphology and static magnetic properties were characterized.

1 Materials and methods

1.1 Materials and formulations

The *Sphaerotilus natans* were isolated and cultured from the Xiamen sewage treatment plant [13,14], then the *Leptothrix discophora* was purchased from ATCC (ATCC 43182).

*Corresponding author (email: jun_cai@buaa.edu.cn)

1.2 Experimental process

Ferrite fabricated by the *Sphaerotilus natans* growth: According to Table 1, 0.8 L urea medium was configured. After high-temperature sterilization, the activated *Sphaerotilus natans* were vaccinated. Then the medium was cultured for 24 h at the constant temperature of 27°C so as to obtain a great number of *Sphaerotilus natans*. In order to prevent the bacteria from being killed as a result of a great change of pH, the iron deposition medium, after UV sterilization, needed to be added to the mixture in an equal amount by ten times. In this process, the pH was kept between 6.5 and 7.0 by dropping 1 mol/L of sodium hydroxide and 1 mol/L hydrochloric acid solution to the medium. Place the culture medium in the incubator of 27°C for 50 continuous days. After that remove it and the sediment was obtained for observation and testing by repeated centrifugation, washing, and vacuum drying. In addition, put a small amount of powder into the muffle furnace, and heat-treat it at 900°C for 1 h. After that, cool down the sample in the furnace to the room temperature, and remove it for use.

Ferrite fabricated by the *Leptothrix discophora* growth: The *Leptothrix discophora* was inoculated to pre-configured ATCC medium at 2°C by 150 r/min, activated and enrichment cultured for 72 h, and with the pH value (pH 7) adjusted every 12 h with NaOH; then take 100 mL enriched culture medium and add to the pre-configured MMS medium, adjust pH to 6.0; prepare FeSO₄ and MnSO₄ mixed solution (Fe²⁺, Mn²⁺ ion ratio is 3:1, 2:1, and 1:1, Mn²⁺ ions concentration of 1 mol/L), and after filter-sterilized,

20 mL solution was added to the three culture medium respectively to adjust pH to 6; add every 24 h for 10 consecutive days; the medium was left to stand at 27°C for a week, and then it was repeatedly centrifugated, washed, and vacuum dried in order to obtain sediment; finally, place the sediment into the muffle furnace, and heat-treat it at 900°C for 1 h. After that, cool down the sample in the furnace to the room temperature, and remove it for use.

1.3 Properties characterization

XSY-1 optical microscope plus Cannon 450 photographic camera, CS3400 scanning electron microscope (SEM), and Apollo300 Field emission scanning electron microscope (FESEM) were used to observe and study the cell form, while Oxford Link 860 energy dispersive spectrometer (EDS) and D/Max 2200 PC automatic X-ray diffraction (XRD) were used respectively to analyse the composition and the phase structure. The magnetic properties were evaluated on a vibrating sample magnetometer (VSM, JDM-13).

2 Results and discussion

To fabricate the fiber and iron oxide using the growth of iron bacteria, the *Sphaerotilus natans* and the *Leptothrix discophora* first needed enrichment culturing.

Figure 1 shows the optical photograph of *Sphaerotilus natans* (1.8–2.3 μm × 10–250 μm). In Figure 1a, with a few sheath adhering in bundles, pseudo-single-branch and cell vacancy appeared. In Figure 1b, single iron oxidizing bacteria cells (A), short rod-shaped (1.0–2.5 μm × 1.5–5.8 μm), were found around the clothing sheath with more cells vacancy (B).

Figure 2 shows the optical photograph of *Leptothrix discophora* (0.5–1 μm) with the ATCC medium enrichment culture. Figure 2a shows that the single bacterial cells account for a large percentage, with obvious agglomeration; while in Figure 2b, there is a marked absence of clothing and cell sheath. Although *Leptothrix discophora* (ATCC43182) itself does not have clothes sheath [9], but in the latter part of the training process, there will be variations in the cell, thus forming clothing sheath.

Figure 1 Optical photograph of *Sphaerotilus natans*. a, Enrichment culture for 24 h; b, enrichment culture for 72 h; A, a single cell of *Sphaerotilus natans*; B, a cell vacancy.

Table 1 The formula table of different medium^{a)}

Name	ATCC medium (mg)	MMS medium (mg)	Urea medium (mg)	Iron deposition medium (mg)
Peptone	500			
Urea			500	500
Yeast extract	500			
Glucose	500		1000	1000
Pyruvate		240		
MgSO ₄ · 7H ₂ O	600	35	50	50
CaCl ₂ · 2H ₂ O	70	30		
HEPES	3570			
MnSO ₄ · H ₂ O	17.0			
FeSO ₄		0.15		
(NH ₄) ₂ SO ₄		120		
KNO ₃		15		
NaHCO ₃		0.84		
KH ₂ PO ₄		0.70		
Vitamin B ₁₂		0.002		
K ₂ HPO ₄			100	100
Fe ₂ (SO ₄) ₃				2280
Distilled water (L)	1	1	1	1

a) With NaOH to adjust pH: ATCC medium, pH 7.3; MMS medium, pH 6.0; urea medium, pH 7.0; and iron deposition medium, pH 6.5.

Figure 2 Optical photograph of *Leptothrix discophora*. a, Cell-free state; b, clothing sheath cells forming.

The iron deposition medium and MMS media were used further to inoculate the *Sphaerotilus natans* and the *Leptothrix discophora*, respectively. After 24 h, yellow or brown sediment began to appear in the culture medium. Several weeks later, the sediment was basically stable. Figure 3 shows the overview of the sediment sample which was separated from the iron deposition medium, filamentous fibers (A) occupying most of the view with some micro particulate particles (B). Figure 3a is low magnification SEM micrographs of *Sphaerotilus natans* sediment, in which the proportion of iron fibers (A) in products is more than 90%. The fibers maintained the shape of the *Sphaerotilus natans* (fibrous and curved), and most of the iron fibers did not have a smooth outer layer. Figure 3b is high magnification SEM image of one single ferrite fiber, and from its scale we can see that the fiber diameter was 2.18 µm, and that the fiber aspect ratio was about 50. Figure 3c gives the enlarged image of the broken section of the fiber. It is clear that the fiber was hollow inside, which was consistent with the deposition theory of iron bacteria. Figure 4 is SEM micrographs of manganese and iron oxide which was deposited by *Leptothrix discophora*. Figure 4a shows that the obtained oxides were nano-particles with the diameter changing from tens to hundreds of nanometers (C). It was observed that *Leptothrix discophora* (D) cells remained in the nano-particles, which is the further evidence of *Leptothrix discophora* growth leading to iron and manganese oxides in the surrounding sediments; while Figure 4b shows the obtained

Figure 3 SEM images of iron oxide fiber prepared by *Sphaerotilus natans*. a, Gathered fibers; b, single fiber; c, the broken section of the fiber.

Figure 4 SEM images of manganese and iron oxide prepared by *Leptothrix discophora*. a, Manganese and iron oxide; b, mixture of manganese and iron oxide fiber and particles.

oxide composite still contained a small number of hollow fibers (E), with the diameter of 1–2 µm, and the aspect ratio of more than 50, indicating that variation bacteria with sheath ability could form a hollow ferromanganese oxide fibers, and *Leptothrix discophora* cell was out of the sheath.

EDS was used to analyze the chemical composition of the SEM sample, the results showed that the major component of the *Sphaerotilus natans* sediments was iron oxide, and the main content of the *Leptothrix discophora* sediments was ferromanganese oxides, whose EDS spectra was shown in part in Figure 5. Figure 5a is the EDS spectra of iron fibers which were the deposition of the *Sphaerotilus natans*. The result showed that the main components of the fibers were Fe and O, with the Fe/O atomic ratio of 0.77 throughout the statistical analysis on the whole region of iron oxides and a point of the fiber. It is inferred that fiber deposited by the *Sphaerotilus natans* was an iron oxide fiber, Figure 5b and c is the average EDS spectra of the sediment produced by *Leptothrix discophora* growing (Fe^{2+} : Mn^{2+} for the 2:1 and 1:1). The result showed that the main component of sediments were Fe, Mn and O. By comparing the deposition product of Fe, Mn atomic ratio and the addition of iron, manganese ion concentration ratio, they were found to be basically the same (add the ratio of the original 3:1, 2:1, 1:1; and test the average ratio of 3.1:1, 1.9:1, 1:1). Further, it is confirmed that the use of *Leptothrix discophora* could not only prepare ferromanganese oxides, but also control the ratio of manganese and iron in the product.

Figure 6 is the typical XRD diffraction pattern of iron bacteria deposits before and after treatment. There are two broad peaks in the graph in Figure 6a, with d -values corresponding to $d = 0.25$ nm and $d = 0.15$ nm. The major components were considered to be poor crystalline iron oxide. The XRD pattern of the hydrated ferric oxide fibers is similar to Lepidocrocite, $\text{FeO}(\text{OH})$. Figure 6b is the XRD diffraction pattern of the amorphous iron oxide fiber which was heat-treated at 900°C for 1 h. There are many obvious crystallization peaks, indicating that high-temperature treatment results in crystalline phase-change of the amorphous hydrated ferric oxide fibers to crystalline iron oxide fibers. As is shown in Figure 6b, the sample showed strong diffraction peaks matching well with the standard data for $\alpha\text{-Fe}_2\text{O}_3$ (JCPDS79-1741). According to Scherrer formula, their average grain size is 41 nm. And Figure 6c is the XRD

Figure 5 EDS analysis of iron bacteria sediment. a, Iron oxide fiber prepared by *Sphaerotilus natans*; b, c, the manganese and iron oxide prepared by *Leptothrix discophora*.

diffraction pattern of ferromanganese oxide deposited by *Leptothrix discophora* which was heat-treated at 900°C for 1 h. Figure 6c shows obvious characteristic diffraction peaks, indicating good crystalline. As is shown in Figure 6c, the strong diffraction peaks of the sample matched well with MnFe_2O_4 . According to Scherrer formula, their average grain size is 45 nm. The XRD results indicated that the use of iron bacteria deposits to fabricate spinel ferromanganese oxide is feasible.

After heat treatment, the static magnetic properties of iron oxide fiber and manganese ferrite were characterized by vibrating magnetometer respectively. Figure 7a shows the saturation magnetization of iron oxide fibers was 0.4 emu/g, while the coercivity was 1.18×10^5 A/m, which belongs to anti-ferromagnetic material; while Figure 7b shows that the manganese ferrite saturation magnetization reached to 24 emu/g and the coercivity was 796 A/m, with good soft magnetic properties. Initial analysis showed that the performance difference of iron oxide fiber and manganese ferrite prepared by the deposition of iron bacteria was mainly due to the fact that the iron oxide belongs to $\alpha\text{-Fe}_2\text{O}_3$ with the no magnetic body-centered cubic structure. With the

Figure 6 XRD diffraction pattern of iron bacteria sediment. a, Iron oxide fiber prepared by *Sphaerotilus natans*; b, the iron oxide fiber with heat-treatment; c, the manganese and iron oxide with heat-treatment.

deposition of Mn ions, the overall structure of the lattice changed into a spinel-type, resulting in the production of soft magnetic properties, which are consistent with characterized results by XRD and are consistent with the reported properties of manganese ferrite as well [5]. It proved that the feasibility of ferromanganese oxidizing bacteria in preparing manganese ferrite by co-deposition.

3 Conclusions and perspectives

In this paper, the fibrous amorphous iron oxides, with the diameter of 1–3 μm , the aspect ratio of over 50, and the Fe/O atomic ratio of 0.77, were obtained by culturing the sheath iron bacteria. After heat treatment, their main component was $\alpha\text{-Fe}_2\text{O}_3$ and the average grain diameter was 41 nm. Furthermore the composite, with the content of iron and manganese controlled, was prepared successfully by bio-manufacturing using *Leptothrix discophora* with the manganese and iron oxidative capacity. After heat treatment, soft magnetic manganese ferrite was obtained with saturation

Figure 7 Hysteresis loops of iron bacteria sediment. a, Iron oxide fiber; b, manganese ferrite.

magnetization of 25 emu/g and coercivity of 796 A/m. This paper extends the application field of iron bacteria and provides a new method to preparing light and special shaped ferrite. The next step will be in-depth study of the control method of ferromanganese content, the relationship between static magnetic properties and the dynamic magnetic properties, and a try to fabricate soft ferrite and hollow fiber tube by use of iron bacteria.

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