Microaerobic Conditions Are Required for Magnetite Formation Within *Aquaspirillum magnetotacticum*

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Abstract The amount of magnetite (Fe_3O_4) within magnetosomes of the microaerophilic bacterium *Aquaspirillum magnetotacticum* varies with oxygen and nitrogen supply. The development of optical methods for directly measuring cell magnetism in culture samples has enabled us to quantitate bacterial Fe_3O_4 yields. We measured final cell yields, average cell magnetic moments, and magnetosome yields of growing cells. Cultures were grown with NO_3^-, NH_4^+, or both, in sealed, unshaken vials with initial headspace Po_2 values ranging from 0 (trace) to 21 kPa.

More than 50% of cells had detectable magnetosomes only when grown in the range of 0.5–5.0 kPa O_2. Optimum cell magnetism (and Fe_3O_4 formation) occurred under microaerobic conditions (initial headspace Po_2 of 0.5–1 kPa) regardless of the N source. At optimal conditions for Fe_3O_4 formation, denitrifying cultures produced more of this mineral than those growing with O_2 as the sole terminal electron acceptor. This suggests that competition for O_2 exists between processes involving respiratory electron disposal and Fe_3O_4 formation. Oxygen may also be required for Fe_3O_4 formation by other species of magnetotactic bacteria.

Bacterial Fe_3O_4 appears to persist in sediments after death and lysis of cells. The presence of bacterial Fe_3O_4 in the fossil and paleomagnetic records may be of use as a retrospective indicator of sedimentation that has occurred in microaerobic waters.

Introduction

Magnetotactic bacteria contain intracellular, enveloped, magnetic particles or "magnetosomes" (Fig. 1). The magnetosomes of strains that have been studied consist of Fe_3O_4, the iron oxide magnetite (Frankel et al., 1983; Towe and Moench, 1981).

Aquaspirillum magnetotacticum strain MS-1, the most thor-

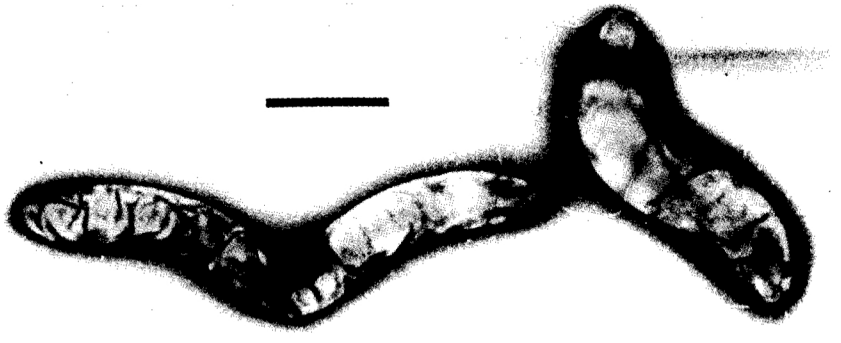

Fig. 1. Transmission electron micrograph of negatively stained cells of *A. magnetotacticum*. Cells contain chains of magnetosomes which are often bisected by division planes. Bar = 1 μm.

oughly studied magnetic bacterium, is an obligate microaerophile. Cells denitrify microaerobically and concomitantly consume O_2. However, unlike most other denitrifiers, they do not grow under strictly anaerobic conditions, even with NO_3^- present (Blakemore et al., 1979; Bazylinski and Blakemore, 1983*a*). Rates of growth and intracellular Fe_3O_4 formation (Blakemore et al., 1979), denitrification (Bazylinski and Blakemore, 1983*a*), and nitrogen fixation (Bazylinski and Blakemore, 1983*b*) by this organism are noticeably depressed when the initial O_2 tension in the culture headspace is greater than 6 kPa. Cultures growing with O_2 as the terminal electron acceptor (e.g., with NH_4^+ in lieu of NO_3^- as the sole N source) contain fewer magnetic cells than those which are denitrifying. However, the effect of O_2 and N source on Fe_3O_4 formation by cells has not previously been quantitated due to lack of a suitable method to measure cell magnetism.

We have previously estimated cell magnetism in liquid cultures by microscopically noting the extent to which suspended cells align with an applied magnetic field, or by qualitative estimates of their differential light scattering when cultures are held in a continuously rotating magnetic field (e.g., over a laboratory magnetic stirrer). The cells exhibit optical birefringence, however, and methods have been developed to quantitate cell magnetic moments (values of μ) from measurements of magnetic field–dependent birefringence (Rosenblatt et al., 1982).

We undertook the present study to clarify the relationship between available O_2 and cell magnetism in cultures grown with NO_3^- and/or NH_4^+ as the sole N source. Our findings are relevant to the biogeochemistry of iron, and, if representative of other species, indicate that bacteria produce Fe_3O_4 only with O_2 available but under microaerobic conditions.

Materials and Methods

A. magnetotacticum was cultured in a chemically defined medium (Blakemore et al., 1979) containing tartaric acid in lieu of succinic as a carbon source, and either 2 mM $NaNO_3$,

$(NH_4)_2SO_4$, or both N sources, each at 1 mM. Cells were inoculated into 155-ml sealed serum vials, each containing 55 ml of culture medium under a gas atmosphere of known composition. Cultures were prepared in triplicate using seven different initial headspace gas compositions: 0.0 (trace) 0.5, 1.0, 2.5, 5.0, 10, and 21% (vol/vol) of O_2 in N_2. It is important to note that the culture system used lacked extensive redox buffering. Medium containing resazurin was colorless prior to inoculation but was faintly pink just afterward. Thus, trace amounts of O_2 added with the inoculum were sufficient to allow some cell growth. No sample in this study was strictly anaerobic prior to incubation. In confirmation of earlier published findings, *A. magnetotacticum*, when tested under stringent conditions of anoxia, failed to grow even with NO_3^- present. Cultures were each inoculated to 1.3×10^6 cells/ml and incubated at 30°C without shaking. At the end of growth the following were evaluated:

1. cells/ml (by means of direct microscopic counts)
2. magnetosomes per cell (by means of direct transmission electron microscopic examination; magnetosomes within 100 cells from each culture were counted)
3. average cell magnetic moment (by measuring magnetic field–dependent culture birefringence)

The apparatus used to measure magnetic field–dependent culture birefringence has been described (Rosenblatt et al., 1982). Bacteria in 5 ml of culture fluid were fixed with a drop of 10% glutaraldehyde. Fixed cells in suspension were placed in a 3-ml cuvette (1-cm path length) situated within a Helmholtz coil pair that was used to vary the magnetic field applied to the sample. This entire assembly was placed within a Mumetal canister to cancel the ambient laboratory magnetic field on the cells. The optic axis of the sample was perpendicular to the applied magnetic field. Increases in the field strength over the range 0.1–25.0 Oe produced a corresponding increase in measured birefringence. Only magnetic cells contributed to the measured effect. The data were analyzed to yield the average value and distribution of μ for those cells.

Results

We did not observe total inhibition of growth by high O_2 in this study. Cells exhibited a growth lag at high O_2 values but, despite their microaerophilic character, they eventually grew. We attribute this to the use of sealed, static cultures, because cells never grew from small inocula on a culture in free exchange with air. Additional studies using systems with constant Po_2 are expected to define the precise O_2 tension for optimal cell growth and Fe_3O_4 formation.

Nevertheless, the results show that more than 50% of cells in cultures had magnetosomes only when the initial headspace Po_2 was between 0.5 and 5 kPa (Tables 1–3; Figs. 2–4). Outside of this narrow range, Fe_3O_4 production diminished markedly regardless of the N source, even though cells grew. The optimal Po_2 for Fe_3O_4 formation was 1 kPa with NO_3^- present (Tables 1, 3), or 0.5 kPa with NH_4^+ alone (Table 2). At the optimal Po_2, the largest percentage of cells in the population contained magnetosomes (Tables 1–3) and the average number of magnetosomes per cell was also highest (Figs. 2–4). Figs. 2–5 also illustrate the effect of both low and high O_2 in inhibiting Fe_3O_4 formation by cells. Values of cell magnetic moment were also optimal at approximately 1 kPa O_2 (Tables 1–3), illustrating the correlation between cell magnetosome content and intrinsic magnetic moment as determined from birefringence measurements. The average magnetic moments measured were those expected from Fe_3O_4 grains in the 400–500 Å range.

A somewhat higher value of μ was obtained for cells grown with trace amounts of O_2 and with NH_4^+ as the sole N source (Table 2) than for those cells provided with NO_3^- (Tables 1 and 3). Since the cells did not grow well on NH_4^+ with trace O_2, the relatively high value of μ (Table 2) was strongly influenced by that of the inoculum, which consisted of magnetic cells. The magnetic moment measured was not representative of Fe_3O_4 formation during incubation.

At the optimal Po_2 for Fe_3O_4 formation, the average μ values for cells cultured with NO_3^- (Tables 1 and 3) were roughly com-

Table 1

Effect of O_2 on *A. magnetotacticum* growth and Fe_3O_4 synthesis with NO_3^-

Initial O_2 Concentration (kPa in headspace)	*Final Cell Concentration (cells/ml)*[a]	*Average No. of Magnetosomes (± SD*[b]*/Cell)*	*Percent of Cells with Magnetosomes*[c]	*Magnetic Moment (μ) ($\times 10^{-13}$ emu)*[d]
Trace	$1.8 \pm 0.0 \times 10^8$	2.0 ± 3.5	41	0.6–0.7
0.5	$1.8 \pm 0.2 \times 10^8$	10.0 ± 10.0	88	0.8–1.0
1.0	$2.5 \pm 0.3 \times 10^8$	17.2 ± 7.8	100	2.9–3.3
2.5	$2.4 \pm 0.0 \times 10^8$	14.9 ± 7.7	94	2.8–3.1
5.0	$2.5 \pm 0.3 \times 10^8$	13.0 ± 15.9	82	2.7–2.9
10.0	$1.1 \pm 0.0 \times 10^8$	1.6 ± 4.8	14	1.0–1.2
21.0	$8.7 \pm 0.3 \times 10^7$	0.5 ± 2.6	4	ND[e]

[a] Values indicate means and standard deviations, respectively, obtained from triplicate cultures. Initial cell concentration was $1.3 \pm 0.2 \times 10^6$ cells/ml.

[b] The average number of magnetosomes per cell ± the standard deviation. The magnetosomes from 100 cells cultured at each Po_2 value were enumerated.

[c] Values corrected for percent of cell yield as inoculum, assuming the inoculum consisted entirely of magnetic cells.

[d] Measured by field-dependent birefringence. Values indicate ranges from duplicate cultures.

[e] ND = None Detected.

Table 2

Effect of O_2 on *A. magnetotacticum* growth and Fe_3O_4 synthesis with NH_4^+

Initial O_2 Concentration (kPa in headspace)	*Final Cell Concentration (cells/ml)*[a]	*Average No. of Magnetosomes (± SD*[b]*/Cell)*	*Percent of Cells with Magnetosomes*[c]	*Magnetic Moment (μ) ($\times 10^{-13}$ emu)*[d]
Trace	$1.8 \pm 0.3 \times 10^6$	9.0 ± 9.1	19	3.3
0.5	$2.6 \pm 0.5 \times 10^7$	11.5 ± 8.0	87	3.1–3.7
1.0	$7.5 \pm 3.1 \times 10^7$	11.9 ± 9.7	84	3.2–3.6
2.5	$9.7 \pm 1.0 \times 10^7$	6.0 ± 10.0	59	1.3–1.7
5.0	$1.4 \pm 0.1 \times 10^8$	3.7 ± 4.8	54	1.7–2.3
10.0	$1.8 \pm 0.1 \times 10^8$	3.1 ± 5.4	35	1.3
21.0	$2.5 \pm 0.1 \times 10^8$	2.6 ± 4.4	48	0.6

[a] Values indicate means and standard deviations, respectively, obtained from triplicate cultures. Initial cell concentration was $1.3 \pm 0.2 \times 10^6$ cells/ml.

[b] The average number of magnetosomes per cell ± the standard deviation. The magnetosomes from 100 cells cultured at each Po_2 value were enumerated.

[c] Values corrected for percent of cell yield as inoculum, assuming the inoculum consisted entirely of magnetic cells.

[d] Measured by field-dependent birefringence. Values indicate ranges from duplicate cultures.

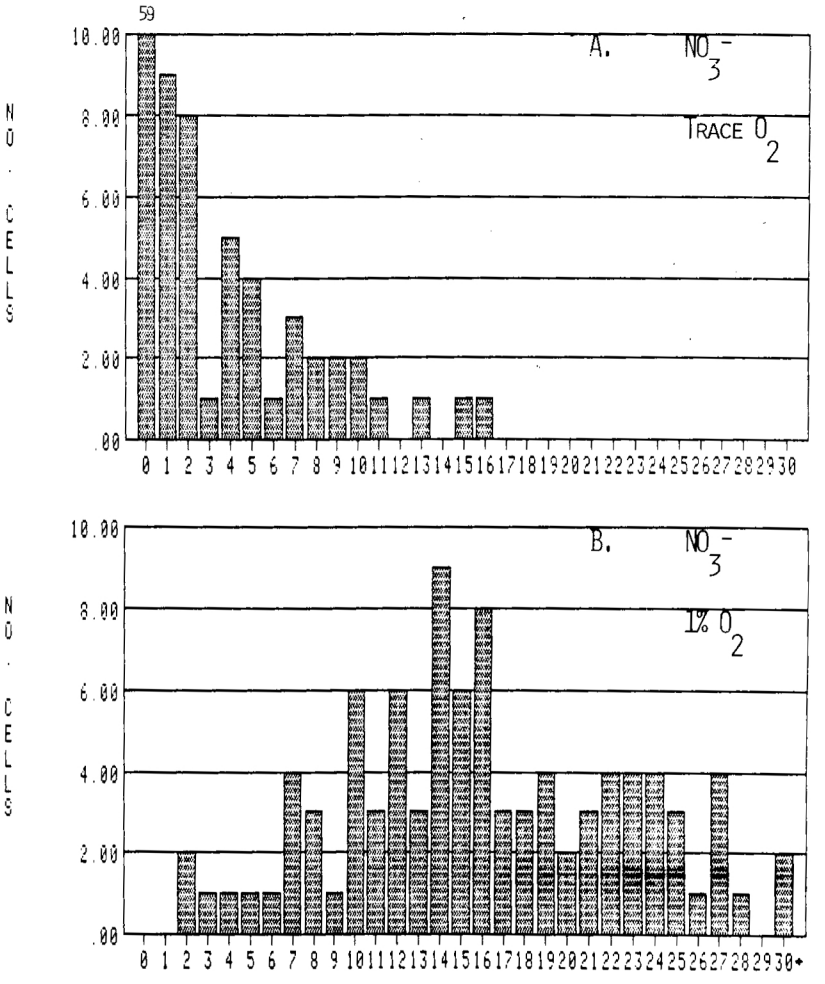

Fig. 2. Magnetosome distributions within *A. magnetotacticum* grown with NO_3^- and (A) trace, (B) 1%, and (C) 21% [kPa] O_2. Magnetosomes within 100 cells were counted by means of direct electron microscopy using cultures grown at each O_2 tension. Small numbers above the "zero" columns indicate the numbers of cells observed with no magnetosomes. Values of "30 +" in abscissas indicate numbers of cells observed that contained 30 or more magnetosomes.

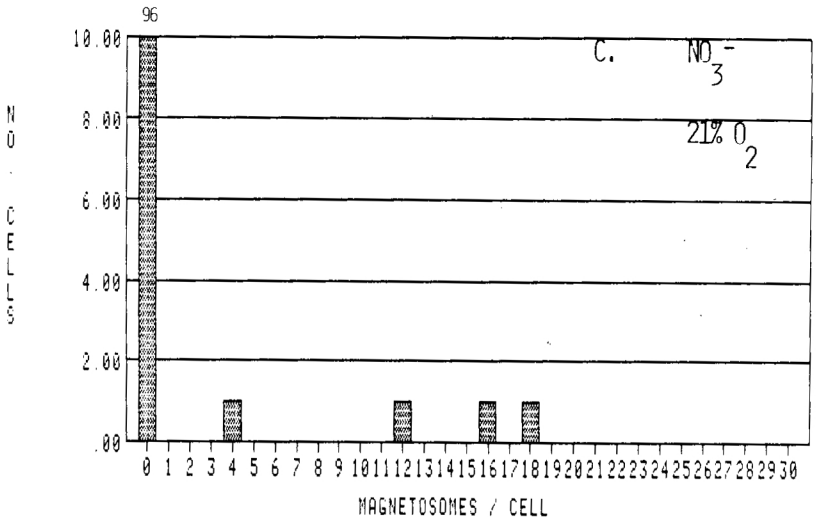

Fig. 2. *(Continued)*

parable to that of cells grown on NH_4^+ alone (Table 2). However, at the optimal Po_2, 100% of cultured cells grown with NO_3^- alone (Table 1; Fig. 2) possessed magnetosomes, whereas only 84 to 87% of cultured cells grown with NH_4^+ alone (Table 2; Fig. 3) contained magnetosomes. Furthermore, at their optimum Po_2, cells grown with NH_4^+ alone (Table 2) may have produced fewer magnetosomes than cells with NO_3^- alone (Table 1). The large standard deviations associated with these measurements reflect difficulty in our ability to differentiate between smaller magnetosomes and non-magnetosomes in electron micrographs of whole cells. In addition, during growth in the closed system used, cells may have exhibited variable magnetosome production as O_2 and Fe concentrations changed. The stimulatory effect of NO_3^- was especially evident, however, when the total Fe_3O_4 yield of cultures was evaluated for each value of O_2 (Fig. 5). Since NH_4^+ at the concentration used does not repress denitrification (Bazylinski and Blakemore, 1983*b*), these results verify that Fe_3O_4 was produced in greatest quantity by denitrifying cells. This effect appeared to be the result of

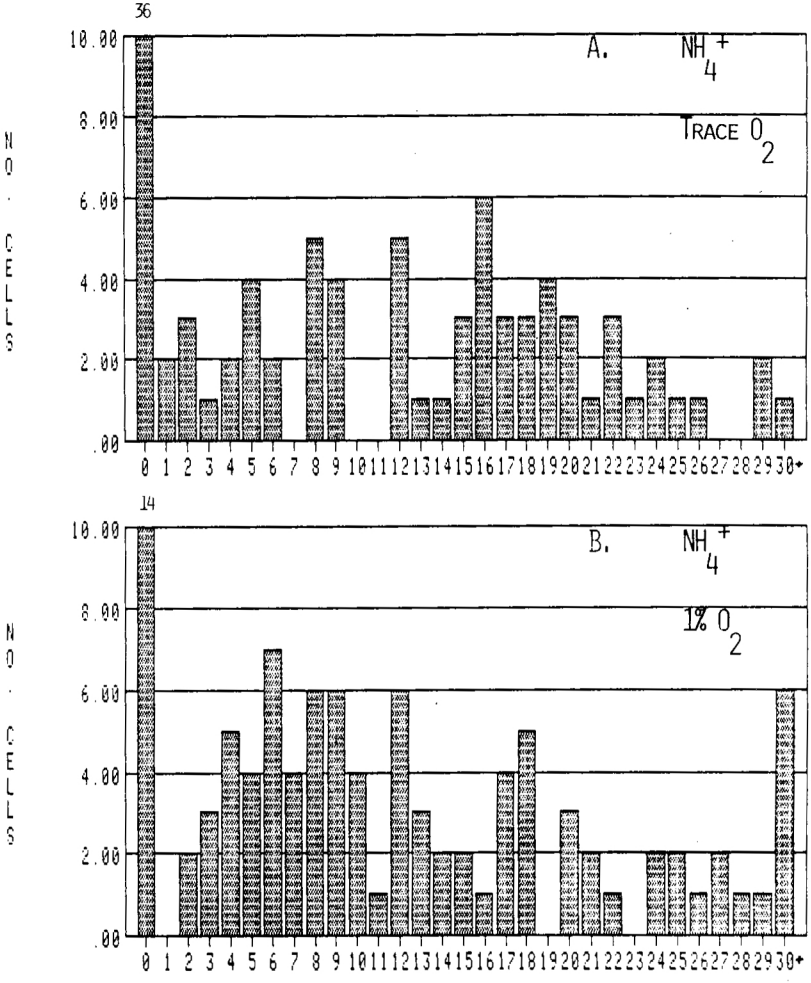

Fig. 3. Magnetosome distributions within *A. magnetotacticum* grown with NH_4^+ and (A) trace, (B) 1%, and (C) 21% [kPa] O_2. Magnetosomes within 100 cells were counted by means of direct electron microscopy using cultures grown at each O_2 tension (see also legend to Fig. 2).

stimulation by NO_3^- rather than inhibition by NH_4^+ because at the Po_2 optimal for Fe_3O_4 formation, the combined effect of the two (Table 3; Fig. 4) was similar to that of NO_3^- alone (Table 1; Fig. 2).

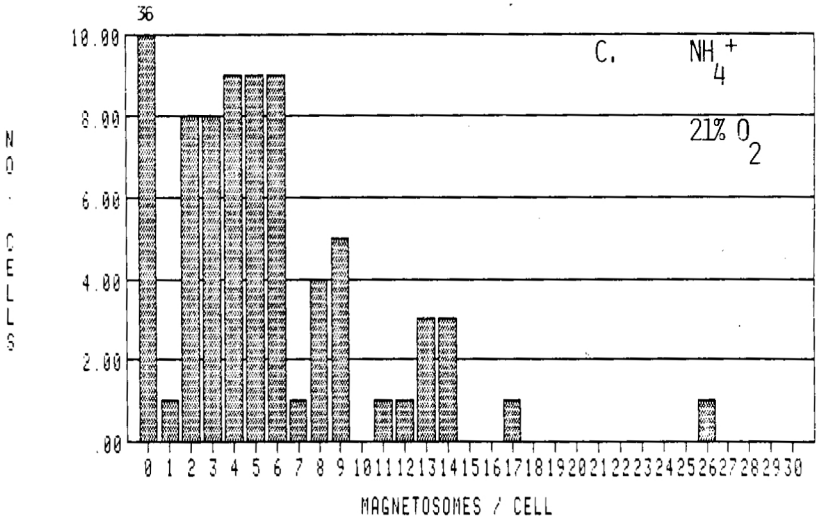

Fig. 3. *(Continued)*

Discussion

Lower final growth yields at < 1 kPa O_2, regardless of the N source, illustrate the specific requirement of this organism for O_2. The nature of this requirement is unknown but may relate to a need for O_2 in biosynthesis. With NH_4^+ as sole N source, cells at < 1kPa O_2 were also limited by electron acceptor (O_2). They grew to higher cell yields at low Po_2 when supplied with the alternate electron acceptor, NO_3^-.

The reason for depressed growth of cells with NO_3^- present at high (10–21 kPa) O_2 is unknown, but may have been due to O_2 inhibition of NO_2^- reduction. The resulting accumulation of NO_2^- would be toxic for cells (Bazylinski and Blakemore, 1983*b*).

The data indicate that bacterial Fe_3O_4 was produced optimally under microaerobic conditions. More than 50% of cells had magnetosomes only when the initial headspace Po_2 of a sealed culture was between 0.5 and 5 kPa. Thus, cells require O_2 to produce Fe_3O_4. Moreover, since atomic oxygen was always abundant in phosphate and nitrate of the culture medium, only dioxygen satisfied this requirement.

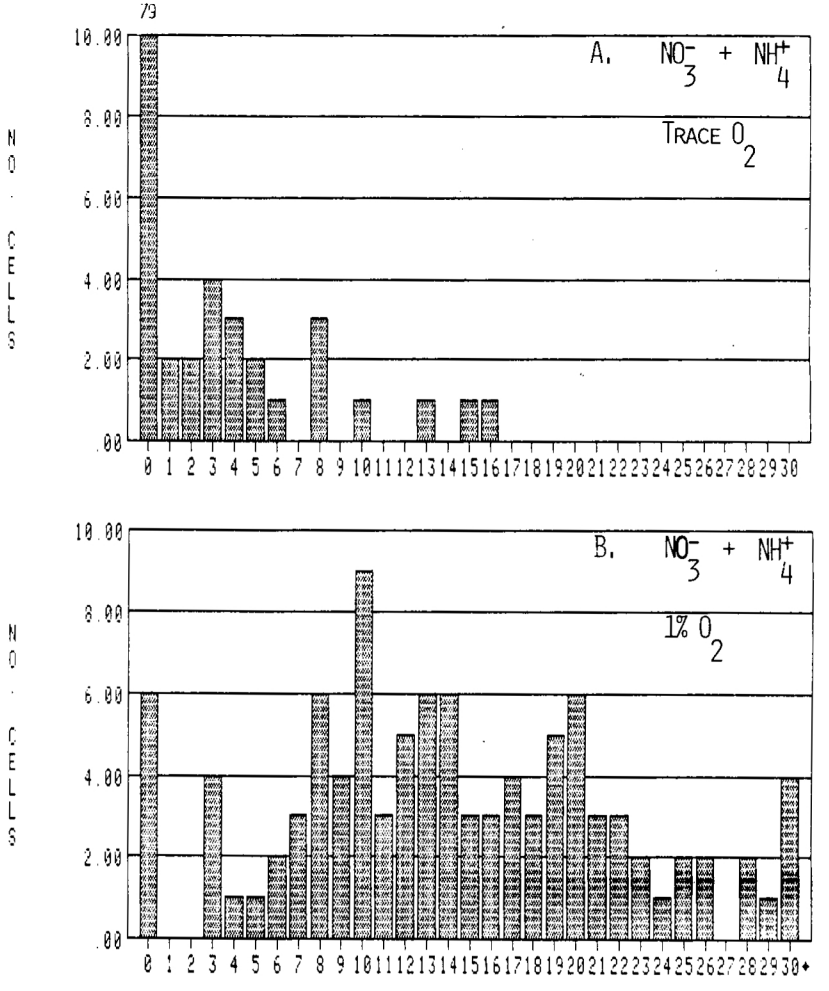

Fig. 4. Magnetosome distributions within *A. magnetotacticum* grown with NO_3^- plus NH_4^+ and (A) trace, (B) 1%, and (C) 21% [kPa] O_2. Magnetosomes within 100 cells were counted by means of direct electron microscopy using cultures grown at each O_2 tension (see also legend to Fig. 2).

Lower magnetism at low Po_2 of cells grown with NH_4^+, compared to those grown with NO_3^-, may indicate competition between reactions employing O_2 as a terminal electron acceptor and others requiring O_2 for formation of the iron oxide, Fe_3O_4. Since the bulk of O_2 consumed by growing cells is consumed in

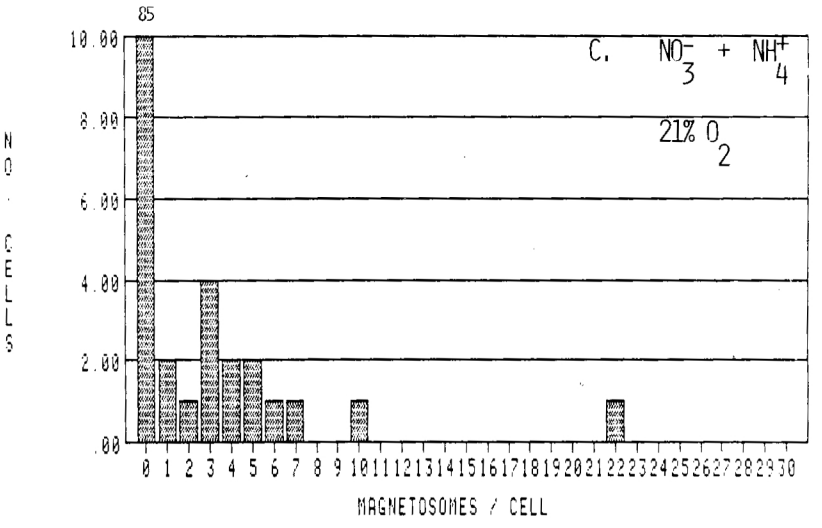

Fig. 4. *(Continued)*

respiration (unpublished results), the effect of such competition in decreasing the Fe_3O_4 yield is most evident with cells grown on NH_4^+ under microaerobic conditions.

The O_2 requirement for the formation of Fe_3O_4 by bacteria is of considerable physiological interest because cells of this and of at least some other species of magnetotactic bacteria (Spormann and Wolfe, 1984) are aerotactic. As a result of their aerotactic response, cells are directed to, and accumulate in, microaerobic zones optimal for growth and for Fe_3O_4 formation.

We believe our findings may have broader implications in biogeochemistry and geology. Magnetotactic bacteria produce Fe_3O_4 particles with a narrow size range and with morphologies unique to various species (Matsuda et al., 1983; Mann et al., 1984). Structures with the unique morphology and size of bacterial Fe_3O_4 grains have been observed in sediments by several investigators (Blakemore, 1975; W. Ghiorse, personal communication; Kirschvink and Chang, 1984). They apparently persist as magnetic microfossils in sediments after their release from dead bacteria (Fig. 6). Because they are single magnetic domains and have permanent remanent moments, bacterial Fe_3O_4 grains might contribute a biological remanent magnetization to sedi-

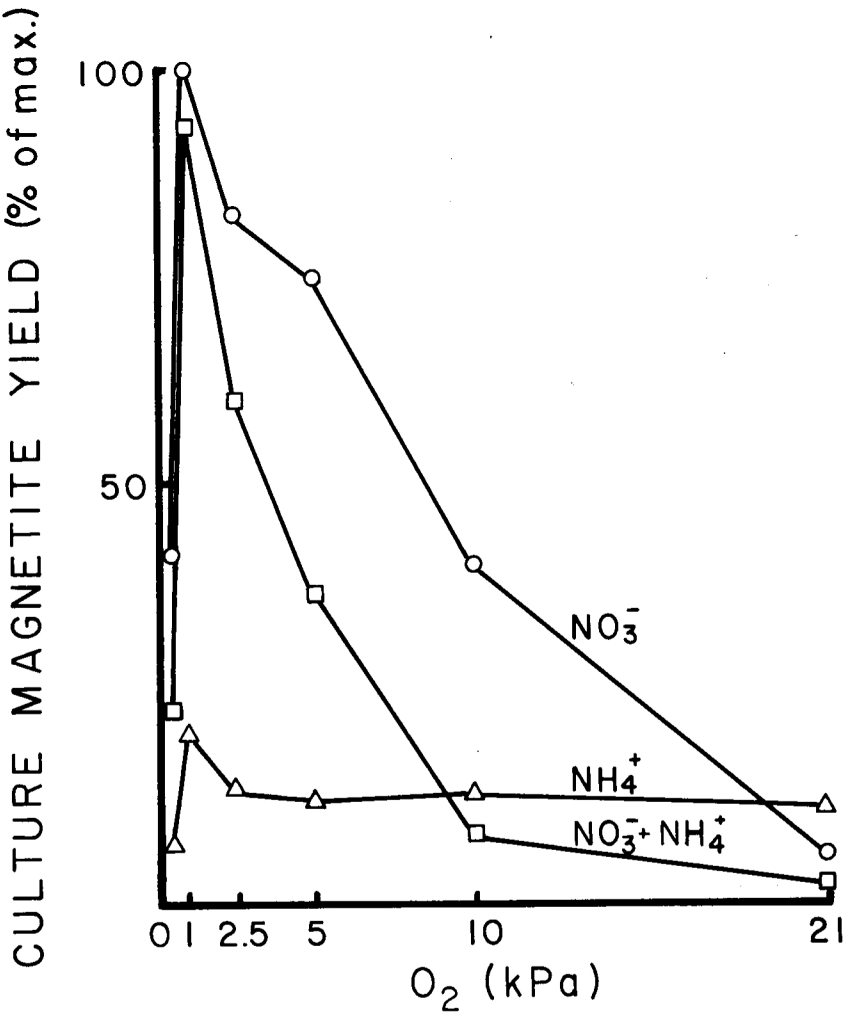

Fig. 5. Total magnetite yield of cultures grown with various sole nitrogen sources at different initial headspace O_2 concentrations. Values were obtained by multiplying the average final cell yield by the corresponding average number of magnetosomes per cell. Values are normalized to the maximum yield obtained.

ments containing them. J. L. Kirschvink and S.-B. R. Chang (1984) have determined that the stable, natural remanent magnetization they detected in marine calcareous oozes appeared to

Table 3

Effect of O_2 on *A. magnetotacticum* growth and Fe_3O_4 synthesis with NO_3^- and NH_4^+

Initial O_2 Concentration (kPa in headspace)	*Final Cell Concentration (cells/ml)*[a]	*Average No. of Magnetosomes (± SD*[b]*/Cell)*	*Percent of Cells with Magnetosomes*[c]	*Magnetic Moment (μ) ($\times 10^{-13}$ emu)*[d]
Trace	$1.2 \pm 0.1 \times 10^8$	1.2 ± 3.2	21	0.7–0.8
0.5	$2.7 \pm 0.1 \times 10^8$	3.7 ± 4.4	55	1.0–1.1
1.0	$2.8 \pm 0.1 \times 10^8$	14.4 ± 8.3	94	2.9–3.1
2.5	$2.2 \pm 0.3 \times 10^8$	11.8 ± 10.6	89	2.1–2.6
5.0	$1.5 \pm 0.5 \times 10^8$	10.6 ± 10.0	84	1.9–2.2
10.0	$1.1 \pm 0.2 \times 10^8$	2.9 ± 6.9	36	1.0–2.6
21.0	$1.2 \pm 0.0 \times 10^8$	0.8 ± 2.7	15	0.7–1.5

[a] Values indicate means and standard deviations, respectively, obtained from triplicate cultures. Initial cell concentration was $1.3 \pm 0.2 \times 10^6$ cells/ml.

[b] The average number of magnetosomes per cell ± the standard deviation. The magnetosomes from 100 cells cultured at each Po_2 value were enumerated.

[c] Values corrected from percent of cell yield as inoculum, assuming the inoculum consisted entirely of magnetic cells.

[d] Measured by field-dependent birefringence. Values indicate ranges from duplicate cultures.

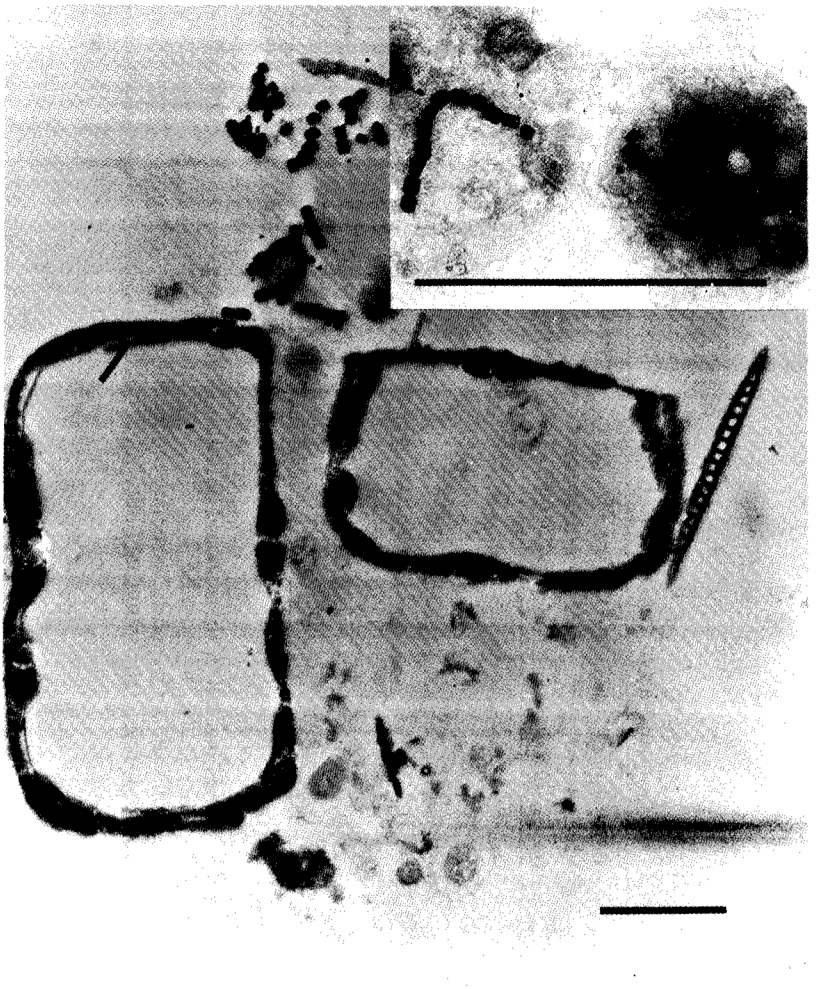

Fig. 6. Transmission electron micrograph of sediment collected from a brackish environment containing magnetotactic bacteria. Thin section stained with uranyl acetate. Note the transverse sections through diatoms as well as through clumps of extracellular particles having a size and morphology of magnetosomes found within bacteria collected at the same site. Inset shows negatively stained magnetotactic bacteria collected from the sediment sample before it was prepared for thin sectioning. Bar = 1 μm.

be, in large part, due to single-domain sized Fe_3O_4 crystals. The unique size and shape of these grains and their similarity to Fe_3O_4 of bacterial origin led these workers to suggest that the magnetotactic bacteria are a prime source of an important component of the paleomagnetic record.

Studies of the occurrence and distribution of other species of magnetotactic bacteria found in natural environments and in enrichments (Blakemore, 1975, 1982; Moench and Konetzka, 1978; Spormann and Wolfe, 1984) lead us to believe that other types of magnetotactic bacteria are also microaerophilic and require O_2 for Fe_3O_4 formation. For instance, bacterial Fe_3O_4 is formed from hydrous ferric oxide precursors produced, in turn, by oxidation of ferrous ions (Frankel et al., 1983). The presence in the fossil record of biogenic structures known to be produced only under microaerobic conditions might provide a useful tool for examining the occurrence of free O_2 in the early earth.

Our data, if applicable to other species, demonstrate that bacterial Fe_3O_4 formation (hence magnetotaxis) could have evolved only after free O_2 became available to cells. This may at first have been in microhabitats shared by oxygenic photoautotrophs, such as in algal mats. Do Precambrian stromatolites or Archean–Early Proterozoic banded iron formations, in particular, preserve evidence of magnetic bacteria? Current thinking suggests that from 1.7 to 2.3 billion years before the present, evolving O_2 apparently saturated its principal Archean sinks (Fe^{2+} and reduced gases) and began to accumulate in the atmosphere (Walker et al., 1983). During this transition to an oxidizing global atmosphere, the earth's entire atmosphere became, by today's standards, microaerobic. The "microaerophiles," including magnetotactic bacteria, had a thermodynamic edge over cells unable to use O_2 as a terminal electron acceptor and may have become the most prevalent physiological group on earth during the Early Proterozoic. As the O_2 tension of the atmosphere increased toward its present level, extant species of microaerophiles apparently failed to evolve suitable means of detoxifying damaging products of O_2 metabolism. They appear to have once again become restricted in their distribution; this time, however, to microhabitats containing suitably low Po_2.

The acquisition of magnetotaxis and aerotaxis would have helped ensure their localization in these microaerobic zones. It would be interesting if prokaryotic magnetosomes in the fossil and paleomagnetic records provided evidence to test these possibilities and to probe further the details of Earth's early evolution.

Acknowledgments

We gratefully acknowledge the technical assistance of Nancy Blakemore and Ali Geshnizgani. We are also grateful to S.-B. R. Chang, J. L. Kirschvink, A. M. Spormann, and R. S. Wolfe for sharing unpublished results. This work was supported by a National Science Foundation grant PCM 82–15900. The Francis Bitter National Magnet Laboratory is supported by the National Science Foundation. R.B.F., C.R., K.A.S. and R.P.B. were partially supported by the Office of Naval Research.

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