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Magnetic Characterization of Bacterial Magnetic Particles

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Abstract. Mössbauer measurement, chemical analysis (ratio of Fe^{2+} to total Fe content), analyses of the rotational hysteresis loss (W_r) and the magnetic viscosity coefficient (S_v) for bacterial magnetic particles covered with organic thin films (BMPs) were investigated and compared with the results for Fe_3O_4 fine particles prepared by a chemical coprecipitation method. The calculated value of the saturation magnetization (J_s) of BMPs was found to be 1.06×10^{-4} Wb m/kg (84.7 emu/g) using Mössbauer spectroscopy and chemical analysis. The W_r/J_s versus H curve for BMPs had a stronger peak than that of Fe_3O_4 fine particles (Sample C1). The rotational hysteresis integrals (R_h) for BMPs and Fe_3O_4 fine particles were 1.12 and 0.62, respectively. It is believed that they have different magnetization reversal mechanisms. The S_v value of BMPs at 127 A/m was 50% smaller than that of the Fe_3O_4 fine particles (C1). The diameter (D_{act}) of the activation volume (minimum volume for magnetization reversal) calculated from S_v , and the critical diameter (D_c) obtained by considering the superparamagnetic behavior for BMPs, were 39 and 22 nm, respectively. As the Fe_3O_4 fine particles (C1) have smaller D_{act} (31 nm) and D_c (18 nm), it may be concluded that BMPs is magnetically more stable than Fe_3O_4 fine particles (C1).

1. INTRODUCTION

Magnetic bacteria contain crystalline magnetic particles which cause them to orient in the presence of an external magnetic field and swim along the field lines. *Magnetospirillum* sp. strain AMB-1 is a freshwater magnetic bacterium which synthesizes intracellular magnetic particles [1]. The ecological significance of these biological magnets remains unclear and the mechanism of magnetic particles synthesis in magnetic bacteria has not been elucidated, although it has been proposed that sensitivity to the geomagnetic field allows oxygen sensitive magnetic bacteria to swim downward into oxygen-poor sediments, their preferred habitat. Purified bacterial magnetic particles (BMPs) are smaller in size (30-100 nm) and disperse very well because they are covered with organic thin films which consist of stable lipid membrane. Large quantities of bioactive substances may be immobilized on BMPs due to the presence of this membrane. For this reason, BMPs have been investigated for enzyme, antibody and DNA carriers [2]. It is particularly important to study the saturation magnetization (J_s) without the organic thin film, the mechanism of magnetization reversal and the magnetic stability in order to enhance the application of BMPs. This paper reports on studies of Mössbauer measurement, chemical analysis (ratio of Fe^{2+} to total Fe content), analyses of the rotational hysteresis loss (W_r), the magnetic viscosity coefficient (S_v) and the activation volume (V_{act} : minimum volume of magnetization reversal) for BMPs covered with organic thin films. Also, the results of Fe_3O_4 fine particles having various particle diameters prepared by a chemical coprecipitation method will be compared.

2. EXPERIMENTAL

AMB-1 was cultured anaerobically in a MSGM (pH 6.75) at 25°C. The cells grown to stationary phase were harvested by centrifugation at 5 kG for 20 min. The harvested cells (about 10 g wet weight) were washed with Lysis buffer (pH 8.0) and treated with lysozyme (37°C, 40 min) and proteinase (55°C, 8 h) in order to isolate BMPs. The BMPs were collected from the enzyme treated cell fraction using a Sm-Co magnet (0.4 T). They were collected at the bottom of the tube using the magnet and the supernatant was removed. The BMPs covered with organic thin films (about 13 mg) were washed with distilled water twice.

Table 1 shows particle diameter (D), the saturation magnetization (J_s) and coercive force (H_c) for Fe_3O_4 single crystal, BMPs and Fe_3O_4 fine particles (C1, C2, C3 and C4) prepared by a chemical coprecipitation method. D was observed using TEM and SEM. The values of J_s and H_c were measured using a vibrating sample magnetometer (VSM). The value of J_s for BMPs cannot be obtained using a VSM because they are placed on approximately 2.5 nm thick organic thin films. In this study, the existence of γ - Fe_2O_3 in the samples was suspected. The value of J_s for BMPs was calculated from the ratio of Fe^{2+} to total Fe content obtained using Mössbauer spectrum and the results of chemical analysis.

Mössbauer measurements at 25°C were performed using a conventional, constant acceleration spectrometer with a ^{57}Co source in a Rh matrix. The spectra were least-squares fitted using a computer, assuming Lorentzian line shapes. When the recoil-free fraction is a constant, the number of atoms of Fe per unit volume in the absorber is proportional to the effective thickness[3]. The ratio of the number of Fe ions for $(\text{Fe}^{2+}/\text{Fe}^{3+})$ in Fe_3O_4 to the total number of Fe ions in a sample comprising a mixture of Fe_3O_4 and $\gamma\text{-Fe}_2\text{O}_3$ is given by the equation:

$$R = 3/2 (1 + S_A/S_B) \quad (1)$$

where S_A and S_B are the peak areas at -7.7 and -6.7 mm/s. Therefore, $(1-R)$ corresponds to the ratio of the number of ions of Fe in $\gamma\text{-Fe}_2\text{O}_3$ to the total in the sample. In this experiment, J_s values measured using a VSM for Fe_3O_4 single crystal and $\gamma\text{-Fe}_2\text{O}_3$ particles were 1.14 and 0.96×10^4 Wb m/kg (90.7 and 76.4 emu/g), respectively.

The chemical analysis was carried out as follows. The samples were dissolved in HCl under N_2 flow. Fe^{2+} was determined by spectrophotometry using O-phenanthroline. The total amount of Fe was also determined by spectrophotometry using O-phenanthroline after reducing with hydroxylamine hydrochloride.

The magnetic field (H) dependence of the rotational hysteresis loss (Wr) was measured using a torque magnetometer to study the distribution of the anisotropy field (H_A), the average anisotropy field ($H_{A,ave}$) and the mechanism of magnetization reversal. The Wr and the rotational hysteresis integrals (Rh), which corresponds to the mechanism of magnetization reversal, can be expressed as[4,5]:

$$\text{Wr} = \int L d\theta = J_s/2 \int H_A \cdot \sin 2\phi \cdot d\theta \quad (2)$$

$$\text{Rh} = \int \text{Wr}/J_s d(1/H) \quad (3)$$

where L is the torque, θ and ϕ are angles between the easy axis and, H and J_s . $H_{A,ave}$ corresponds to half of the peak value of the Wr versus $1/H$ curve at high magnetic field[6]. The critical volume (V_c) for superparamagnetic behavior can be expressed as follows[7]:

$$V_c = 50 \text{ kT}/J_s H_{A,ave} \quad (4)$$

The magnetic viscosity coefficient (S_v), which had been described as the fluctuation field (H_f) [8], is an important factor which essentially determines the magnetic stability. It is known to depend on S_v , the irreversible susceptibility (X_{irr}) and the logarithmic time ($\ln t$) [8,9]. When the change in magnetization from time t_1 to t_2 (ΔJ) is considered, S_v can be expressed using the following equation[8-10]:

$$S_v = \Delta J / X_{irr} (\ln t_1 - \ln t_2) = kT / (\partial E / \partial H) T = kT / V_{act} J_s \quad (5)$$

where E is the activation energy and V_{act} is the activation volume. After the application of 1.6 MA/m, the samples were subjected to a constant magnetic field (H) for 1000 s and ΔJ was measured using a VSM[11]. The reversible susceptibility (X_{rev}) and the total differential susceptibility (X_{tot}), obtained by differentiating the demagnetizing curve with respect to H, were also measured using a VSM. X_{irr} can be expressed by $X_{tot} - X_{rev}$.

3. RESULTS AND DISCUSSION

As shown in Table 1, Fe_3O_4 fine particles (C1, C2, C3 and C4) prepared by a chemical coprecipitation method had saturation magnetization (J_s) about 3-9 % smaller than that of the Fe_3O_4 single crystal. In order to clarify this reason, powder X-ray diffraction was measured by step scanning method as shown in Figure 1. It is apparent from the XRD pattern that the $\gamma\text{-Fe}_2\text{O}_3$ phase existed in C4, though this phase was not observed in C2 and C3. From the XRD pattern for C1, it seems that the $\gamma\text{-Fe}_2\text{O}_3$ phase was present in small quantities, and the broadness of peak patterns was influenced by peak splitting due to the mixture of $\gamma\text{-Fe}_2\text{O}_3$ and Fe_3O_4 . However, due to insufficient resolution, the existence of $\gamma\text{-Fe}_2\text{O}_3$ could not be confirmed. Therefore, Mössbauer measurement and chemical analysis were performed in order to obtain the ratio of Fe^{2+} to total Fe content. The Mössbauer spectra of all samples shown in Table 1 were measured at 25°C and the results for BMPs and C1 are given in Figures 2 and 3. A paramagnetic doublet was not found and these spectra consist of magnetic hyperfine lines between approximately +8 and -8 mm/s. In general, magnetite (Fe_3O_4) has a cubic spinel structure with iron in both tetrahedral (A sites) and octahedral (B sites) crystal lattice sites: $(\text{Fe}^{3+})_A(\text{Fe}^{2+}\text{Fe}^{3+})_B\text{O}_4$. Thus, the stoichiometric Fe_3O_4 has two distinct sextets in 1:2 intensity ratio at room temperature, corresponding to A sites with hyperfine field $H_{hf} = 39.0$ MA/m, and B sites with $H_{hf} = 36.6$ MA/m [12]. Maghemite ($\gamma\text{-Fe}_2\text{O}_3$) is obtained by completely oxidizing Fe_3O_4 . It also contains iron in tetrahedral A and octahedral B sites, but there are vacancies (usually B sites) to compensate for the increased positive charge. Thus, in analogy to Fe_3O_4 , the stoichiometry can be represented as $(\text{Fe}^{3+})_A(\square)_{1/3}\text{Fe}^{3+}_{5/3}_B\text{O}_4$, and the H_{hf} value is 39.7 MA/m [12], which is almost the same value in the A sites of Fe_3O_4 .

The curve-fitting parameter of line width (Γ) and H_{hf} , which are obtained from Mössbauer spectra, are shown in Table 2 along with the peak area of S_A and S_B for all samples. The magnetic sextet for

Table 1 Particle diameter D, the saturation magnetization J_s and coercive force H_c of Fe_3O_4 single crystal, bacterial magnetic particles (BMPs) and Fe_3O_4 particles (C1, C2, C3 and C4) prepared by the chemical coprecipitation method.

Sample	D(nm)	$J_s(\times 10^4 \text{ Wb m/kg})$	$H_c(\text{kA/m})$
Fe_3O_4 single crystal		1.14	0.1
BMPs	25 - 65(Dave 44)	...	6.4
C1	18 - 45(Dave 26)	1.06	4.8
C2	300 - 800	1.08	9.6
C3	300 - 800	1.10	11.9
C4	40 - 250	1.03	15.1

Figure 1 X ray diffraction patterns of Fe_3O_4 fine particles prepared by the chemical coprecipitation method.

Figure 2 Mössbauer spectrum for bacterial magnetic particles (BMPs).

C2 and C3 had Hhf of 39.2 (A sites) and 36.6 MA/m (B sites), corresponding to Fe_3O_4 single crystal[12].

The line width (Γ) in B sites had especially broad values for C1 and C4. Moreover, Hhf for C1 was 35.9 MA/m, the smallest value in B sites. These results showed the possible existence of a small amount of superparamagnetic particles in C1. The ratio of wt% Fe of Fe_3O_4 and $\gamma\text{-Fe}_2\text{O}_3$ obtained from Eq.(1) using the values of peak area (S_A and S_B) are shown in Table 3 along with the saturation magnetization (J_s, mos) calculated from wt% Fe. For C1, C2, C3 and C4, the values of J_s, mos were consistent with those of J_s measured using a VSM as shown in Table 1. The calculated value of J_s, mos for BMPs was found to be $1.06 \times 10^{-4} \text{ Wb m/kg}$ (84.7 emu/g).

Table 4 shows the ratio of Fe^{2+} to total Fe content obtained using both Mössbauer spectroscopy and

Figure 3 Mössbauer spectrum for Fe_3O_4 fine particles (C1) prepared by the chemical coprecipitation method.

Table 2 The curve-fitting parameters for Fe_3O_4 single crystal, bacterial magnetic particles (BMPs) and Fe_3O_4 particles (C1, C2, C3 and C4) prepared by the chemical coprecipitation method.

Sample	Sites	Γ (mm/s)	Hhf(MA/m)	S(%)
Fe_3O_4 single crystal	A	0.24 ± 0.01	38.8	33 ± 2
	B	0.33 ± 0.01	36.4	67 ± 2
B M P s	A	0.47 ± 0.04	39.4	61 ± 2
	B	0.60 ± 0.09	36.5	39 ± 2
C1	A	0.49 ± 0.01	38.8	59 ± 2
	B	0.76 ± 0.03	35.9	41 ± 2
C2	A	0.41 ± 0.01	39.2	52 ± 2
	B	0.64 ± 0.03	36.6	48 ± 2
C3	A	0.37 ± 0.01	39.2	47 ± 2
	B	0.37 ± 0.01	36.6	53 ± 2
C4	A	0.50 ± 0.01	39.2	68 ± 2
	B	0.70 ± 0.07	36.6	32 ± 2

Table 3 The value of wt%Fe and saturation magnetization J_s, mos for Fe_3O_4 single crystal, $\gamma\text{-Fe}_2\text{O}_3$ fine particles, bacterial magnetic particles (BMPs) and Fe_3O_4 particles (C1, C2, C3 and C4) prepared by the chemical coprecipitation method using Mössbauer spectroscopy.

Sample	wt%Fe		J_s, mos
	Fe_3O_4	$\gamma\text{-Fe}_2\text{O}_3$	($\times 10^{-4} \text{ Wb} \cdot \text{m/kg}$)
Fe_3O_4 single crystal	100	0	1.14
$\gamma\text{-Fe}_2\text{O}_3$	0	100	0.96
BMPs	59.7	40.3	1.06
C1	62.8	37.2	1.07
C2	73.8	26.2	1.09
C3	81.7	18.3	1.10
C4	47.2	52.8	1.04

Table 4 The value of $\text{Fe}^{2+}/\text{Total Fe}$ for Fe_3O_4 single crystal, $\gamma\text{-Fe}_2\text{O}_3$ fine particles, bacterial magnetic particles (BMPs) and Fe_3O_4 particles (C1, C2, C3 and C4) prepared by the chemical coprecipitation method using Mössbauer spectroscopy and chemical analysis.

Sample	$\text{Fe}^{2+}/\text{Total Fe}$ (%)	
	Mössbauer	chemical analysis
Fe_3O_4 single crystal	33	33
$\gamma\text{-Fe}_2\text{O}_3$	0	0
BMPs	20	19
C1	21	17
C2	25	23
C3	27	27
C4	16	17

chemical analysis. The results of Mössbauer spectroscopy agree well with those obtained using chemical analysis. The increase of Fe^{2+} and vacancies is considered to be indicative of the existence of $\gamma\text{-Fe}_2\text{O}_3$ in solid solution with Fe_3O_4 in BMPs and C1. The J_s values for BMPs and C1 therefore decreased compared with that of the Fe_3O_4 single crystal.

The rotational hysteresis loss (W_r/J_s) as a function of H is plotted in Figure 4. It may be understood from Eq.(2) that the W_r/J_s versus H curve represents the distribution of HA. The W_r/J_s versus H curve of BMPs had a stronger peak than that of Fe_3O_4 particles (C1, C2, C3 and C4). The distribution of HA for BMPs seems to be excellent.

Since the half-value width of the W_r/J_s versus H curve of C4 was broad, the latter may comprise particles with various HA values. The rotational hysteresis integrals (R_h) obtained from Eq.(3), using the values shown in Figure 4, were calculated to be 1.12, 0.62, 0.67 and 0.67 for BMPs, C1, C2 and C3, respectively (Table 5). The R_h for BMPs is closer to the value calculated from the chain-of-spheres with fanning model in random alignment ($R_h=1.02$) [4]. However, R_h values for C1, C2 and C3 are intermediate between coherent rotation model ($R_h=0.38-0.42$) [4] and the chain-of-spheres with fanning model in random alignment. It is believed that they have different magnetization reversal mechanisms. The $H_{A,ave}$ obtained from the W_r/J_s versus $1/H$ curve at high field [6], were 35.8 and 68.5 kA/m for BMPs and C1, respectively (Table 5). The critical volumes (V_c) for superparamagnetic behavior for BMPs and C1 calculated from Eq.(4) using J_s, mos shown in Table 3, were found to be 11×10^{-24} ($D_c=22$ nm) and 6×10^{-24} m³ ($D_c=18$ nm), respectively (Table 5). It was inferred from the results of Mössbauer spectroscopy and from the critical diameter (D_c) for superparamagnetic behavior that a small amount of very fine superparamagnetic particles exists in C1. As the size of the particles decreases, the magnetic viscosity increases, and further reduction in size finally results in superparamagnetism.

The magnetic field dependences of $\Delta J/(\ln t_1 - \ln t_2)$ and X_{irr} were measured at 25°C for BMPs, C1, C2, C3 and C4. A comparison of the magnetic field dependences of $\Delta J/(\ln t_1 - \ln t_2)$ and X_{irr} shows that the shape of the curve for all samples is very similar.

Figure 5 shows the magnetic field dependence of the magnetic viscosity coefficient (S_v) for BMPs, C1, C2, C3 and C4 obtained from Eq.(5). This experiment shows that the range in which S_v stays at constant values, which

are independent of the magnetic field (H), is very wide. The S_v value of BMPs was 50 % smaller than that of C1 which had the lowest coercive force ($H_c = 4.8$ kA/m) of these samples. Barbier's relation [13], which establishes that the logarithm of S_v varies in proportional to $\ln H_c$ from soft to hard magnetic materials is generally known. The S_v value of C1 was large compared to that of γ -Fe₂O₃ fine particles ($S_v = 96$ A/m) used for recording media with higher H_c (28 kA/m) [11]. The time dependence of magnetization for C1 was found to be -7 % after 1000 h at 60 °C. The values of V_{act} at 25 °C for BMPs and C1 calculated from Eq.(5) using S_v and $J_{s,mos}$, were found to be 61×10^{-24} and 29×10^{-24} m³, respectively (Table 5). The diameters (D_{act}) of V_{act} for BMPs and C1 were 39 and 31 nm, respectively (Table 5). D_{act} values for BMPs and C1 were very close to that of the average particle diameter ($D_{ave} = 44$ and 26 nm) shown in Table 1. As C1 has a smaller D_c and D_{act} , it is concluded that BMPs is magnetically more stable than C1. Typical magnetic properties obtained in this study are shown in Table 7.

Figure 4. Plots of the rotational hysteresis loss Wr/J_s versus magnetic field H for bacterial magnetic particles (BMPs), Fe₃O₄ particles (C1, C2, C3 and C4) prepared by the chemical coprecipitation method.

Figure 5. Magnetic field dependence of the magnetic viscosity coefficient S_v for bacterial magnetic particles (BMPs), Fe₃O₄ particles (C1, C2, C3 and C4) prepared by the chemical coprecipitation method.

Table 5 Average anisotropy field $H_{A,ave}$, the rotational hysteresis integral R_h , the critical volume V_c and its diameter D_c for superparamagnetic behavior, the magnetic viscosity coefficient S_v , the activation volume V_{act} and its diameter D_{act} for bacterial magnetic particles (BMPs) and Fe₃O₄ particles (C1, C2, C3 and C4) prepared by the chemical coprecipitation method.

Sample	$H_{A,ave}$ (kA/m)	R_h	V_c ($\times 10^{-24}$ m ³)	D_c (nm)	S_v (A/m)	V_{act} ($\times 10^{-24}$ m ³)	D_{act} (nm)
BMPs	35.8	1.12	11	22	127	61	39
C1	68.5	0.62	6	18	255	29	31
C2, C3	109.8	0.67	4	15	88	85	44
C4	127.4	0.95	3	14	151	49	37

4. CONCLUSIONS

The calculated value of the saturation magnetization for BMPs was found to be 1.06×10^4 Wb m/kg (84.7 emu/g) using Mössbauer spectroscopy and chemical analysis. The rotational hysteresis integrals for BMPs and Fe₃O₄ particles (C1, C2 and C3) were 1.12, 0.62, 0.67 and 0.67, respectively. They are considered to have different magnetization reversal mechanisms. The magnetic viscosity coefficient of BMPs was 50 % smaller than that of Fe₃O₄ particles (C1) which has a smaller activation volume (V_{act}). It is concluded that BMPs is magnetically more stable than C1. The relations $V \geq V_{act}$ and $V > V_c$ (V : particle volume, V_c : critical volume for superparamagnetic behavior) are of significant importance for the magnetic stability of fine particles.

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