Research

Cite this article: Byrne JM, Coker VS, Moise S, Wincott PL, Vaughan DJ, Tuna F, Arenholz E, van der Laan G, Patrick RAD, Lloyd JR, Telling ND. 2013 Controlled cobalt doping in biogenic magnetite nanoparticles. *J R Soc Interface* 10: 20130134.

<http://dx.doi.org/10.1098/rsif.2013.0134>

Received: 11 February 2013

Accepted: 25 March 2013

Subject Areas:

nanotechnology, biomaterials,
environmental science

Keywords:

coercivity, anisotropy, hyperthermia, *Geobacter*, Fe(III) reduction, X-ray magnetic circular dichroism

Author for correspondence:

J. M. Byrne

e-mail: james.byrne@uni-tuebingen.de

Controlled cobalt doping in biogenic magnetite nanoparticles

J. M. Byrne^{1,3}, V. S. Coker¹, S. Moise⁴, P. L. Wincott¹, D. J. Vaughan¹, F. Tuna², E. Arenholz⁵, G. van der Laan^{1,6}, R. A. D. Patrick¹, J. R. Lloyd¹ and N. D. Telling⁴

¹School of Earth, Atmospheric and Environmental Sciences, Williamson Research Centre for Molecular Environmental Science, and ²School of Chemistry, University of Manchester, Manchester M13 9PL, UK

³Geomicrobiology, Center for Applied Geoscience, University of Tuebingen, Sigwartstrasse 10, 72076 Tuebingen, Germany

⁴Institute for Science and Technology in Medicine, Keele University, Stoke-on-Trent ST4 7QB, UK

⁵Advanced Light Source, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA

⁶Diamond Light Source, Didcot, Oxfordshire OX11 0DE, UK

Cobalt-doped magnetite ($\text{Co}_x\text{Fe}_{3-x}\text{O}_4$) nanoparticles have been produced through the microbial reduction of cobalt–iron oxyhydroxide by the bacterium *Geobacter sulfurreducens*. The materials produced, as measured by superconducting quantum interference device magnetometry, X-ray magnetic circular dichroism, Mössbauer spectroscopy, etc., show dramatic increases in coercivity with increasing cobalt content without a major decrease in overall saturation magnetization. Structural and magnetization analyses reveal a reduction in particle size to less than 4 nm at the highest Co content, combined with an increase in the effective anisotropy of the magnetic nanoparticles. The potential use of these biogenic nanoparticles in aqueous suspensions for magnetic hyperthermia applications is demonstrated. Further analysis of the distribution of cations within the ferrite spinel indicates that the cobalt is predominantly incorporated in octahedral coordination, achieved by the substitution of Fe^{2+} site with Co^{2+} , with up to 17 per cent Co substituted into tetrahedral sites.

1. Introduction

Research into magnetic nanoparticles (MNPs) of the form $M_x\text{Fe}_{3-x}\text{O}_4$ ($M = \text{Co}$, Zn, Ni, Cr, etc.) has developed into an area of widespread activity in recent years in response to a broad range of potential applications, such as remediation of contaminated land and water [1–3], catalysis [4,5], targeted drug delivery [6], magnetic data storage [7] and magnetic hyperthermia targeting cancerous tumours [8–10].

Targeted cancer therapies include magnetic hyperthermia in which a heating effect is induced by MNPs (localized within a tumour) under the influence of an oscillating magnetic field. Using this effect, once the temperature at the localized region reaches 40–45°C [10], cancerous cells will be destroyed without causing extensive damage to healthy tissue (cancerous cells are more sensitive to heat than normal cells [11]). The heating effect is dominated either by the physical rotation of MNPs within solution (Brownian relaxation), or by the magnetization reversal within the particles (e.g. Néel relaxation or magnetic hysteresis losses). Both processes lead to a release of heat; however, the effect is dependent on the size and anisotropy of the MNPs [12]. The overall heating produced by the MNPs under the influence of an AC field is defined by the *specific loss power* (SLP) [13].

Several methods have been developed for producing MNPs, including chemical and mechanical methods such as co-precipitation and ball milling [14]. Many of these processes can be expensive and environmentally damaging owing to the use of high temperatures and toxic materials. An alternative way to produce MNPs at ambient temperatures is via the use of subsurface Fe(III)-reducing bacteria such as *Geobacter sulfurreducens* or *Shewanella oneidensis*, which

Table 1. The composition of the starting materials and nanoparticles. The at% cobalt concentrations in the starting material were measured using ICP-AES ($\%Co_{ICP}$), and the nanoparticles by EPMA and calculated from the XAS spectra. Values of x in the formula $Co_xFe_{3-x}O_4$ were also calculated from both the EPMA (x_{EPMA}) and XAS (x_{XAS}).

sample	designed starting material %Co	ICP-AES starting material %Co _{ICP}	EPMA final material %Co	x_{EPMA}	XAS final material %Co	x_{XAS}
Co0	0	0	0	0	0	0
Co5	5	4.2	4.3	0.13	5.6	0.17
Co15	15	12.0	12.7	0.38	11.7	0.35
Co20	20	14.4	14.0	0.42	14.5	0.44
Co33	33	24.9	23.7	0.71	20.0	0.60
Co50	50	34.3	33.7	1.01	29.8	0.89

can conserve energy through the oxidation of an electron donor such as organic matter or hydrogen, coupled with the reduction of Fe(III) [15,16]. The reduction leads to the release of soluble Fe(II) which is able to recrystallize with solid phase Fe(III) to produce magnetite (Fe_3O_4) and potentially other mineral phases, including siderite, goethite, vivianite, haematite and green rust depending on the conditions of formation (i.e. pH and temperature) [17–19].

Magnetite has a cubic spinel lattice structure with a unit cell containing 32 oxygen ions, eight Fe^{3+} ions in tetrahedral (A) sites, eight Fe^{2+} and eight Fe^{3+} ions in octahedral [B] sites, i.e. $Fe^{3+}(A)Fe^{2+}[B]Fe^{3+}[B]O_4$. The magnetism exhibited by magnetite MNPs is a result of the superexchange interaction between the (A) and [B] site cations, which yields antiparallel magnetic moments between the two sublattices. The distribution of iron within the spinel lattice is such that the magnetic moments of Fe^{3+} (approx. $5 \mu_B$ per atom) on both lattice sites effectively cancel each other, resulting in a net magnetization owing to Fe^{2+} (approx. $4 \mu_B$ per atom). The substitution of Fe^{2+} or Fe^{3+} cations with transition metal dopants such as cobalt, zinc, nickel or chromium in the magnetite serves as a method to change the magnetic properties of the MNPs [20,21].

The aim of this work is to further the understanding of the effect of cobalt substitution into the structure of biogenic magnetite and to demonstrate the potential use of such biologically synthesized cobalt ferrites for magnetic hyperthermia applications. Several techniques have been applied in order to fully characterize the material that was produced through the reduction of Co(II)–Fe(III)-oxyhydroxide starting material by *G. sulfurreducens*.

2. Results and discussion

2.1. Physical characteristics and crystal structure

Inductively coupled plasma (ICP) spectrometer analyses show that the cobalt content in the starting materials (at% Co_{ICP}) is approximately 30 per cent less than the designed starting Co fraction at the beginning of the synthesis process (table 1). This ‘loss’ occurs during the process of producing the cobalt–iron precursor and is considered to be due to incomplete precipitation of cobalt into solid phase oxyhydroxide at pH 7 [22]. Electron probe microanalysis (EPMA) used to determine the relative ratios of cobalt to iron (x_{EPMA}) in the nanoparticles shows that the amount of cobalt incorporated into the nanoparticles closely matches the amount that is available in the starting gels. As EPMA analyses a volume of $2 \mu m^3$,

Figure 1. X-ray diffraction with $10^\circ \leq 2\theta \leq 70^\circ$. Reflections for magnetite are labelled on the Co0 trace (top). Low and intermediate cobalt concentrations exhibit reflections corresponding to magnetite. No magnetite peaks are visible for Co33 and Co50 samples; however, a small green rust peak is observable at $2\theta = 11^\circ$ for sample Co50.

it represents the average nanoparticle composition and gives compositions to a high degree of accuracy (error $\leq \pm 0.5\%$); from the $\%Co$ results, the x_{EPMA} , according to the formula $Co_xFe_{3-x}O_4$, was determined.

Powder X-ray diffraction (XRD) was used to characterize the phases that were produced by the microbial reduction in the Co(II)Fe(III)-oxyhydroxide gel (figure 1). The first three samples (Co0, Co5, Co15) clearly show (220), (311), (400), (511) and (440) reflections characteristic of magnetite, with the Co20 sample displaying only (311) and (440) peaks. Samples with the highest cobalt concentrations (Co33 and Co50) do not exhibit any peaks corresponding to magnetite, which is attributed to low particle sizes, resulting in reflections that are indistinguishable from the background noise. At the highest cobalt concentration (Co50), it is possible to see the emergence of a peak at $2\theta = 11^\circ$ consistent with the main reflection for green rust that appears to be produced as an additional mineral phase.

Analysis of the magnetite (311) reflection enabled the determination of mean crystal diameter for samples Co0–Co20. The incorporation of cobalt has a significant effect on the particle size, with an immediate drop in mean crystallite size from 36.7 (Co0) to 11.3 nm with the addition of just approximately 5 per cent cobalt (Co5). There is little change in particle size for cobalt concentrations higher than this with values of 13.7

Figure 2. TEM images of cobalt-doped magnetite. (a) Co0 shows large crystals of 30–40 nm. (b) Co5, (c) Co15 and (d) Co20 particles have approximately 10 nm diameter. (e) Co33 and (f) Co50 are much smaller (approx. 2–4 nm diameter) nanoparticles with needle features also visible in Co50 samples. (Online version in colour.)

and 9.5 nm determined for Co15 and Co20 samples, respectively. Cobalt has a comparable ionic radius to iron (0.074 and 0.077 nm for Co(II) and Fe(II) in octahedral coordination, respectively [23]), thus the particle size change is not due directly to the substitution of the Co(II) cation.

Transmission electron microscopy (TEM) was performed on the samples to investigate morphology and size changes in the series (figure 2). The results confirm XRD measurements with size varying from 30 to 40 nm (Co0), approximately 10 nm (Co5, Co15 and Co20) and approximately 2–4 nm (Co33 and Co50). Selected area electron diffraction (SAED) indicates that the first four samples in the series (Co0–Co20) contain only cobalt-doped magnetite nanoparticles (figure 2, insets). The

two samples with the higher cobalt concentrations (Co33 and Co50) contained both the small particles, identified as magnetite from SAED, as well as large plate-like and needle features. These larger particles were attributed to green rust that occurs as hexagonal plates [24], as indicated by the XRD (figure 1), although SAED measurements could not confirm this.

Energy dispersive X-ray (EDX) spectroscopy was used to confirm differences in cobalt concentration throughout the series. The amount of cobalt in the nanoparticles was measured at 4.4 per cent, 12.8 per cent, 13.9 per cent, 18.8 per cent and 23.3 per cent for samples Co5, Co15, Co20, Co33, Co50, respectively. The values of the first four samples match very closely with EPMA measurements; however,

Figure 3. Superconducting quantum interference device magnetometry results. (a) Hysteresis loops measured at $T = 300$ K. (b) Hysteresis loops at $T = 5$ K. (c) Coercivity measurements. Note room temperature coercivity measurements shown are $\times 10$ enhanced compared with actual results to show changes more effectively on the same scale as low-temperature measurements. (d) Saturation magnetization. (e) Remanence ratio M_r/M_s . (f–h) Zero-field-cooled (ZFC) and field-cooled (FC) curves showing the changes in blocking temperature. (Online version in colour.)

Co33 is marginally lower, and Co50 is roughly one-third less than expected from electron probe analysis. This variation is due to a high level of Co incorporation into the plate-like green rust phase that would have formed a part of the EPMA. The EDX spectra from the large plate-like phases indicated the presence of chlorine, iron and cobalt in ratios of 3.0 per cent Cl, 42.5 per cent Fe, 54.5 per cent Co and 2.2 per cent Cl, 36.0 per cent Fe, 61.8 per cent Co for samples Co33 and Co50, respectively, which suggests the plates to be an $\text{Fe}^{2+}\text{--Co}^{2+}$ phase similar to green rust I (chloride) group [17]. High cobalt concentrations are known in green rust (general formulae $(\text{Fe}^{2+})_6\text{Fe}^{3+}(\text{OH}, \text{Cl})_{18}\cdot 4\text{H}_2\text{O}$), with cobalt replacing the Fe(II) [25,26].

It is not clear from the results presented why the particle size decreases so significantly with increasing cobalt concentration. Several factors could have an effect, including (i) the rate of Fe(III) reduction by *G. sulfurreducens* [27]; (ii) heavy metal toxicity to the micro-organism; and (iii) the initial amount of cobalt-doped ferrihydrite nanoparticles at the start of the culture experiment. This varies across the samples as the initial total Fe(III) concentration (50 mmol l^{-1}) was constant; consequently assuming that all the cobalt–ferrihydrite

particles have the same size, more particles are required to ensure an equal Fe(III) content. (iv) The size of the ferrihydrite nanoparticle starting materials might also be affected by the introduction of cobalt and lead to overall changes in the sizes of cobalt-doped magnetite nanoparticles produced. The size of magnetite nanoparticles produced by *G. sulfurreducens* has previously been shown to be controllable by the amount of bacteria introduced at the start of the experiment with high concentrations of microbial cells yielding smaller nanoparticles than when fewer cells are used. It would be possible to apply similar methods in the case of cobalt-doped nanoparticles and might provide a possible route through which the particle sizes could be made more uniform with increasing cobalt concentration.

2.2. Magnetic properties and hyperthermia

Measurements of the magnetization with respect to applied magnetic field were carried out at room temperature (RT = 300 K) and low temperature (5 K) with results for coercivity (H_c), saturation magnetization (M_s) and remanence (M_r) obtained from hysteresis loops presented in figure 3a–e.

Table 2. Structural and magnetic properties of cobalt-doped magnetite nanoparticles. d , particle size; M_s , saturation magnetization; H_c , coercivity; M_r , remanence; K , anisotropy.

sample	x_{EPMA}	d (nm) XRD	d (nm) TEM	M_s (emu g ⁻¹)		H_c (kOe)		M_r (emu g ⁻¹)		K (erg cm ⁻³)
				300 K	5 K	300 K	5 K	300 K	5 K	5 K
Co0	0	36.7	30–40	81.3	89.6	0.05	0.3	5.8	19.41	1.4×10^5
Co5	0.13	11.3	~10	77.6	84.6	0.35	5.6	17	69.1	2.4×10^6
Co15	0.38	13.7	~10	74	82.9	0.59	8.7	22.2	69.1	3.7×10^6
Co20	0.42	9.5	~10	74.6	81.4	0.39	11.5	13	65.9	4.8×10^6
Co33	0.71	—	2–4	68.9	77.6	0.39	11.5	12.1	60.5	4.6×10^6
Co50	1.01	—	2–4	36.8	56.3	0	10.2	0.3	32.3	2.9×10^6

These measurements indicate a very large increase in the low-temperature coercivity (H_c) as the cobalt dopant concentration increases and, importantly, only relatively small decreases in overall saturation magnetization (M_s).

The increase in H_c at 5 K reaches a maximum at Co20 (11.5 kOe), with Co33 exhibiting the same value, before a small decrease for Co50 (10.2 kOe). M_s decreases (with increasing Co doping) at both RT and low temperature, although the most significant reduction is observed for Co50. An important result that can be taken from these measurements is that at Co20 (low temperature), there is a very significant increase in coercivity compared with Co0 (37× larger), while the saturation magnetization only decreases by 6 emu g⁻¹, corresponding to an 8 per cent decrease. Maintaining a high M_s is important in order to minimize the amount of material required to achieve the desired magnetic response for different applications, in particular those focused on medical therapies. Room temperature measurements also show increases in the coercivity of the samples as cobalt concentration increases although the maximum is reached at Co15 where H_c is 12× larger than that measured for Co0. Changes in saturation magnetization parallel those seen at 5 K although approximately 8 emu g⁻¹ lower, excluding Co50, for which the difference is 20 emu g⁻¹.

Additionally, figure 3e shows that M_r/M_s ratio initially increases with increasing cobalt concentration at both 5 and 300 K, with a value of zero at 300 K for Co50, corresponding to zero coercivity (i.e. superparamagnetism). This superparamagnetic state is reached through the reduction in particle size to less than 4 nm for the highest Co concentrations.

Information about the blocking temperature (T_B) of the samples is obtained through examination of the field-cooled (FC) and zero-field-cooled (ZFC) magnetization collected at 100 Oe (figure 3f–h). The T_B is defined as the point above which the sample exhibits superparamagnetism such that the nanoparticle magnetization is free to align in random orientations. This is best interpreted as the peak of the ZFC curve at the point where it intersects with the FC curve. The data show that at low %Co (Co0, Co5), intermediate %Co (Co15, Co20) and Co33 samples, the T_B is above room temperature with no discernible peak visible below 300 K. A clear peak is visible for Co50 at 220 K at which point the gradient of the ZFC curve is zero as it joins the FC curve. This point corresponds to the T_B , above which the sample becomes superparamagnetic. This is confirmed by an

examination of the H_c values that indicate Co50 has zero coercivity at room temperature.

A lower limit for the anisotropy (K) of the samples can be estimated from the expression $H_c = 0.958 K/M_s$ [28] (assuming uniaxial anisotropy), where H_c and M_s correspond to coercivity and saturation magnetization at 5 K, respectively. However, the presence of larger particles in the distribution is likely to increase the anisotropy above this threshold. The size of the anisotropy determines how strongly the particle's magnetic moment is fixed to an easy direction within the particle. It should be noted, however, that such values underestimate the true anisotropy when the samples are not fully saturated (as is the case here for samples with higher Co concentrations). The results presented in table 2 indicate that the anisotropy significantly increases by an order of magnitude through minor additions of cobalt (Co5). Thus, the inclusion of Co in the ferrite spinel leads to an increase in the magnetic anisotropy of the nanoparticles comparable to previously observed values of first-order anisotropy constants of synthetic bulk Co-ferrites (CoFe₂O₄; $|K_1| \approx 2 \times 10^6$ erg cm⁻³) [29]. The effect appears to be the largest for the Co20 and Co33 samples that exhibit the highest-recorded coercivity.

The potential use of the cobalt-doped magnetite nanoparticles for hyperthermia measurements was assessed from results shown in figure 4. The SLP of the nanoparticles was determined from the rate of change of temperature of a suspension containing a known concentration of cobalt-ferrite nanoparticles under the influence of an AC magnetic field, as discussed extensively elsewhere [12] (i.e. $\text{SLP} \propto dT/dt$). A relatively low magnetic field of 205 Oe was applied at a frequency of 87 kHz for the experiments reported here. The temperature rise observed (normalized to the mass of iron in the samples measured in mg) is shown in figure 4 together with the determined SLP for the biogenic magnetite and three of the cobalt ferrite nanoparticle samples (Co20, Co33 and Co50). The results demonstrate significant heating effects at a frequency and applied field that would be suitable for clinical hyperthermia applications. Several studies have also attempted to explore the use of biogenically derived magnetite nanoparticles using magnetosomes (intracellularly produced magnetite grains) for magnetic hyperthermia [30–32] and reported high values of SLP (up to 1 kW g⁻¹); however, it is difficult to make a direct comparison between those results and the values reported here owing to the alternative conditions applied in that study (10 kA m⁻¹; 410 kHz). Other difficulties

Figure 4. Hyperthermia experiments indicate the rate of temperature change of a solution containing MNPs Co0, Co20, Co33 and Co50 under the influence of an AC magnetic field (87 kHz). The specific loss power (SLP) describes the power achievable per gram of iron in the material. (Online version in colour.)

associated with using magnetosomes include large crystal size that probably results in magnetically blocked particles that would be difficult to control at room temperature, hence biomedical applications, owing to residual magnetization. Another advantage of using the approaches presented within this paper instead of magnetosomes is the ability to generate much larger quantities of material relatively easily.

An enhanced heating effect can be clearly observed for the cobalt–ferrite biogenic nanoparticles in comparison with the undoped biogenic magnetite nanoparticles. For superparamagnetic particles, the SLP is highly dependent on the strength and frequency of the applied magnetic field, and the effective relaxation time (τ_{eff}) of the nanoparticles in suspension [33]. In turn, τ_{eff} depends on the intrinsic size and anisotropy of the nanoparticles, and also on their hydrodynamic size (i.e. the effective size in fluid suspension) and solution viscosity. For the nanoparticles measured here, the combination of a reduction in particle size and an increase in anisotropy will alter τ_{eff} with respect to the undoped particles. The observed enhancement in heating effect for the cobalt-doped samples could thus be caused by a better matching of τ_{eff} with the applied magnetic field frequency. However, given the very large anisotropy values generated in these particles, it is also possible that hysteresis losses could play a role. Additional heating effects could be induced for the low-cobalt-containing samples by decreasing their particle size. Simple modifications of the procedures used throughout this study could be applied to yield Fe_3O_4 nanoparticles with diameters of 10 nm by increasing the number of bacteria used to initiate the microbial Fe(III) reduction in ferrihydrite as previously shown [27]. It is anticipated that this modification to the technique would also yield smaller cobalt-doped magnetite. An additional benefit of applying this modification to the methods procedure is a decrease in the size distribution of the nanoparticles. The smallest Fe_3O_4 nanoparticles produced within that study have very narrow size distributions that are comparable to those generated via synthetic methods [34,35]. Further investigation of the hyperthermia properties of these particles is in progress and will be presented in a future publication.

2.3. Distribution of cations within the crystal structure

2.3.1. X-ray absorption spectra and X-ray magnetic circular dichroism

X-ray absorption spectra (XAS) and X-ray magnetic circular dichroism (XMCD) spectra were collected to understand the changes in the magnetic structure of the MNPs as cobalt is incorporated (figure 5). The XAS spectra obtained in total-electron yield (TEY) mode for both Fe and Co $L_{2,3}$ edges are shown in figure 5a, with the absorption intensity of the Fe L_3 maximum normalized to 1, and the Co signal scaled accordingly. The peak intensity of Co with respect to Fe provides the relative cobalt concentration in the structure of the nanoparticles. The values match closely with those found using EPMA (table 1). Examination of the leading Fe L_3 peak also shows the emergence of a shoulder feature (marked by a dashed vertical line) as more cobalt is incorporated. Such a feature is characteristic of ‘oxidized’ magnetite (a composition relatively deficient in $\text{Fe}^{2+}[\text{B}]$) [2,36]; a result that will serve to confirm that cobalt is entering the magnetite in place of the Fe^{2+} on the [B] site.

Figure 5b shows the XMCD spectra obtained for the Fe $L_{2,3}$ edges. The spectra show a systematic variation in cation distributions within the magnetite as the cobalt concentration increases, with the leading $\text{Fe}^{2+}[\text{B}]$ peak clearly reducing in magnitude with respect to the peaks corresponding to Fe^{3+} . The relative intensities of the XMCD spectra provide information on the magnetization of the samples relative to each other. It is clear that sample Co50 has the smallest XMCD amplitude in all cation sites, reflecting its lower saturation magnetization as shown in figure 3d.

The cations in the magnetite structure produce unique XMCD signatures determined by site location, valence state and magnetization direction. Atomic multiplet calculations [37,38] have been applied to the Fe $L_{2,3}$ XMCD spectra and were fitted to obtain the most accurate description of the experimental data to determine the relative occupancy and oxidation state of the Fe cations in the three sites in the lattice. Stoichiometric magnetite has a total iron occupancy of 3 (i.e. $\text{Fe}^{2+}[\text{B}] : \text{Fe}^{3+}(\text{A}) : \text{Fe}^{3+}[\text{B}] = 1 : 1 : 1$); however, this decreases as cobalt is introduced, towards a ferrite spinel with a formula of CoFe_2O_4 with a total iron occupancy of 2 distributed between the three sites. The sum of the Fe cations in table 3 has been normalized to account for cobalt in the spinel such that

$$x + \text{Fe}^{2+}[\text{B}] + \text{Fe}^{3+}(\text{A}) + \text{Fe}^{3+}[\text{B}] = 3, \quad (2.1)$$

where $x = x_{\text{XAS}}$ (EPMA is a bulk technique, whereas XAS and XMCD are surface measurements, and thus the XAS results are more relevant for examination of XMCD data) and where $\text{Fe}^{2+}[\text{B}]$, $\text{Fe}^{3+}(\text{A})$ and $\text{Fe}^{3+}[\text{B}]$ represent the respective site occupancies in the unit formula.

The results in table 3 confirm a strong decrease in $\text{Fe}^{2+}[\text{B}]$ site occupancy with increasing Co; however, they also point to a decrease in both $\text{Fe}^{3+}(\text{A})$ and $\text{Fe}^{3+}[\text{B}]$, although less severely. These results show that the majority of the cobalt is incorporated into the octahedral site in place of ferrous iron; however, there is also substitution into Fe^{3+} tetrahedral and octahedral sites for all Co concentrations (although the results for Co5 are within the error of ± 0.02 from the results of Co0).

Figure 5c graphically shows the results from table 3 for the changing Fe cation occupancy as a function of Co. The plots indicate almost no deviation from linear fits as Co content increases with the slopes corresponding to the probability of Co entering a particular site. A linear fit of

Figure 5. XAS and XMCD measurements. (a) Both Fe $L_{2,3}$ (700–730 eV) and Co $L_{2,3}$ (770–810 eV) XAS, (b) Fe $L_{2,3}$ XMCD, (c) change in cation occupancy with Co concentration, x_{XAS} , determined from fitting Fe $L_{2,3}$ XMCD spectra and (d) Co $L_{2,3}$ XMCD. (Online version in colour.)

Table 3. Results of XMCD peak fitting. The Fe site occupancies normalized using equation (2.1) to include Co determined from x_{XAS} .

	x_{XAS}	$\text{Fe}^{2+}[\text{B}]$	$\text{Fe}^{3+}(\text{A})$	$\text{Fe}^{3+}[\text{B}]$
Co0	0	1.11	0.92	0.97
Co5	0.17	0.97	0.90	0.96
Co15	0.35	0.84	0.87	0.94
Co20	0.44	0.78	0.86	0.93
Co33	0.60	0.68	0.82	0.90
Co50	0.89	0.46	0.77	0.88

the data gives $\text{Fe}^{2+}[\text{B}]:\text{Fe}^{3+}(\text{A}):\text{Fe}^{3+}[\text{B}] = 0.72:0.17:0.11$ which would suggest that 17 per cent of the Co goes in place of $\text{Fe}^{3+}(\text{A})$ which is in close agreement with Patrick *et al.* [39].

Further analysis of the Co XMCD (figure 5d) is useful in determining where cobalt is entering the crystal structure. The Co L_3 XMCD signal has negative sign which is indicative for the magnetic alignment of the octahedral site. The highest cobalt-containing samples (Co50) have the most intense XAS signal, corresponding to higher Co concentration; however, these also have the least intense Co XMCD, reflecting the overall reduction in M_s and also the presence of a non-magnetic phase (most likely unconverted $\text{Co(II)Fe(III)-oxyhydroxide}$;

see Mössbauer results below) that contains Co. The Co XAS and XMCD spectra obtained for all samples show very little differences in spectral shape, indicating the same cobalt composition is present in all samples. The cobalt species can be identified through a comparison of experimental and calculated spectra [37,38]. The spectra observed most closely match that of calculated Co^{2+} octahedral (as identified by Coker *et al.* [20]), a result that confirms the incorporation of cobalt in place of ferrous iron in the octahedral site and agreeing well with results for CoFe_2O_4 thin films [40]. However, the complex multiplet structure of the various Co species fails to exclude a small amount of tetrahedral Co substitution. In fact, simulations by Hocheplid *et al.* [41] would suggest that between 10 and 20 per cent Co exists as tetrahedral Co in cobalt–ferrite nanoparticles.

Using the results in figure 5c, we can speculate on the several possible scenarios to describe the incorporation of cobalt into the magnetite crystal structure. Clearly, the majority of the cobalt (72%) substitutes as Co^{2+} in the $\text{Fe}^{2+}[\text{B}]$ site, which preserves the overall neutral charge balance of the unit cell. However, also the other Fe site occupancies can be seen to change in figure 5c. It seems that part of the cobalt (11%) substitutes in $\text{Fe}^{3+}[\text{B}]$, or that some $\text{Fe}^{2+}[\text{B}]$ has changed its oxidation state to $\text{Fe}^{3+}[\text{B}]$. Also, it appears that 17 per cent of the cobalt substitutes into the $\text{Fe}^{3+}(\text{A})$ site. However, replacement of Fe^{3+} by Co^{2+} results in an overall negatively charged unit cell. Alternatively, 17 per cent cobalt could substitute as Co^{3+} into the $\text{Fe}^{3+}(\text{A})$ site, conserving the charge balance.

Figure 6. Mössbauer spectra obtained for the cobalt-doped magnetite samples at (a) room temperature and (b) low temperature. The different types of sextet visible correspond to (A) sites (dark grey), [B] sites (light grey) and [B2] sites (black).

However, there is insufficient evidence to suggest that the Co^{2+} from the starting compound has been oxidized to Co^{3+} either through chemical or microbial oxidation. Moreover, the 2+ and 3+ charges of the Fe and Co cations should not be regarded as integral numbers but represent only nominal values, of which the precise value is still a matter of debate [42]. Therefore, any charge balance arguments should be considered with caution.

It is possible that the substitution of Co for Fe^{3+} cations in addition to the expected substitution on Fe^{2+} (A) sites could be related to the preservation of the high saturation magnetization of the biogenic cobalt-doped magnetite nanoparticles observed here. However, further work is required to confirm this.

2.3.2. Mössbauer spectroscopy

Mössbauer analysis was carried out to characterize the bulk cation properties of the materials at room temperature (RT = 300 K) and low temperature (LT = 110 K; figure 6 and table 4). The spectra were fitted to obtain the isomer shift (IS), quadrupole splitting (QS) and magnetic hyperfine field (B_{hf}). Almost all samples exhibit B_{hf} spectra except Co50 at RT that displays a paramagnetic doublet. This is superparamagnetic behaviour, the result of the $T_{\text{B}} < \text{RT}$ as observed using superconducting quantum interference device (SQUID) magnetometry ($T_{\text{B}} \approx 220$ K). All hyperfine field spectra were fitted with two sextets, the first corresponding to Fe^{3+} (A) and the second to Fe^{2+} – Fe^{3+} [B]. Analysis of cobalt-doped samples (i.e. Co5 to Co50) showed that an additional sextet (denoted [B2]) is required to provide an accurate fit. Normally, electron hopping between octahedral Fe^{2+} and Fe^{3+} is too fast to distinguish different spectra for each cation [43,44]; however, the incorporation of Co^{2+}

eliminates some of the Fe^{2+} – Fe^{3+} [B] interactions available owing to a decrease in the amount of Fe^{2+} present for pairing (assuming Fe^{2+} substitution by Co^{2+}). It is therefore reasonable to anticipate the extra sextet arising as a result.

Room temperature measurements of Co50 indicate the presence of two doublets, which based on IS and QS correspond to an Fe^{2+} -containing mineral determined to have similar parameters to green rust and was identified as an Fe^{2+} – Co^{2+} structure from TEM results [45], and an unidentified Fe^{3+} -containing mineral purportedly unreduced Co(II)Fe(III)-oxyhydroxide. The low-temperature spectrum is best fitted with five different components that correspond to the (A), [B] and [B2] sites, the Fe^{2+} – Co^{2+} double-layered phase and non-reduced Co(II)Fe(III)-oxyhydroxide starting material.

A decrease in the LT IS can be observed in the [B] spectra from 0.73 (Co0) to 0.44 mm s^{-1} (Co50). No significant change is observable in IS for (A) or [B2] spectra that display values of approximately 0.40 ± 0.01 and 0.56 ± 0.02 mm s^{-1} , respectively. The QS observed shows the general trend for the (A) and [B2] sites to remain relatively unchanged throughout the series; however, there is seen to be a linear decrease in [B] site splitting until sample Co50, for which the value more closely matches that of Co5. Only unpaired electrons contribute to B_{hf} . The B_{hf} determined for the pure magnetite sample at room temperature is calculated at 486.6 kOe at the tetrahedral (A) site, and 451.1 kOe at the octahedral [B] site, which closely matches established data [46]. Low-temperature measurements of 506.5 and 478.8 kOe for (A) and [B] sites, respectively, also match pre-existing data closely [45,46]. There is an observable overall decrease in the B_{hf} of all three sextets through the series that supports the interpretation of the XMCD data that suggests that some Co enters the tetrahedral site in place of Fe^{3+} (A).

Table 4. Mössbauer fitting parameters determined for cobalt-doped ferrite spinel samples at room temperature (RT) and low temperature (LT). Parameter values are given for the isomer shift (IS), quadrupole splitting (ΔE_Q), hyperfine field (B_{hf}) and population (pop.).

sample	temp.	site	IS (mm s^{-1})	ΔE_Q (mm s^{-1})	B_{hf} (kOe)	pop. (%)
Co0	RT	Db	0.74	2.32	—	2.7
		(A)	0.33	0.04	486.6	37.6
		[B]	0.64	0.06	451.1	59.7
	LT	Db	0.80	2.47	—	3.2
		(A)	0.42	−0.02	506.5	38.7
		[B]	0.73	0	478.8	58.1
Co5	RT	Db	0.43	2.81	—	2.4
		(A)	0.36	0.04	486.3	47.9
		[B]	0.58	0.02	447.2	44.6
		[B2]	0.49	−0.60	488.3	5.1
	LT	Db1	0.39	2.84	—	2.5
		Db2	−0.40	0.99	—	1.3
		(A)	0.41	0	507.2	50.5
		[B]	0.61	−0.22	471.9	24.4
		[B2]	0.54	−0.24	525.8	21.3
Co15	RT	Db	0.18	0.02	—	8.7
		(A)	0.34	0.06	477.4	36.9
		[B]	0.54	−0.02	439.8	46.5
		[B2]	0.50	−0.36	492.1	7.9
	LT	Db1	0.44	2.79	—	1.9
		Db2	0.19	0.24	—	2.0
		(A)	0.39	0.04	503.2	42.8
		[B]	0.60	−0.28	474.9	29.7
		[B2]	0.59	−0.26	523.7	23.5
Co20	RT	Db	0.40	2.53	—	9.4
		(A)	0.34	0.06	475.0	29.8
		[B]	0.54	−0.04	438.2	39.0
		[B2]	0.54	−0.40	491.1	21.8
	LT	Db1	0.43	2.75	—	2.8
		Db2	0.08	0.26	—	0.8
		(A)	0.39	0.04	503.3	41.1
		[B]	0.62	−0.28	475.8	30.0
		[B2]	0.58	−0.26	523.5	25.4
Co33	RT	Db	0.40	2.53	—	4.2
		(A)	0.33	0.06	472.0	47.2
		[B]	0.49	−0.08	436.3	41.4
		[B2]	0.49	−0.30	492.9	7.3
	LT	Db	0.45	2.80	—	3.2
		(A)	0.39	0.02	501.4	45.6
		[B]	0.56	−0.40	467.3	20.0
		[B2]	0.55	−0.20	524.7	31.3
Co50	RT	Db1	0.32	0.34	—	52.1
		Db2	1.07	1.92	—	48.0
	LT	Db1	−0.38	1.06	—	4.8
		(A)	0.42	0	497.5	43.8
		[B]	0.44	−0.20	464.7	30.4
		[B2]	0.54	−0.16	520.1	15.6
		Db2	1.35	0.85	23.4	5.4

3. Conclusions

This work has explored the magnetic properties of biogenic cobalt-doped magnetite nanoparticles formed by the reduction of Co(II)Fe(III)-oxyhydroxide starting material by an Fe(III)-reducing bacterium at ambient temperature and pressure. The nature of the techniques that we have used allows studies of the surface layers of the nanoparticles to be compared against the bulk measurements, with differences from the original biogenic magnetite observable using both. The Fe XMCD and Mössbauer results suggest that the majority of Co substitution occurs into octahedral coordination in place of Fe^{2+} [B]. From examination of the Fe XMCD, it is possible that there is also incorporation of Co in place of Fe^{3+} (A). Further analysis based on the calculated multiplet fits for the Fe presented in figure 5c indirectly suggests that up to 17 per cent Co is incorporated into the tetrahedral site. Changes in the hyperfine field detected using Mössbauer spectroscopy also lend support to the likelihood of tetrahedral cobalt substitution.

The overall coercivity of the particles is found to increase strongly with increased cobalt doping, with a concurrent minor decrease in saturation magnetization. We have demonstrated that a biological method can be used to obtain ferrite nanoparticles with tailored magnetic anisotropy values through Co doping. For applications in magnetic hyperthermia, this ability to control anisotropy, together with the possibility of controlling nanoparticle sizes as demonstrated previously [27], could provide a method to optimize the nanoparticle relaxation time for a specific applied field frequency, thus greatly enhancing the heating effect. Large heating effects are essential to realize the therapeutic potential of the technique where the applied field amplitude and frequency are limited by practical and clinical considerations.

4. Experimental methods

Six different cobalt–iron suspensions (Co(II)Fe(III)-oxyhydroxide) were prepared through the co-precipitation of a mixture of iron(III) chloride (FeCl_3) and cobalt(II) chloride (CoCl_2) via the addition of sodium hydroxide (10 N) acting as the hydrolysing agent until the suspension reached a pH of 7.0 [15]. The starting concentrations of FeCl_3 and CoCl_2 were varied to produce six different ‘gels’ corresponding to 0 per cent, 5 per cent, 15 per cent, 20 per cent, 33 per cent and 50 per cent Co (by molar percentage) referred to as samples Co0, Co5, Co15, Co20, Co33 and Co50, respectively. After precipitation, the chloride ions were removed by centrifugation at 17 000g for 20 min and washed with deionized water, with this step repeated six times. The total concentration of iron was determined by Ferrozine assay [47]. ICP atomic emission spectroscopy (ICP-AES), after extraction using 3 M HNO_3 , was used to determine the relative amounts of cobalt and iron in the starting oxyhydroxide material.

Cell cultures were prepared in deionized water to a total volume of 9.9 ml containing an electron donor (sodium acetate; 20 mM), an electron acceptor (Co(II)Fe(III)-oxyhydroxide; 50 mmol l^{-1}), sodium bicarbonate buffer (NaHCO_3 ; 30 mM) and an electron shuttle, anthraquinone 2,6-disulfonate (10 μM), to accelerate Fe(III) reduction. All manipulations were carried out under a N_2 : CO_2 (80:20) gas line to ensure anaerobic conditions.

Geobacter sulfurreducens was grown in anaerobic conditions in the dark at 30°C in modified fresh water medium [48] (25 mM sodium acetate as electron donor, 40 mM fumaric acid as electron acceptor). Late log-phase cultures of *G. sulfurreducens* were harvested by centrifugation at 5000g and 4°C, for 20 min and washed twice in bicarbonate buffer (30 mM; pH 7) under a N_2 : CO_2 (80:20) gas line. Cells were transferred into bicarbonate buffer, forming a total volume of 30 ml bacterial suspension. Optical density at a wavelength of 600 nm (OD_{600}) was measured using an M501 single beam scanning UV/visible spectrophotometer. The cell suspension was diluted with buffer to achieve an OD_{600} of 0.4 in 10 ml volume (0.2 ml cells, 9.8 ml deionized H_2O). Each culture was then inoculated with 0.2 ml *G. sulfurreducens* suspension (0.132 mg ml^{-1} protein, determined by reference to a standard curve prepared with bovine serum albumin) and incubated in the dark at 30°C for one week. The end product was washed twice with deionized water to remove cells and buffer solution.

The chemical composition of the material was obtained using EPMA using a Cameca SX100 microprobe equipped with wavelength dispersive spectrometers. The operating voltage was 15 kV with a specimen current of 20 mA. Pressed pellets of nanoparticles were compared against pure metal standards.

XRD measurements were carried out using a Bruker D8 Advance with a $\text{Cu K}\alpha_1$ source. Data were acquired over a 2θ range of 10–70° with a step size of 0.02°. When magnetite formation was observed in the XRD trace, the average particle size was determined by fitting a Lorentzian curve to the most intense reflection (311) in order to determine full width at half maximum and Bragg angle. These values were used to determine the particle size via input into the Scherrer equation [49,50].

TEM was carried out using a Technai F20 electron microscope equipped with a field emission gun, EDX detector (Oxford Instruments) and Gatan SC600 CCD camera. All images were obtained using an operating beam voltage of 200 kV.

Magnetic measurements were performed on polycrystalline samples restrained in eicosane using a Quantum Design MPMS-XL SQUID magnetometer equipped with a 7 T magnet. ZFC and FC magnetization curves were recorded over a 5–300 K temperature range with an applied magnetic field of 100 Oe. The diamagnetism of the sample holder and eicosane was measured and extracted from the raw magnetic data.

For hyperthermia experiments, stable aqueous suspensions of nanoparticles were prepared by coating with citric acid using a procedure similar to that described elsewhere [51]. Briefly, 25 mg of biogenic nanoparticles was added to 5 ml of dH_2O and the pH lowered to less than 3 using concentrated HCl. The solution was bath sonicated for 2 min before adding dry citric acid to give a concentration in solution of 15 mg ml^{-1} , and the pH was subsequently increased to 5.2 using concentrated ammonia. The nanoparticle plus citric acid solution was then heated to more than 80°C for 30 min. Excess citric acid was removed by magnetic decantation, and the nanoparticles washed with acetone several times. The citric-acid-coated nanoparticles were then resuspended in dH_2O and subjected to several cycles of vortexing, sonication and centrifugation in order to obtain an optimum stable suspension. ICP measurements were used to obtain iron concentrations in citric-acid-coated nanoparticle suspensions, following complete digestion in high-purity nitric acid. Briefly, the nanoparticle suspensions were mixed with concentrated

nitric acid (70%) and heated at 60°C for 48 h. Complete digestion of the nanoparticles was confirmed by dynamic light scattering measurements performed before and after digestion. The resulting samples were diluted with ultrapure water (giving a final 2% nitric acid concentration) and 0.2 µm filtered, prior to ICP analysis.

The solution properties of the nanoparticles were determined using a Malvern Zetasizer 3000. Following citric acid coating, the hydrodynamic cluster (particle) sizes in the aqueous suspensions were determined to be in the range of 40–75 nm for the magnetite and cobalt–ferrite nanoparticles. The measured zeta potential was –45 mV at pH 7.0 reducing to zero at pH ≈ 2.5 in agreement with previous studies [51].

Magnetic hyperthermia measurements were performed using a commercially available unit supplied by nanoTherics Ltd. The heating effects were determined in an AC magnetic field of 205 Oe at a frequency of 110 kHz. An equivalent volume of dH₂O was measured to confirm that no heating occurred in the absence of nanoparticles.

XAS at the Fe and Co *L*_{2,3} edges were measured at beamline 4.0.2 of the Advanced Light Source, Lawrence Berkeley National Laboratory, CA, USA. Samples were dried and ground in an anaerobic cabinet and mounted onto carbon tape attached to the sample probe with transportation to the beamline taking place in sealed N₂ containers. The sample probe was loaded into the chamber in a backflow of N₂ to limit potential exposure to oxygen. Measurements were made in TEY mode with an effective probing depth of approximately 3–4 nm, where the signal decreases exponentially with increasing depth, hence most signal originates from the first few nanometres. XMCD spectra were obtained as the difference between two XAS spectra recorded with two opposite applied magnetic fields of ±0.6 T (parallel and anti-parallel to the beam direction). The XMCD is dependent on

the magnetic moments of the ferrite spinel sublattices in the magnetite structure (i.e. spin up and spin down), valence state (i.e. number of d electrons) and site symmetry (crystal field). The distribution of Fe cations within the magnetite structure can be determined by comparison of the XMCD with atomic multiplet calculations [37,38]. In the case of Fe₃O₄, each peak in the *L*₃ edge of the spectrum corresponds primarily to a different component of the magnetite, the lowest energy negative peak corresponds to octahedral Fe²⁺[B], the positive peak to tetrahedral Fe³⁺(A) and highest energy negative peak to octahedral Fe³⁺[B] [39].

Mössbauer spectra were recorded with a FAST ComTek 1024-multichannel analyser system using a constant acceleration drive (RT, γ-source approx. 25 mCi ⁵⁷Co/Rh matrix). Samples were sealed between two layers of Kapton tape that were glued together in an anoxic glove bag to prevent oxidation. Measurements at low temperatures were obtained using a liquid nitrogen cryostat. For line fitting, the Lagarec/Rancourt recoil fitting routine was used (Intelligent Scientific Applications Inc.). Spectra were fitted using Lorentzian line shape symmetrical doublets/sextets. IS data were calibrated with reference to metallic Fe foil recorded at RT. The absorber thickness was less than 4 mg Fe cm^{–2}.

All data associated with this publication are available at: <http://doi.pangaea.de/10.1594/pangaea.808948>.

This work was carried out with the financial support of an NERC PhD studentship awarded to J.M.B. The Advanced Light Source is supported by the Director, Office of Science, Office of Basic Energy Sciences, of the US Department of Energy (under contract no. DE-AC02-05CH11231). We acknowledge NERC Envirosync II for providing support for this work. Additional thanks to Dr Michael Ward for assistance with and the provision of access to transmission electron microscope by Leeds Nanoscience and Nanotechnology Facility (LENNF). We also thank Dr Eva Cespedes for assistance with the hyperthermia measurements.

References

- Cutting RS *et al.* 2010 Optimizing Cr(VI) and Tc(VII) remediation through nanoscale biomineral engineering. *Environ. Sci. Technol.* **44**, 2577–2584. (doi:10.1021/es902119u)
- Telling ND, Coker VS, Cutting RS, van der Laan G, Pearce CI, Patrick RAD, Arenholz E, Lloyd JR. 2009 Remediation of Cr(VI) by biogenic magnetic nanoparticles: an X-ray magnetic circular dichroism study. *Appl. Phys. Lett.* **95**, 163701. (doi:10.1063/1.3249578)
- Hend V, Mucha P, Orlikova A, Leskova D. 1995 Utilization of ferrites for water treatment. *Water Res.* **29**, 383–385. (doi:10.1016/0043-1354(94)E0112-J)
- Coker VS *et al.* 2010 Microbial engineering of nanoheterostructures: biological synthesis of a magnetically recoverable palladium nanocatalyst. *ACS Nano* **4**, 2577–2584. (doi:10.1021/nn9017944)
- Polshettiwar V, Luque R, Fihri A, Zhu H, Bouhrara M, Basset J-M. 2011 Magnetically recoverable nanocatalysts. *Chem. Rev.* **111**, 3036–3075. (doi:10.1021/cr100230z)
- Alexiou C, Arnold W, Klein RJ, Parak FG, Hulin P, Bergemann C, Erhardt W, Wagenpfeil S, Lübke AS. 2000 Locoregional cancer treatment with magnetic drug targeting. *Cancer Res.* **60**, 6641–6648.
- Zeng H, Black CT, Sandstrom RL, Rice PM, Murray CB, Sun SH. 2006 Magnetotransport of magnetite nanoparticle arrays. *Phys. Rev. B* **73**, 020402(R). (doi:10.1103/PhysRevB.73.020402)
- Johannsen M *et al.* 2005 Clinical hyperthermia of prostate cancer using magnetic nanoparticles: presentation of a new interstitial technique. *Int. J. Hyperthermia* **21**, 637–647. (doi:10.1080/02656730500158360)
- Johannsen M *et al.* 2007 Thermotherapy of prostate cancer using magnetic nanoparticles: feasibility, imaging, and three-dimensional temperature distribution. *Eur. Urol.* **52**, 1653–1662. (doi:10.1016/j.eururo.2006.11.023)
- Goya GF, Grazu V, Ibarra MR. 2008 Magnetic nanoparticles for cancer therapy. *Curr. Nanosci.* **4**, 1–16. (doi:10.2174/157341308783591861)
- Wust P, Hildebrandt B, Sreenivasa G, Rau B, Gellermann J, Riess H, Felix R, Schlag PM. 2002 Hyperthermia in combined treatment of cancer. *Lancet Oncol.* **3**, 487–497. (doi:10.1016/S1470-2045(02)00818-5)
- Fortin J-P, Wilhelm C, Servais J, Ménager C, Bacri J-C, Gazeau F. 2007 Size-sorted anionic iron oxide nanomagnets as colloidal mediators for magnetic hyperthermia. *J. Am. Chem. Soc.* **129**, 2628–2635. (doi:10.1021/ja067457e)
- Piepenbrock A, Dippon U, Porsch K, Appel E, Kappler A. 2011 Dependence of microbial magnetite formation on humic substance and ferrihydrite concentrations. *Geochim. Cosmochim. Acta* **75**, 6844–6858. (doi:10.1016/j.gca.2011.09.007)
- Majewski PTB. 2007 Functionalized magnetite nanoparticles: synthesis, properties, and bio-applications. *Crit. Rev. Solid State Mater. Sci.* **32**, 203–215. (doi:10.1080/10408430701776680)
- Lovley DR, Phillips EJP. 1986 Availability of ferric iron for microbial reduction in bottom sediments of the fresh-water tidal Potomac River. *Appl. Environ. Microbiol.* **52**, 751–757.
- Coker V, Patrick R, van der Laan G, Lloyd J. 2007 Formation of magnetic minerals by non-

- magnetotactic prokaryotes. In *Magnetoreception and magnetosomes in bacteria* (ed. D Schüller), pp. 275–300. Heidelberg, Germany: Springer.
17. Cornell RM, Schwertmann U. 2003 *The iron oxides: structure, properties, reactions, occurrences and uses*, pp. 388–401. Weinheim, Germany: Wiley-VCH.
 18. Hansel CM, Benner SG, Fendorf S. 2005 Competing Fe(II)-induced mineralization pathways of ferrihydrite. *Environ. Sci. Technol.* **39**, 7147–7153. (doi:10.1021/es050666z)
 19. Hansel CM, Benner SG, Neiss J, Dohnalkova A, Kukkadapu RK, Fendorf S. 2003 Secondary mineralization pathways induced by dissimilatory iron reduction of ferrihydrite under advective flow. *Geochim. Cosmochim. Acta* **67**, 2977–2992. (doi:10.1016/S0016-7037(03)00276-X)
 20. Coker VS, Telling ND, van der Laan G, Patrick RAD, Pearce CI, Arenholz E, Tuna F, Winpenny REP, Lloyd JR. 2009 Harnessing the extracellular bacterial production of nanoscale cobalt ferrite with exploitable magnetic properties. *ACS Nano* **3**, 1922–1928. (doi:10.1021/nn900293d)
 21. Staniland S, Williams W, Telling N, van der Laan G, Harrison A, Ward B. 2008 Controlled cobalt doping of magnetosomes *in vivo*. *Nat. Nanotechnol.* **3**, 158–162. (doi:10.1038/nnano.2008.35)
 22. Esmadi F, Simm J. 1995 Sorption of cobalt(II) by amorphous ferric hydroxide. *Colloids Surf. A* **104**, 265–270. (doi:10.1016/0927-7757(95)03289-4)
 23. Shannon RD, Prewitt CT. 1969 Effective ionic radii in oxides and fluorides. *Acta Crystallogr. B* **25**, 925–946. (doi:10.1107/S0567740869003220)
 24. Bernal JD, Dasgupta DR, Mackay AL. 1959 The oxides and hydroxides of iron and their structural inter-relationships. *Clay Miner. Bull.* **4**, 15–30. (doi:10.1180/claymin.1959.004.21.02)
 25. Chaves LHG, Curry JE, Stone DA, Carducci MD, Chorover J. 2009 Nickel incorporation in Fe(II, III) hydroxysulfate green rust: effect on crystal lattice spacing and oxidation products. *Rev. Bras. Cienc. Solo* **33**, 1115–1123. (doi:10.1590/S0100-06832009000500005)
 26. Refait P, Abdelmoula M, Trolard F, Génin JMR, Ehrhardt JJ, Bourrié G. 2001 Mössbauer and XAS study of a green rust mineral; the partial substitution of Fe²⁺ by Mg²⁺. *Am. Miner.* **86**, 731–739.
 27. Byrne JM, Telling ND, Coker VS, Patrick RAD, van der Laan G, Arenholz E, Tuna F, Lloyd JR. 2011 Control of nanoparticle size, reactivity and magnetic properties during the bioproduction of magnetite by *Geobacter sulfurreducens*. *Nanotechnology* **22**, 455709. (doi:10.1088/0957-4484/22/45/455709)
 28. Craik DJ. 1975 *Intrinsic and technical magnetic properties of oxides*, pp. 1–96. London, UK: Wiley.
 29. López JL, Pfannes HD, Paniago R, Sinnecker JP, Novak MA. 2008 Investigation of the static and dynamic magnetic properties of CoFe₂O₄ nanoparticles. *J. Magn. Magn. Mater.* **320**, e327–e330. (doi:10.1016/j.jmmm.2008.02.065)
 30. Alphandéry E, Carvallo C, Menguy N, Chebbi I. 2011 Chains of cobalt doped magnetosomes extracted from AMB-1 magnetotactic bacteria for application in alternative magnetic field cancer therapy. *J. Phys. Chem. C* **115**, 11 920–11 924. (doi:10.1021/jp201274g)
 31. Alphandéry E, Guyot F, Chebbi I. 2012 Preparation of chains of magnetosomes, isolated from *Magnetospirillum magneticum* strain AMB-1 magnetotactic bacteria, yielding efficient treatment of tumors using magnetic hyperthermia. *Int. J. Pharm.* **434**, 444–452. (doi:10.1016/j.ijpharm.2012.06.015)
 32. Hergt R, Hiergeist R, Zeisberger M, Schüller D, Heyen U, Hilger I, Kaiser WA. 2005 Magnetic properties of bacterial magnetosomes as potential diagnostic and therapeutic tools. *J. Magn. Magn. Mater.* **293**, 80–86. (doi:10.1016/j.jmmm.2005.01.047)
 33. Gunnar G, Rudolf H, Matthias Z, Silvio D, Stefan N, Werner W. 2006 The effect of field parameters, nanoparticle properties and immobilization on the specific heating power in magnetic particle hyperthermia. *J. Phys. Condens. Matter* **18**, S2935. (doi:10.1088/0953-8984/18/38/S27)
 34. Liu C, Zou B, Rondinone AJ, Zhang ZJ. 2000 Chemical control of superparamagnetic properties of magnesium and cobalt spinel ferrite nanoparticles through atomic level magnetic couplings. *J. Am. Chem. Soc.* **122**, 6263–6267. (doi:10.1021/ja000784g)
 35. Sun S, Zeng H, Robinson DB, Raoux S, Rice PM, Wang SX, Li G. 2003 Monodisperse MFe₂O₄ (M = Fe, Co, Mn) nanoparticles. *J. Am. Chem. Soc.* **126**, 273–279. (doi:10.1021/ja0380852)
 36. Brotton SJ, Shapiro R, van der Laan G, Guo J, Glans P-A, Ajello JM. 2007 Valence state fossils in proterozoic stromatolites by L-edge X-ray absorption spectroscopy. *J. Geophys. Res.* **112**, G03004. (doi:10.1029/2006JG000185)
 37. van der Laan G, Kirkman IW. 1992 The 2p absorption spectra of 3d transition metal compounds in tetrahedral and octahedral symmetry. *J. Phys. Condens. Matter* **4**, 4189–4204. (doi:10.1088/0953-8984/4/16/019)
 38. van der laan G, Thole BT. 1991 Strong magnetic X-ray dichroism in 2p absorption spectra of 3d transition-metal ions. *Phys. Rev. B* **43**, 13 401–13 411. (doi:10.1103/PhysRevB.43.13401)
 39. Patrick RAD, van der Laan G, Henderson CMB, Kuiper P, Dudzik E, Vaughan DJ. 2002 Cation site occupancy in spinel ferrites studied by X-ray magnetic circular dichroism: developing a method for mineralogists. *Eur. J. Miner.* **14**, 1095–1102. (doi:10.1127/0935-1221/2002/0014-1095)
 40. Islam FS, Gault AG, Boothman C, Polya DA, Charnock JM, Chatterjee D, Lloyd JR. 2004 Role of metal-reducing bacteria in arsenic release from Bengal delta sediments. *Nature* **430**, 68–71. (doi:10.1038/nature02638)
 41. Islam FS, Pederick RL, Gault AG, Adams LK, Polya DA, Charnock JM, Lloyd JR. 2005 Interactions between the Fe(III)-reducing bacterium *Geobacter sulfurreducens* and arsenate, and capture of the metalloid by biogenic Fe(II). *Appl. Environ. Microbiol.* **71**, 8642–8648. (doi:10.1128/AEM.71.12.8642-8648.2005)
 42. Ahmed IAM, Benning LG, Kakonyi G, Sumoondur AD, Terrill NJ, Shaw S. 2010 Formation of green rust sulfate: a combined in situ time-resolved x-ray scattering and electrochemical study. *Langmuir* **26**, 6593–6603. (doi:10.1021/la903935j)
 43. Daniels JM, Rosencwaig A. 1969 Mössbauer spectroscopy of stoichiometric and non-stoichiometric magnetite. *J. Phys. Chem. Solids* **30**, 1561–1571. (doi:10.1016/0022-3697(69)90217-0)
 44. Franke H, Rosenberg M. 1976 Mössbauer effect and charge transfer in cobalt–iron ferrites. *J. Magn. Magn. Mater.* **4**, 186–192. (doi:10.1016/0304-8853(77)90032-4)
 45. Murad E. 1987 Mössbauer spectra of nontronites: structural implications and characterization of associated iron oxides. *Z. Pflanzen. Bodenk.* **150**, 279–285. (doi:10.1002/jpln.19871500503)
 46. Murad E. 2010 Mössbauer spectroscopy of clays, soils and their mineral constituents. *Clay Miner.* **45**, 413–430. (doi:10.1180/claymin.2010.045.4.413)
 47. Stookey LL. 1970 Ferrozine: a new spectrophotometric reagent for iron. *Anal. Chem.* **42**, 779–781. (doi:10.1021/ac60289a016)
 48. Lovley DR, Phillips EJP. 1988 Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. *Appl. Environ. Microbiol.* **54**, 1472–1480.
 49. Patterson AL. 1939 The Scherrer formula for X-ray particle size determination. *Phys. Rev.* **56**, 978–982. (doi:10.1103/PhysRev.56.978)
 50. Scherrer P. 1918 Estimation of size and internal structure of colloidal particles by means of Röntgen rays. *Nachr. Ges. Wiss. Göttingen* **2**, 96–100.
 51. Campelj S, Makovec D, Drofenik M. 2008 Preparation and properties of water-based magnetic fluids. *J. Phys. Condens. Matter* **20**, 204101. (doi:10.1088/0953-8984/20/20/204101)