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A simple and accurate method for quantification of magnetosomes in magnetotactic bacteria by common spectrophotometer

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Abstract

A simple apparatus for measuring the magnetism of magnetotactic bacteria was developed with a common laboratory spectrophotometer, which was based on measuring the change in light scattering resulting from cell alignment in a magnetic field. A multiple coils were built around the cuvette holder of the spectrophotometer to compensate geomagnetic field and to generate two mutually perpendicular magnetic fields. In addition, we defined a novel magnetism parameter, R_{mag}, by modifying the definition of C_{mag} to a normalized parameter with the culture absorbance obtained without application of magnetic field. The number of magnetosomes in each cell was determined by transmission electron microscopy to assess the relationship between the two magnetism parameters and the distribution of magnetosomes in the cells. We found that both R_{mag} and C_{mag} were linearly correlated rather with the percentage of magnetosome-containing bacteria than with the average magnetosome numbers, and R_{mag} exhibited a better linearity than C_{mag} with respect to the percentage of magnetosome-containing bacteria.

Keywords: *Magnetospirillum* sp. AMB-1; Magnetic field; Magnetosomes; Spectrophotometer

1. Introduction

Magnetotactic bacteria (MTB) comprise a group of Gram-negative aquatic prokaryotes that can be found from both fresh water and marine environments. They possess magnetosomes that are intracellular magnetic iron nanocrystals surrounded by lipid bilayer membrane. Magnetosomes form a chain inside the cell, which enables the bacterium to migrate along the geomagnetic field and to maintain its position within the boundary of the oxic–anoxic transition zone (OATZ), a behavior known as magnetotaxis [1,2]. Each bacterial species synthesizes unique highly homogenous nanoscaled magnetic crystals, which can be widely used in biotechnology, iatrology, magnetic separation, effluent disposal and magnetic storage [3,4]. Thus, an accurate quantification of the bacterial magnetism appears to be essential for both fundamental research of the magnetotactic bacteria and their application.

Several methods for the determination of magnetism in the magnetotactic bacteria have been reported. A common method is to assay swimming behavior and magnetic responses of individual living cells by simple microscopic inspection or image analysis techniques [5,6]. Although the method can be easily practiced [7], it is not directly related to the magnetism of magnetosomes.

Since 1980s, optical method has been applied to detect the magnetic moment of the magnetic bacteria [8,9]. In 1995, Schüler et al. initiated a simple light scattering method to obtain the magnetism of the magnetic bacteria [10], which utilized the effects of different magnetic orientations to the incident light beam on the light scattering of a suspension of magnetic bacteria (except the magnetotactic cocci). The maximum scattering (E_{max}) occurs if the applied magnetic field is directed parallel to the light beam, whereas perpendicular orientation with respect to the light results in minimal scattering (E_{min}). They defined C_{mag} as the ratio of the value of the light scattering in the direction of the magnetic field parallel to the light path and perpendicular to the light path, and expressed as shown in formula (1):

$$C_{mag} = \frac{E_{max}}{E_{min}} \quad (1)$$

A light scattering measurement system using 637 nm light emitting diode (LED) as a light source was established to measure

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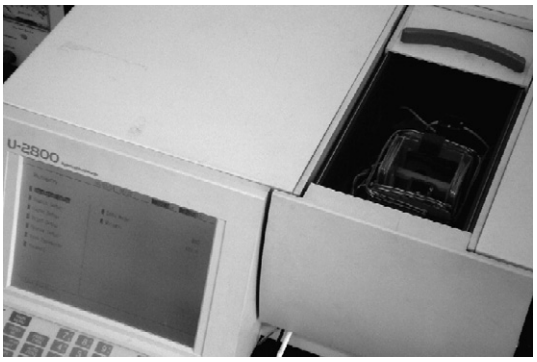

Fig. 1. Magnetism measuring system. It was composed of a spectrophotometer and an electromagnetic system. Coils were placed in the sample cell of the spectrophotometer to produce magnetic fields around the cuvette in the spectrophotometer.

E_{max} and E_{min}, which included a pair of permanent magnets to produce 70 mT homogenous magnetic fields. They found that the C_{min} value was generally correlated with the average number of magnetosomes in the bacteria, especially showing a nearly linear correlation at low magnetosome numbers. Occasionally, an alternative definition was used in order to zero out the C_{mag} value of nonmagnetic cells, as shown in formula (2) [11].

$$C_{mag} = \frac{E_{max}}{E_{min}} - 1 \quad (2)$$

The method has been successfully applied in the studies of the magnetotactic bacteria [11,12]. However, it is based on a homemade optical system, which might restrict its general usage. In this study, we estimated the feasibility of using a common laboratory spectrophotometer to set up a simple apparatus for measuring bacterial magnetism in any microbiological laboratory. Furthermore, we defined a new parameter R_{mag} aiming at a better characterization of the magnetism of magnetotactic bacteria. We assessed the relationship between the magnetism parameters and the distribution of magnetosomes in the cells by transmission electron microscopy (TEM) to determine the number of magnetosomes in each cell.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Cells of *Magnetospirillum* sp. AMB-1 (ATCC 700264) were used in all the experiments. The growth of bacteria was routinely performed in the enriched magnetic spirillum growth medium (EMSGM) [13] under the condition of micro-aerobic at 28 °C. To prepare pure magnetic bacteria, the culture media was kept under the above conditions for 24 h. Two pieces of permanent magnets (Nd-Fe-B, their surface magnetic flux densities were 350 mT) were fixed at each side of the culture bottles. After the cells accumulated at the wall facing the magnets, they were scratched off and put into fresh culture media. As checked by TEM, the magnetically collected bacterial cells all contained magnetosomes. To prepare pure nonmagnetic bacteria, the bacterial media was cultured by shaking at 80 rpm, 28 °C under aerobic growth and deprived of ferric citrate for three growth cycles. The cultures were checked by TEM to confirm that there were no magnetosomes in the cells. For assessing the relationship between magnetism parameters and distribution of magnetosomes, the pure magnetic cells were mixed with the pure nonmagnetic cells at different ratio and measured for the C_{mag} and R_{mag} values. The distribution of magnetosomes was checked by TEM.

2.2. Magnetism measuring system

The magnetism measuring system was composed of a spectrophotometer (HITACHI U-2800, Japan) and an electromagnetic system (Fig. 1). The compartment of the cuvette has a dimension of 55 mm × 70 mm × 60 mm (height).

The electromagnetic system, which can produce adjustable magnetic field around the cuvette in the spectrophotometer, was composed of compensating coils, main coils, current powers and a switch. All the coils were placed in the sample cell of the spectrophotometer. Metal components which would interfere with the magnetic field were replaced by nonmagnetic materials. Two pairs of coils with two individually adjustable

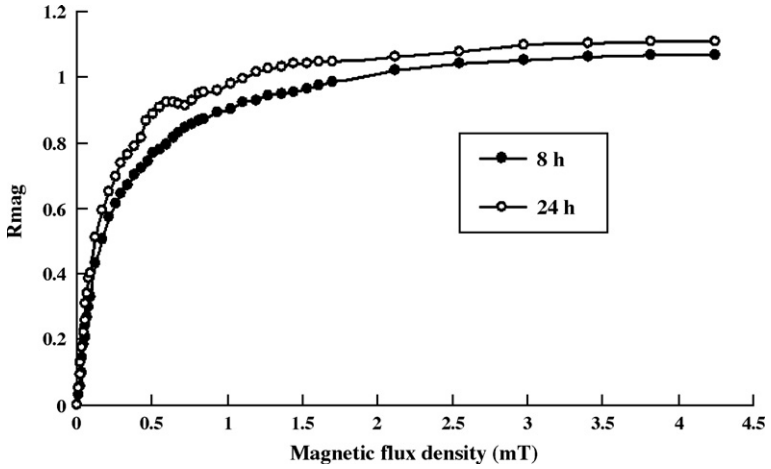

Fig. 2. Relationship between the magnetic flux density of applied magnetic field and R_{mag}. The black dots show the results of bacteria cultured for 8 h, and the white dots show the results for 24 h.

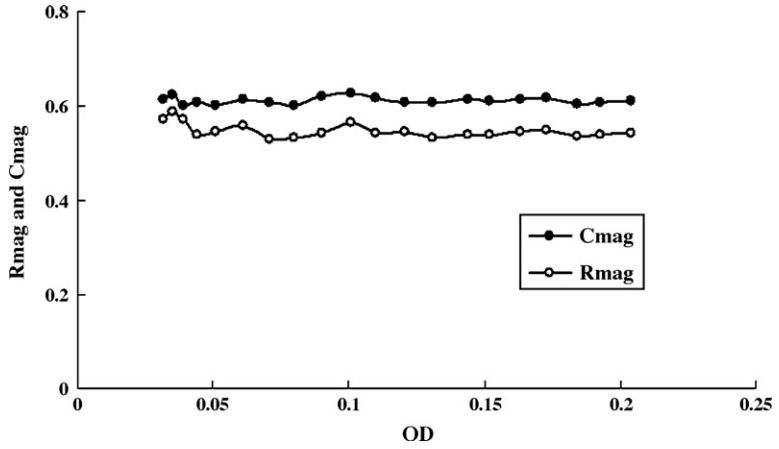

Fig. 3. Relationship between OD_{600} value and R_{mag} or C_{mag}. The black dots show the results of C_{mag}, and the white dots show the results of R_{mag}. The applied magnetic field strength was 4.25 mT.

power supply were used as compensating coils to offset the downward and northward component of geomagnetic field for more precision (the transmeridional component is very small in Beijing, China). The precision of the current powers was 0.1 mA. The magnetic field intensity around the cuvette was reduced to 3 μT by the compensating coils, and the error in three directions was less than 5%. Main coils consisted of two pairs of coils placed perpendicularly with each other to generate magnetic fields parallel or orthogonal to the light path of the spectrophotometer. An adjustable current power supply controlled by a switch was used for the main coils. There were three states for the switch: turn off, turn on to parallel magnetic field and turn on to orthogonal magnetic field. When the current across the main coils was 5 A, the magnetic field around the cuvette was about 4.25 mT with uniformity less than 5%.

For each state of the switch, the extinction value of optical density (OD) of the cultures was recorded at the wavelength of 600 nm. The $OD_{//}$ value was measured for parallel magnetic field, the $OD_{\perp}$ value for orthogonal magnetic field and the OD value alone for normal measurements (without external magnetic field applied).

According to formula (2), the C_{mag} was calculated by:

$$C_{mag} = \frac{OD_{//} - OD_{\perp}}{OD_{\perp}} \tag{3}$$

We defined a novel magnetism parameter R_{mag}, which was expressed as:

$$R_{mag} = \frac{OD_{//} - OD_{\perp}}{OD} \tag{4}$$

2.3. Electron microscopy

A TEM (HITACHI H -600) was used to determine the number of the magnetosomes in the bacterial cells. The acceleration voltage of TEM was 100 kV and the magnification

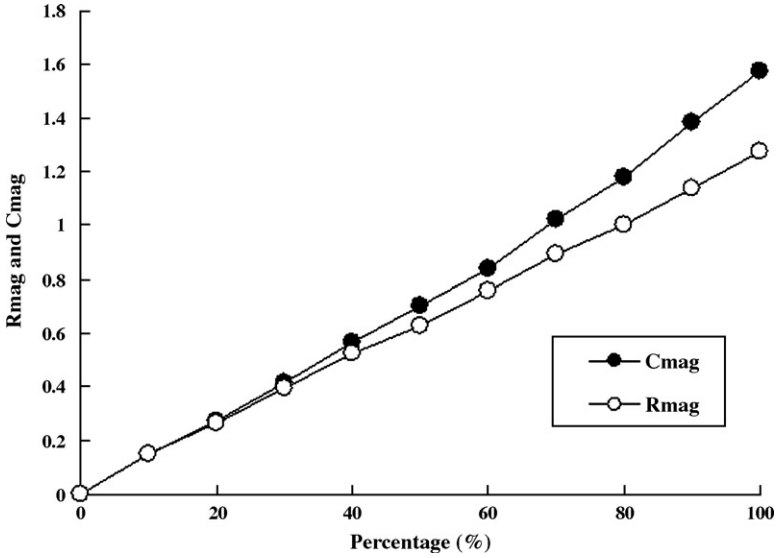

Fig. 4. Relationship between the percentage of magnetosome-containing bacteria and R_{mag} or C_{mag}. The black dots show the results of C_{mag}, and the white dots show the results of R_{mag}. The measurement is under 4.25 mT.

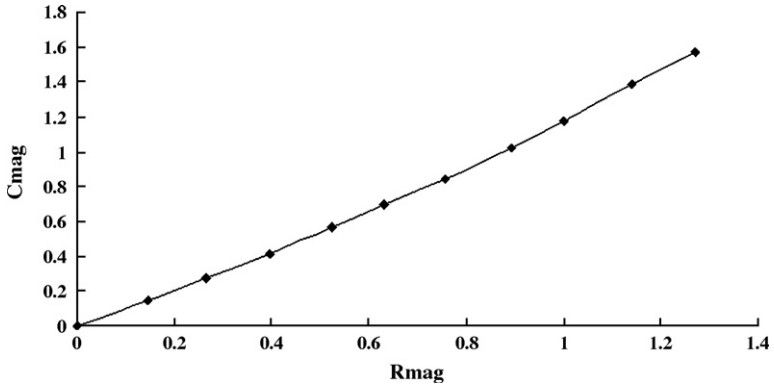

Fig. 5. Relationship between R_{mag} and C_{mag}.

times were 20,000. For electron microscopy, bacterial culture was directly dropped onto carbon-coated copper grids and inspected without staining.

3. Results

3.1. Relationship between R_{mag} and applied magnetic field

The strength of applied magnetic field can be adjusted by the current of main coils for the magnetism measuring system. The R_{mag} values of the bacterial media (cultured for 8 h or 24 h) were measured with an increasing magnetic flux density (Fig. 2). It is shown that, when the applied magnetic field intensity is higher than 3 mT, the R_{mag} value come into a platform stage, indicating a threshold of magnetic field intensity at 3 mT sufficient to align the magnetic bacteria.

3.2. Correlation of R_{mag} with the percentage of magnetosome-containing bacteria

In order to verify the reliability of the method, the bacterial culture was diluted to different concentration by culture media. The OD values were measured using the magnetism measuring system under 4.25 mT applied magnetic field. The relationship between bacterial culture concentration (denoted by OD_{600} value) and R_{mag} was obtained as shown in Fig. 3. From the results, it can be seen that, when the bacterial cultures were diluted to different concentrations, the values of R_{mag} and C_{mag} kept to be invariant.

In order to assess the relationship between R_{mag} and the percentage of magnetosome-containing bacteria, pure magnetic bacterial cultures and non-magnetic bacterial cultures, both adjusted to the same OD_{600} values (0.13), were mixed at the ratio indicated. As shown in Fig. 4, the linearity fitting for the relationship can be expressed as:

$$R_{mag} = 1.2517x + 0.0126,\ R = 0.9996$$

$$C_{mag} = 1.5461x - 0.0363,\ R = 0.9951$$

where x is the percentage of magnetosome-containing bacteria in whole bacterial cultures and R is correlation coefficient.

Furthermore, the relationship between R_{mag} and C_{mag} was also investigated as shown in Fig. 5.

3.3. Magnetosome number inspected by TEM

The *Magnetospirillum* sp. AMB-1 cultured in geomagnetic field for 8 h or 24 h were inspected by TEM, respectively. 100 bacterial cells were selected randomly for each group and the number of the magnetosomes in each cell was counted. The results are shown in Fig. 6. The average number of magnetosomes per cell and the percentage of magnetosome-containing bacteria in whole cultures are shown in Table 1.

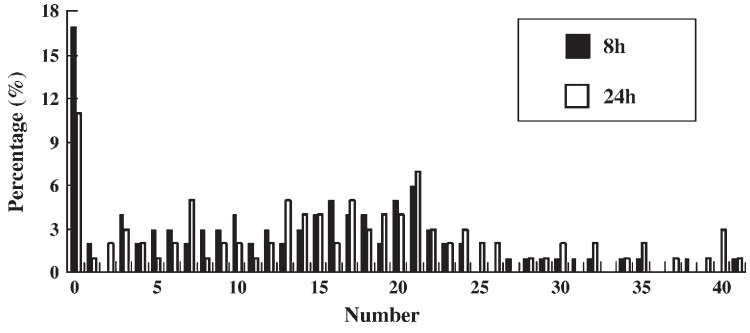

Fig. 6. Percentage distribution of the bacteria containing different numbers of magnetosomes by TEM. The black is the result of the bacteria cultured for 8 h, and the white is for 24 h.

Table 1
Results of TEM inspection with AMB-1 bacteria cultured in geomagnetic field for 8 h and 24 h

	8 h	24 h
Average magnetosome numbers per bacteria	13.2	16.2
Percentages of the bacteria containing magnetosomes	83%	89%

For pure nonmagnetic bacteria, which average number of magnetosomes and the percentage of magnetosome-containing bacteria were zero, the values of R_{mag} or C_{mag} should be zero according to their definitions. Combined with the R_{mag} and C_{mag} values recorded at 8 h and 24 h cultures, the relationship between the R_{mag} or C_{mag} values, average magnetosome numbers and percentage of magnetosome-containing bacteria were investigated as shown in Fig. 7.

4. Discussion

The working principle of spectrophotometer is based on Lambert-Beer theory, which is applicable for detecting the turbidity of the solution with single substance. Extinction value of optical density is linearly correlated with the concentration of the solution in a moderate range. According to optical theory, if the number of particles in solution is invariant, small particles may possess higher OD values than big particles. As *Magnetospirillum* sp. AMB-1 is a kind of spirillum, the OD value of bacterial culture would be the biggest ($OD_{//}$) or the smallest

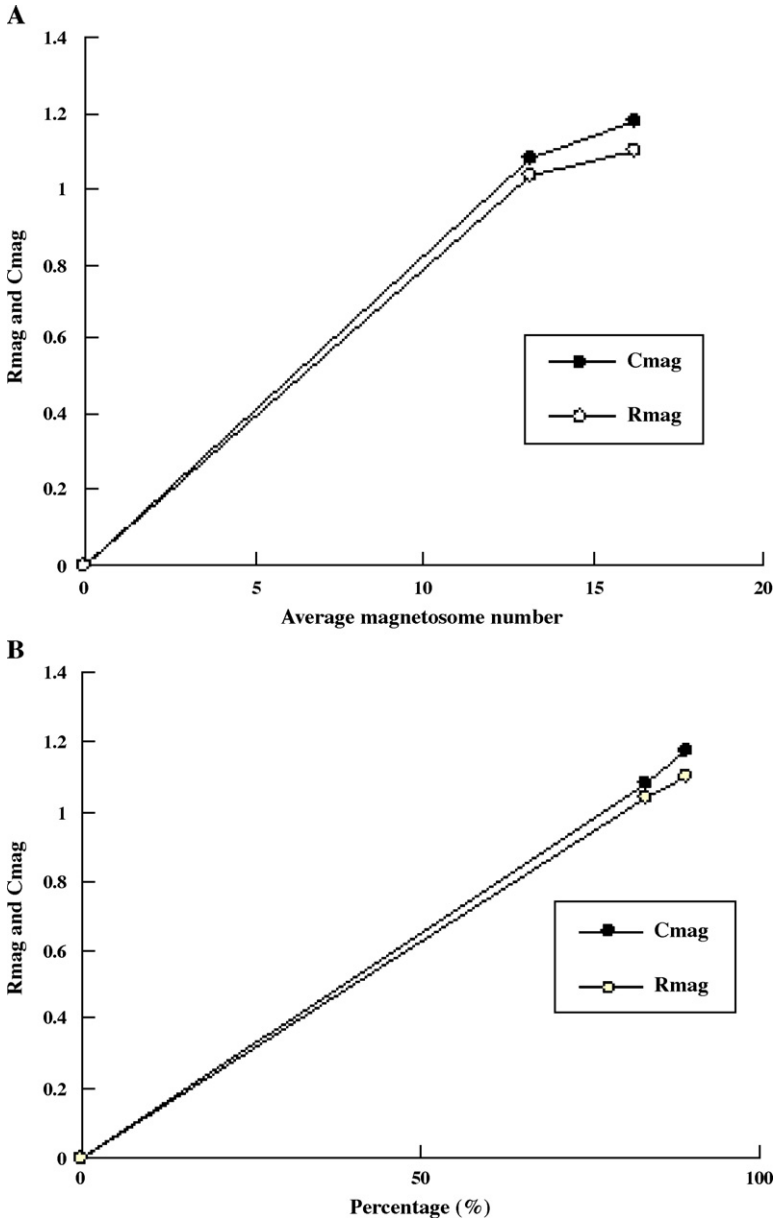

Fig. 7. Relationship between the statistic results and experimental results of R_{mag} and C_{mag}. (A) Relationship between the average number of magnetosomes per bacterium and R_{mag} or C_{mag}; (B) relationship between the percentage of the magnetosome-containing bacteria in the culture and R_{mag} or C_{mag}. The black dots show the results of C_{mag}, and the white dots show the results of R_{mag}.

($OD_{\perp}$) while the applied magnetic field parallel or perpendicular to the light path, respectively. The same principle had been described by Schüler et al. [10]. So the numerator ($OD_{//}$–$OD_{\perp}$) of C_{mag} as shown in formula (3) is correlated with the number of magnetic bacteria that could align under applied magnetic field. In formula (4), we substituted the denominator of C_{mag} by the OD values without external magnetic field applied, which is directly correlated with the total number of bacteria, to obtain the R_{mag} values. We theoretically speculated that the R_{mag} values would positively correlate with the percentage of the magnetosome-containing bacteria in whole bacterial cultures as long as a strong magnetic field applied. It is confirmed by our experiments shown in Figs. 4 and 7(B).

According to Langevin theory of classical paramagnetism [14], the orientation of one bacterium or an ensemble of bacteria in the applied magnetic field of H can be calculated as:

$$\langle \cos\theta \rangle = L(MH/kT) \tag{5}$$

where $\langle \cos\theta \rangle$ is the thermal average of $\cos\theta$ which denotes the orientation, M is the magnetic moment per bacterium, H is applied magnetic field, θ is the angle between M and H, kT is thermal energy, $L\left(\frac{MH}{kT}\right) = \mathrm{ctnh}\left(\frac{MH}{kT}\right) - \left(\frac{MH}{kT}\right)^{-1}$.

The magnetosomes of *Magnetospirillum* sp. AMB-1 are roughly octahedral, 50 nm along each major axis by our TEM observation. For the bacteria containing only one magnetosome, the thermal average of $\cos\theta$ under 3 mT applied magnetic field is 0.98 calculated by formula (5), indicating that 3 mT applied magnetic field was strong enough to align all the magnetosome-containing bacteria (Fig. 2). Although the orientation is also related to the magnetic moment per bacterium, which is linearly correlated with the number of magentosomes, the orientation does not rely on the average number of magnetosomes if the applied magnetic field is strong enough. So the R_{mag} is not directly correlated with the average number of magnetosomes, if the applied magnetic field is stronger than 3 mT (Fig. 7(A)).

Although the R_{mag} values exhibit a better linearity than the C_{mag} values regarding the percentage of magnetosome-containing bacteria (Figs. 4 and 5), the difference between them appears negligible at a low percentage. Therefore, with a lower percentage of magnetosome-containing bacteria in the culture, both the R_{mag} values and the C_{mag} values can be used for the characterization of bacterial magnetism, while the R_{mag} values seem to be more accurate than the C_{mag} values especially at a higher percentage.

From the fitting analysis, the relationship between the R_{mag} values and the percentage of magnetosome-containing bacteria can be described as:

$$R_{mag} = k_0 x \tag{6}$$

where $k_0 = 1.25$ is a constant and x is the percentage of magnetosome-containing bacteria in the culture. Thus, the percentages for *Magnetospirillum* sp. AMB-1 cultured at 8 h or 24 h can be calculated as 82.6% and 87.9%, respectively, which approximate well to the TEM observation (Table 1).

It was an important conclusion that the C_{mag} values were correlated well with the average number of magnetosomes according to Schüler et al. [10,11]. However, it conflicted with our deduction and experimental data. In Schuler's study, the bacterial culture used to investigate the relationship between the C_{mag} values and the average number of magnetosomes was sampled at different stages with initially nonmagnetic cells. The average number of magnetosomes and the percentage of magnetosome-containing bacteria may correlate linearly in that case, especially at low average number of magnetosomes. From our study, the C_{mag} or R_{mag} values correlated actually better with the percentage of magnetosome-containing bacteria (Fig. 7(B)) than with the average number of magnetosomes (Fig. 7(A)). Interestingly, from the view of physics, it is the percentage of the magnetosome-containing bacteria that directly correlate with the C_{mag} or R_{mag} values by a good linearity.

It is noticed that the R_{mag} and C_{mag} must be measured at an applied magnetic field more than 3 mT. Because the geomagnetic field intensity is only about 0.05 mT, which is a weak magnetic field compared with the magnetic field applied ($\geq$3 mT), it is not necessary to use the compensating coils to offset geomagnetic field except a more accurate value is required.

5. Simplified description of the method and its application

In this study, a highly sensitive and rapid method for quantifying the magnetism of the magnetotactic bacteria was established based on a magnetism measuring system and a new parameter R_{mag}. The magnetism measuring system was composed of a common laboratory spectrophotometer and an electromagnetic system. A multiple coils were built around the cuvette holder of the spectrophotometer to compensate geomagnetic field and to generate two mutually perpendicular magnetic fields. The extinction values of the cultures for normal measurements and for applying magnetic fields parallel and orthogonal to the light path were recorded respectively by the spectrophotometer at the wavelength of 600 nm. The parameter R_{mag} value, which is linearly well correlated with the percentage of magnetosome-containing bacteria, was calculated by the three extinction values. Thus, an accurate quantification of the bacterial magnetism was established for both fundamental research of the magnetotactic bacteria and their application. The method may be used to monitor the dynamics of magnetite formation in magnetotactic bacteria under different growth conditions, which may lead to a better understanding of biomineralization control in the magnetotactic bacteria. Also it can be used to study the bacterial magnetism for the potential utilization of magnetosomes.

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