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Probing the site occupancies of Co, Ni and Mn substituted biogenic magnetite using XAS and XMCD

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Abstract

Ferrimagnetic nanoparticles have many uses in industry including in magnetic recording media and transformers, however these particles are often expensive to synthesise. In this study the Fe(III)-reducing bacteria *Geobacter sulfurreducens* and *Shewanella oneidensis* were used to synthesise spinel ferrite nanoparticles of the general chemical formula $M_x\text{Fe}_{3-x}\text{O}_4$, where M is either Co, Ni, Mn, Zn or a combination of Mn and Zn. This was done at ambient temperatures through the dissimilatory reduction of Fe(III)-oxyhydroxides containing the appropriate substitutional cations. A combination of L -edge and K -edge X-ray absorption spectroscopy (XAS) and L -edge X-ray magnetic circular dichroism (XMCD) was used to determine the site occupancies, valence and local structure of the Fe and substitutional cations within the spinels. The Ni and Co ferrites produced using each bacterium were very similar and therefore this study concludes that despite the difference in reduction mechanism of the bacteria used in this study the end-product is remarkably unaltered. Ni ferrites contained only Ni(II), with at least 80% in O_h coordination. Co ferrites contained only Co(II) but with a significant proportion (up to 45%) in T_d coordination, showing a slight preference for T_d sites. Mn-ferrites contained Mn(II) only on the O_h sites but a mixture of Mn(II) and Mn(III) on T_d sites when the amount of Mn exceeded 3% (cf. to the amount of Fe) or some Zn was also present. This study successfully produced a range of nanoparticulate ferrites that could be produced industrially using relatively environmentally benign methodologies.

Introduction

Extracellular nano-sized magnetic particles of magnetite (Fe_3O_4) are produced by *Geobacter sulfurreducens* and *Shewanella oneidensis*, which are Fe(III)-reducing bacteria capable of respiring using amorphous Fe(III) oxides as electron acceptors in anoxic environments (Caccavo Jr et al., 1994). This study demonstrates the capability of these bacteria not only to produce end-member magnetite but also to include the metals (M) Co, Ni, Mn and Zn within the magnetite structure thus forming nano-spinel ferrites with the chemical formula $M_x\text{Fe}_{3-x}\text{O}_4$ (Coker et al., 2004). The capability of these bacteria to produce nanomagnets using energy efficient methodologies has yet been explored by industry even though these particles have electrical, magnetic, and structural properties that differ from coarser grained materials (Jolivet et al., 2002). As ferrite spinels, and especially magnetite-like compounds, are among the most important ferrimagnetic materials for industrial applications, including data storage and as ferrofluids for use in heat transfer, computer systems or levitation of objects, they have great commercial potential

(Jolivet et al., 2002; Safarik and Safarikova, 2002). The magnetoresistive properties of spinels have also been of recent interest in the area of spin electronics (Coey et al., 1998) and half-metallic spin injectors (Lu et al., 2004).

The development of technological applications for bio-mineral nanomagnets produced by bacteria has concentrated largely on magnetotactic bacteria, which produce membrane-bound intracellular nano-magnetite crystals. This group of bacteria are of interest as they can produce cubic magnetite particles with very well constrained particle size and shape (Yeary et al., 2005). The drawback of magnetotactic bacteria is that they are difficult to culture in large quantities and magnetite yields can be relatively low, whereas extracellular manufacture of magnetite can produce large amounts of the bio-mineral. However, the formation of metal-substituted magnetites by bacteria by either intra or extracellular processes has been little studied with some notable exceptions; the thermophilic extracellular Fe(III)-reducer *Thermoanaerobacter ethanolicus* TOR-39 has been shown to produce Cr, Co and Ni-substituted magnetites (Roh et al., 2001) and *S. oneidensis* CN32 has been shown to produce Ni-substituted magnetite (Fredrickson et al., 2001). The structure of these substituted spinels has not been studied in detail, however.

Ferrite spinels. Magnetite has a cubic spinel structure with one quarter of the tetrahedral (T_d) and one half of the octahedral (O_h) sites filled by iron. The formula for magnetite $(Fe^{3+})[Fe^{2+}Fe^{3+}]O_4^{2-}$ where Fe(III) is equally split between (T_d) and [O_h] sites. Fe(II) occupies only O_h sites resulting in a distribution for stoichiometric magnetite of 1 : 1 : 1 at the Fe d^6 O_h : d^5 T_d : d^5 O_h sites. The Fe(III) in the O_h and T_d sublattices contain cations with antiparallel magnetic moments that compensate each other, but there is a resulting net magnetisation (ferrimagnetism) due to the Fe(II) on the O_h sites.

Cations other than Fe can substitute into the spinel ferrite structure to form spinels with the general chemical formula $M_xFe_{3-x}O_4$, where M is a transition metal cation. The percentage of T_d occupied by $M+Fe(III)$ cations defines the degree of ‘inversion’ of the spinel, such that 100% occupation of the T_d sites by $M+Fe(II)$ species corresponds to a so called ‘normal’ spinel whereas 100% T_d occupancy by $M+Fe(III)$ species defines an ‘inverse’ spinel, such as in magnetite. Spinel ferrites can contain divalent Ni, Co and Zn, trivalent Cr and V, and tetravalent Ti (see e.g. Hill *et al.*, 1979; Waychunas, 1991 for reviews). Crystal field stabilization, oxidation state and ionic radius effects result in the general preference of Ni(II), Co(II), Mg(II) and Fe(II) for the O_h sites, while Zn(II) has a very strong affinity for the T_d sites (see Pattrick et al., 2002 and references therein). The addition of these different cations into the magnetite will change the physical and magnetic properties of the nano-particles making them suitable for customising for a range of different technological applications. An understanding of the substitution and site occupancies is a vital first step in developing these bio-mineral nanomagnets.

Numerous analytical techniques have been used previously to identify the structure and chemical nature of ferrite spinels (Henderson et al., 2007; Pearce et al., 2006; Waychunas, 1991). This paper uses the complementary X-ray absorption methods EXAFS (extended x-ray absorption fine-structure) and XMCD (X-ray magnetic circular dichroism) as collectively these techniques give element specific information on the crystal structure and valence of all the elements used.

Materials and Methods

Previous studies of abiotic synthesis of spinel ferrites from ferrihydrite have demonstrated that more metal cations are substituted into magnetite by coprecipitation of the metal-oxides than by addition of the substituting metal cations after conversion of ferrihydrite to magnetite (Cornell and Schwertmann, 2003). Therefore, in this study, the Fe(III) hydroxide starting materials were made incorporating the substituting transition metals, prior to bacterial reduction .

Hydroxide Formation. Synthetic amorphous Fe(III) oxyhydroxide was produced using the method of Lovley and Philips (1986), where a 0.4 M solution of FeCl₃ is neutralised by 10 N NaOH to pH 7 and the solid then washed until no Cl⁻ ions remain. In this study the additional metal chlorides of either Co, Ni, Mn, Zn or Mn and Zn were added, so that co-precipitates of Fe + *M*-oxyhydroxides formed. Final concentrations of the cations in the hydroxides were determined using ICP-OES (VG Elemental, Winsford, UK) with a 1 m Czerny-Turner spectrometer with a 2400 lines/mm diffraction grating. The material was also characterised using X-ray diffraction to confirm that poorly-crystalline material had formed.

Metal reduction. *G. sulfurreducens* was obtained from our laboratory culture collection and grown under strictly anaerobic conditions at 30°C in modified fresh water medium as described previously (Lloyd et al., 2003). Sodium acetate (20 mM) and fumarate (40 mM) were provided as the electron donor and acceptor, respectively. All manipulations were done under an atmosphere of N₂-CO₂ (80:20). *S. oneidensis* was also obtained from laboratory cultures and grown under anaerobic conditions in ferric citrate media (Lovley and Phillips, 1988). Sodium lactate (20 mM) and ferric citrate (56 mM) were provided as the electron donor and acceptor, respectively.

Late log-phase cultures of *G. sulfurreducens* and *S. oneidensis* were harvested by centrifugation at 4920 g for 20 minutes and washed twice in bicarbonate buffer (NaHCO₃; 30 mM, pH 7.1) under N₂-CO₂ (80:20) gas prior to use. Aliquots of the washed cell suspension (1.5 ml) were added to sealed anaerobic bottles containing 28.5 ml bicarbonate buffer. The final concentration of bacteria was approximately 0.2 mg protein per mL. The following additions were made from anaerobic stocks as required; a Fe(III) oxyhydroxide suspension (10 mmol L⁻¹), sodium acetate (10 mM), sodium lactate (10 mM) and anthraquinone 2,6-disulphonate (10 µM). Bottles were incubated in the dark at 20°C. 100 µl of slurry was removed from each shaken serum bottle periodically, using aseptic and anaerobic technique and analysed for 0.5 M HCl-extractable Fe(II) employing the ferrozine method (Lovley and Phillips, 1986). All experiments, including abiotic controls, were done in triplicate and representative data shown throughout. Standard errors were within ±10% of the mean.

Standards. The chemical magnetite analysed in this study as a comparison to biogenic magnetite was Fe₃O₄ 20-30nm APS Powder, with a surface area >60m²/g (Alfa Aesar Cas. no. 1317-61-9). Synthetic samples of NiFe₂O₄ and CoFe₂O₄ were prepared by mixing stoichiometric amounts of the appropriate high purity metal oxides (Fe₂O₃, NiO or CoO) and heating in a muffle furnace for 100 h at 1050 °C. Each sample was reground after 48 h to help to homogenize the reaction mixture.

Electron microscopy. Samples were washed twice in deionised water to remove cells and buffer solution. Aliquots of the sample suspension were then dropped on carbon coated copper grids and left to dry prior to introduction to the microscope. Imaging of the samples was done using

superSTEM at Daresbury Laboratory on a VG HB501 dedicated scanning transition electron microscope (STEM) fitted with a Nion second generation spherical aberration corrector where bright field (BF) high angle annular dark field (HAADF) images were collected giving good contrast for imaging lattice planes in the spinels.

X-ray Absorption Spectroscopy. X-ray absorption spectra were collected for the Fe, Co, Mn, Zn, Co and Ni *K* edges on beamline 7.1 at the Synchrotron Radiation Source (SRS), Daresbury Laboratory. This beamline is equipped with a sagittally focusing double crystal Si(111) monochromator set at 70% harmonic rejection detune, dynamical focussed using active servos. Data were collected at 77 K in fluorescence mode using a 9 element monolithic Ge detector. Standards of cobalt oxide and nickel oxide were collected in transmission mode.

Background subtracted EXAFS spectra were analyzed in EXCURV98 using full-curved-wave theory. Phase shifts were derived in the programs from *ab initio* calculations using Hedin-Lundqvist potentials and von Barth ground states. Fourier transforms of the EXAFS spectra were used to obtain an approximate radial distribution function around the central absorber atom. The peaks of the Fourier transform can be related to shells of surrounding back scattering atoms characterized by atom type, number of atoms in the shell, the absorber-scatterer distance and the Debye-Waller factor, $2\sigma^2$ (measure of both the thermal motion between the absorber and scatterer, and of the static disorder or range of absorber-scatterer distances). The data were fitted for each sample by defining a theoretical model and comparing the calculated EXAFS spectrum with the experimental data. Shells of backscatterers were added around the absorber atom and by refining an energy correction E_F (the Fermi energy), the absorber-scatterer distance and Debye-Waller factor for each shell, a least squares residual (the *R*-factor) was minimized.

X-ray Magnetic Circular Dichroism. X-ray absorption spectra were collected on SRS beamlines 1.1 and 5U.1 using the Flipper electromagnet endstation (Dudzic et al., 2000), on beamline 4.0.2 at the Advanced Light Source (ALS), Berkeley, CA, using the octopole magnet endstation (Arenholz and Prestemon, 2005), and on beamline ID08 at the European Synchrotron Radiation Source (ESRF), Grenoble, France using the octopole magnet endstation (Telling, 2007). Samples were prepared by washing aliquots of the suspension in deionised water before drying under anaerobic conditions. The powders were then mounted in an anaerobic cabinet on carbon tape attached to the sample manipulator. Samples were kept anaerobic when required by saturating the manipulator in a stream of N₂ gas during insertion into the vacuum chamber. The specimen was positioned central to the magnetic pole pieces, with the X-ray beam entering through the narrow centred hole in one of the pole pieces, parallel to the magnetic field and perpendicular to the sample surface. XAS was monitored in total-electron yield mode, which gives an effective probing depth of ~4.5 nm. At each energy point the XAS was measured for the two opposite magnetisation directions by reversing the applied field of 0.6 T. The XAS spectra of the two magnetisation directions were normalised to the incident beam intensity and subtracted from each other to give the XMCD spectrum (Patrick et al., 2002).

The measured Fe, Co, Ni and Mn $L_{2,3}$ XMCD was used to obtain the site occupancies and oxidation state of the cations in the spinel structure of the magnetite. At the transition metal $L_{2,3}$ edge, the $2p$ core electrons are excited by electric-dipole transitions into (partially) localised unoccupied $3d$ states, so that XMCD provides information about the local electronic and magnetic

structure of the absorbing atom (van der Laan and Thole, 1991). The spectrum is split by the $2p$ spin-orbit interaction into two main structures: the L_3 and L_2 edge. For magnetite, the Fe L edge XMCD has three main peaks in the L_3 edge XMCD with negative, positive and negative intensity which are related to the amounts of Fe $d^6 O_h$, $d^5 T_d$ and $d^5 O_h$, respectively, present in the sample (Patrick et al., 2002).

To obtain the relative amounts of the three Fe sites, the experimental spectra were fitted by means of a non-linear least-squares analysis, using calculated spectra for each of the Fe sites (Fig. 1). In these calculations, as described in (van der Laan and Thole, 1991), the Hartree-Fock Slater integrals for the $3d-3d$ and $2p-3d$ Coulomb and exchange interactions were scaled to 70% and 80%, respectively, and the crystal fields for the O_h and T_d sites were taken to be $10Dq = 1.2$ eV and -0.6 eV, respectively. The calculated spectra were convoluted by a Lorentzian of $\Gamma = 0.3$ (0.5) eV for the L_3 (L_2) edge to account for intrinsic core-hole lifetime broadening and by a Gaussian of $\sigma = 0.5$, 0.35 or 0.2 eV for the data collected at SRS 5U.1, SRS 1.1 or ALS 4.0.2/ESRF ID08, respectively, to account for instrumental broadening. The experimental spectra were fitted over the L_3 main peaks only, which has previously been shown to give meaningful results, although fitting over the L_2 peak does give good qualitative agreement (Patrick et al., 2002; Pearce et al., 2006).

To calculate the errors in the fitting of the XMCD spectra the same sample of bio-magnetite was measured on the three different beamlines used and then the spectra were fitted using the methods above. By taking the standard deviation of the site occupancies according to the three different spectra the error in each of the individual sites was found to be 1.10 ± 0.02 , 0.99 ± 0.01 and 0.98 ± 0.02 for the $d^6 O_h$, $d^5 T_d$ and $d^5 O_h$, respectively. The error in the vacancies, which indicate the degree of departure from stoichiometric magnetite, was found to be ± 0.01 . Biogenic magnetite produced by each of the bacteria had previously been measured by XMCD and compared to chemically produced magnetite (Coker et al., 2007); these data are used here.

Results

Formation of magnetite from Fe(III) oxyhydroxides

Amorphous Fe(III) oxyhydroxides containing Co(II), Ni(II), Mn(II) and Zn(II) were produced (see Table 1). X-ray diffraction (XRD) was used to confirm that each of the starting materials in Table 1 were poorly-crystalline during formation and diffraction traces showed only two broad humps with d -spacings of 2.51 and 1.46 Å (data not shown), which is consistent with the formation of 2-line ferrihydrite, a poorly ordered Fe(III) hydroxide (Cornell and Schwertmann, 2003).

Magnetite was produced by adding cells of *G. sulfurreducens* to preparations of the starting materials listed in Table 1 and in addition by adding *S. oneidensis* to the Co- and Ni-containing Fe-oxyhydroxides. Fig. 2 shows total Fe(II) accumulation in the microbial cultures. All the cultures supplemented with bacteria and an electron donor, with or without an electron shuttle showed formation of a black magnetic precipitate from the orange non-magnetic starting material, except for the 16 at% Zn sample where the increased amount of Zn compared to the 4 at% Zn sample inhibited Fe(III)-reduction. In addition, for *G. sulfurreducens* cultures containing Ni, autoclaving the ferric iron starting material (121 °C, 103 kPa) prior to inoculation with *G. sulfurreducens* was required for a magnetic precipitate to form, otherwise a non-magnetic green rust formed (confirmed by XRD). For each set of cultures, Fe(III)-reduction occurred at an increased rate with the addition

of the electron shuttle, AQDS, consistent with previous results (Fredrickson et al., 2001; Lovley et al., 1996); in the absence of bacteria no Fe(III) reduction was recorded (Fig. 2). Cultures containing the 1 at% Mn samples displayed increased Fe(II) production rates compared to those containing the 6 at% Mn samples. Whereas an increase in the amount of Ni or Co in cultures caused an increase in the rate of reduction and the total amount of Fe(III) reduced for both *G. sulfurreducens* and *S. oneidensis*. This was more apparent in cultures supplemented by AQDS (Fig. 2).

The final product of the reduction process, after cessation of Fe(II) production (Fig. 2; after approximately 200 hrs of incubation), was imaged using superSTEM and examples of *G. sulfurreducens* bio-reduced 12 at% Co and 6.5 at% Ni samples are shown in Fig. 3. The images show the nanoparticulate nature of the products with a size range of 20 to 30 nm and the crystalline nature of the material as demonstrated by the lattice planes which are particularly well defined in the 12 at% Co sample. The lattice images also show the nanoparticles to be single crystals that are clumped, due to their magnetic nature. The development of monodispersed particles would require a capping agent.

The local structural environment of both the Fe and the other transition metals within the magnetite was investigated in all samples using EXAFS, which gives the relative amounts of each in tetrahedral (T_d) and octahedral (O_h) coordination within the spinel structure (Table 2). Figs. 4 and 5 show the EXAFS and corresponding radial distribution function together with best-fit curves for each of the spectra. To complement the EXAFS, the *L* edge XAS and associated XMCD were collected and used to allocate the relative amounts of the different oxidation states of the Fe and the dopant cations within the structure. Figs. 6-11 and Table 3 show these results.

The EXAFS spectra revealed the non-substituted magnetite produced by *G. sulfurreducens* to have 35% Fe cations in tetrahedral sites at a distance of 1.82 Å and 65% in octahedral coordination at a distance of 2.04 Å, within error of the values for chemically precipitated magnetite (Table 2) and consistent with values expected for stoichiometric chemically produced Fe_3O_4 (Finger et al., 1986). From the XMCD data in Table 3, the site occupancy data for pure biogenic magnetite produced by *G. sulfurreducens* and *S. oneidensis* showed a small excess of between 0.01-0.04 Fe cations present as Fe(II) d^6 O_h , as previously reported in (Coker et al., 2007).

Bio-spinel production using Co and Ni containing ferrihydrite was examined using both *G. sulfurreducens* and *S. oneidensis*. Results using EXAFS and XMCD were from cultures not supplemented by AQDS only, as preliminary results showed no difference in the final product despite a variation in the Fe(II) production rate (Fig. 2).

Ni-substituted bio ferrites

Ni-edge. Ni K-edge EXAFS data on bio-spinels produced by *G. sulfurreducens* and *S. oneidensis* from 2 and 6.5 at% Ni samples reveal similar distribution ratios of the Ni in the spinel structure (Fig. 4 and Table 2) of 20% T_d and 80% O_h . The Ni-O distance for the O_h site was similar to the Fe-O O_h distance but the Ni-O T_d distance of ca 1.87 Å was much smaller than that for Fe-O (1.99 Å) in the same site. A similar pattern was seen in the synthetic $NiFe_2O_4$ but with a slightly different Ni distribution of 12.4% T_d and 87.5% O_h , but identical average Ni-O bond lengths of 1.87 Å for T_d sites and 2.03 Å for O_h sites. The short Ni-O distances are characteristic of Ni in tetrahedral coordination and has been recorded for other Ni-spinels (Ma, 1975).

In Fig. 6 representative data for the Ni L edge of a bio-spinel is shown for the 6.5 at% Ni sample produced by *G. sulfurreducens* and it shows the characteristics common to all the biospinel Ni L edge spectra. The XAS has a main L_3 peak with a small peak adjoining at high energy and the L_2 peak shows a doublet structure (van der Laan et al., 1986). The resulting XMCD has a large negative peak at the L_3 edge escorted by a smaller positive peak and an asymmetric doublet at the L_2 edge (van der Laan et al., 1999). The XAS and XMCD can be fitted for Ni(II) using a crystal field atomic multiplet model (van der Laan and Kirkman, 1992; van der Laan and Thole, 1991). The calculated XAS and XMCD spectra for Ni d^8 O_h with a crystal field splitting of $10Dq = 1.2$ eV provide a good fit to the experimental data (Fig. 6(a)). The spectral shape means that the presence of Ni (III) d^7 can be discounted (van der Laan and Kirkman, 1992).

However, the nature of the spectral shape means the sample might contain small amounts of Ni(II) in T_d sites, and this is explored further here. Fig. 6(b) shows the calculated spectrum over the $L_{3,2}$ edge for Ni(II) with a tetrahedral crystal field of $10Dq = -0.6$ eV. The L_3 main peak of the XAS is slightly broader than for O_h because it consists of a doublet structure with an unresolved splitting of 0.5 eV and the 2 eV wide satellite of the L_3 XAS is lower than for O_h symmetry; the L_2 XAS for T_d also consists of a single peak, instead of a doublet for O_h . The calculated XMCD is shown using the same intensity scale for both sites. Although the integrated intensity of the XMCD for T_d is actually larger than for O_h , the peak intensity at the L_3 maximum is only a third of that of O_h . Therefore, the spectral features of the XAS and XMCD make it difficult to fit the precise amounts of O_h and T_d in the experimental spectrum, and a small amount of Ni(II) T_d can go unnoticed due to this low XMCD peak amplitude. Nonetheless, these data provide no conclusive evidence of Ni(II) T_d and in general agreement of the EXAFS assignment of at the very least, the majority of the Ni cations to O_h sites.

Fe edge. The Fe EXAFS data (Fig. 5, Table 2) indicate that between 40-50% of the Fe cations in biogenic Ni-ferrites are T_d coordinated reflecting the marked decrease in Fe occupation of O_h sites compared to Fe_3O_4 . The XMCD spectra in Fig. 7 with corresponding data analyses in Table 3 suggest slightly lower values for Fe T_d occupation of between 34-37% of the total Fe cations. However, these values still shows decreased iron occupation of O_h sites. The synthetic Ni-ferrite, from both EXAFS and XMCD data, shows 45% Fe in T_d sites, which is a similar to the biogenic spinels.

Comparison of the Fe cation site occupancy derived from the XMCD spectrum of the 2 at% Ni sample (Table 3) with standard bacterial Fe_3O_4 for both bacteria shows a large decrease in the Fe(II) d^6 O_h site and a corresponding increase in the Fe(III) d^5 O_h resulting in only a small change in the T_d/O_h ratio compared to stoichiometric magnetite. In the 6.5 at% Ni sample the amount of Fe(II) d^6 O_h decreases further (reflected in the experimental spectra by the first negative peak becoming smaller (Fig. 7), with a corresponding decrease in Fe(III) d^5 T_d .

Coker et al (2007) noted that a range of un-substituted bacterial magnetites produced by *G. sulfurreducens*, *S. oneidensis* and *Geothrix fermentans* displayed a cation (Fe) excess. However, biogenic Ni ferrites have cation-vacancies of between 0.06-0.10 for the lower concentration Ni sample and 0.02-0.06 for the higher concentration Ni sample (Table 3). The chemically synthesised

NiFe₂O₄, in which a third of the cations are Ni has a cation excess, most likely from the higher Ni content of the synthetic samples.

Co-substituted bio ferrites

Co edge. K-edge EXAFS of the four Co-bearing bio-ferrites (Fig. 4 and Table 2) revealed each sample to contain between 35-45 % Co in T_d coordination, displaying a slight preference of Co for the T_d sites. The Co-O bond lengths for the T_d sites (1.90-1.92 Å) and O_h sites (2.04-2.09 Å) are slightly longer than the corresponding Fe-O bonds of 1.78-1.88 Å for T_d and 1.98-1.99 Å for O_h sites. The Fe-O and Co-O O_h bond distances for chemically produced CoFe₂O₄ at 1.93 Å and 2.02 Å, are shorter than for the biogenic materials although the Co-O T_d bond distances are similar while the chemically produced Fe-O T_d bond is longer, at 1.95 Å (Table 2).

Representative Co *L* edge data from the 12 at% Co sample produced by *G. sulfurreducens* is shown in Fig. 8; all Co-samples produced similar data. The *L*-edge data was fitted, as for Ni, using a crystal field atomic multiplet calculation (van der Laan and Kirkman, 1992; van der Laan and Thole, 1991) for the Co(II) *d*⁷ O_h. The calculations with a crystal field splitting of 10*Dq* = 1.2 eV for the isotropic XAS and the XMCD spectra reveal a good fit to the experimental data in Fig. 8. For comparison, the same spectra from a thin film standard sample of CoFe₂O₄ (van der Laan et al., 2007), is also presented as this is representative of a sample produced to contain only Co(II) *d*⁷ O_h. The thin film and biogenic Co ferrite spectra show good agreement and the Co(II) *d*⁷ O_h calculated spectra accounts for most of the features of the two experimental spectra, although the fit is not perfect. The main difference is that the two sets of experimental spectra have a slightly different spectral shape in both the XAS and XMCD at the high-energy side of the *L*₃ edge and at the *L*₂ edges. This component might be Co in either T_d coordination, as the EXAFS in Table 2 suggests or Co(III). To examine this, Fig. 8 shows the calculation for Co(II) in a tetrahedral crystal field of 10*Dq* = -0.6 eV. The main difference with O_h symmetry is that the *L*₃ peak is much narrower, and lacks the sharp peaks at the low and high energy sides. The same is seen in the calculated XMCD for T_d symmetry; only the middle peak is present. Thus O_h and T_d give very different spectra, and if there is any contribution of Co(II) T_d it must be small. The spectral fitting at the *L* edge demonstrates that the vast majority is Co(II) O_h.

Fe edge. Analysis of the EXAFS spectra (Fig. 5 and Table 2) allocates between 25-30% Fe to T_d sites compared to a higher value of 36-39% from the analyses of the XMCD results (Fig. 9 and Table 3). The data are similar for the different bacteria and the different concentration of dopant. Comparing bacterial un-substituted biogenic Fe₃O₄ and the 12 at% Co samples, the most marked decrease in Fe cation occupancy was in the Fe(II) O_h sites from 1.03-1.13 to 0.63-0.68 depending on the bacteria examined; this is similar to the samples containing Ni. This decrease is offset by an increase in the occupancy of the Fe(III) O_h, maintaining a reasonably constant T_d/O_h ratio during incorporation of Co into the spinel structure. As would be predicted the 12 at% Co sample showed a decrease in Fe cation occupancy in all three different Fe cation structural environments compared to that of the 2 at% Co sample, for both of the Fe(III)-reducing bacteria..

The vacancies in the Co ferrites followed a similar pattern to the Ni samples. For the 2 at% Co samples the vacancies were between 0.07-0.08 but this number decreased to become an excess of cations for 12 at% Co samples. Biogenic 12 at% Co samples had an Fe:Co ratio of ~ 3:1, nearly as much as the chemically synthesised analogue which also displays a cation excess .

Zn and Mn-doped bio-ferrites

Zn-edge. Only EXAFS data is available (Fig. 4 and Table 2) as Zn has a filled d^{10} shell, which gives no XMCD signal. The data for 4 at% Zn and 8 at% Zn/2 at% Mn samples are in agreement as both allocate 80% of the Zn cations to the T_d sites. The Zn-O bond distances are 1.99 Å and 2.12 Å for T_d and O_h sites respectively in the 4 at% Zn sample, which is longer than the equivalent Fe-O distances of 1.83 Å (T_d) and 2.04 Å (O_h) for un-substituted magnetite. In the sample containing both Zn and Mn, the Zn-O bond length is 1.94 Å in T_d sites and 2.03 Å in O_h coordination.

Mn-edge. The distribution and oxidation state of Mn cations in 1 at% Mn, 6 at% Mn and 2 at% Mn/8 at% Zn samples were measured using Mn L edge XAS and the data fitted using calculated curves for Mn d^4 and d^5 configuration in T_d and O_h symmetry. These curves were again calculated using the crystal field atomic multiplet model described in van der Laan and Thole (1991) and van der Laan and Kirkman (1992) and are displayed in Fig. 10 along with the experimental spectra for the three samples. In the calculation, crystal field splitting of $10Dq = -0.6$ and 1.0 eV were used for the T_d and O_h sites, respectively (Table 4). The measured spectra for 1.5 and 6 at% Mn samples are similar; both have a low energy shoulder on the L_3 edge that corresponds well to the calculated Mn(II) d^5 O_h spectrum. However, this spectrum alone does not account for all the features of the experimental spectra for these two samples. The spectra for the sample containing 1 at% Mn has a best-fit curve that includes a significant component of Mn(II) d^5 T_d so that the amount of Mn(II) in each site is approximately equal. The spectra for the sample containing 6 at% Mn also requires a contribution from the Mn(II) d^5 T_d calculated spectra, but to account for an intensity deficit on the high energy sites of the L_3 , a small amount of Mn(III) d^4 O_h also improves the fit. This is not a unique solution, as a small addition of the Mn(III) d^4 T_d calculated spectrum can also account for this deficit but as (O'Handley, 2000) revealed Mn(III) has a strong preference for O_h coordination in spinels, Mn(III) O_h is included in the fit here. The Mn L edge XAS spectral shape for the 2 at% Mn/8 at% Zn sample is quite different to the samples containing only Mn. However, to fit this spectrum a combination of Mn(II) d^5 O_h and T_d is again required to account for the low energy shoulder on the L_3 edge and the main peak. However, the large high energy secondary peak is accounted for by a significant amount of Mn(III) d^4 O_h . These data for Mn XMCD are summarised in Table 4 and in all the three samples the Mn shows a preference for the T_d sites, as more than 33% was present in T_d coordination.

The results from fitting the EXAFS Mn K edge data in Fig. 4 are shown in Table 2. These indicate a much higher preference for the T_d sites of 60-67.5% Mn than the L edge data. These data also give the Mn-O bond lengths for both sites as 2.07-2.10 Å for T_d sites and 2.16-2.21 Å for O_h sites, longer than the corresponding Fe-O bond lengths of 1.85-1.88 Å for T_d sites and 2.01-2.03 Å for O_h sites (Table 2).

Fe edge. The Fe edge XMCD data for the three Mn bio-ferrites with calculated curve fitting are shown in Fig. 11, and the site occupancy data for the Fe cations that are a result of the curve fitting are shown in Table 3. These data indicate that each of the bio-spinels contains an excess of cations as totals exceed 3, and the number of vacancies range from -0.05 to -0.08. These data show that the percentage of Fe in T_d and O_h does not change between the three Mn-containing samples reflected by a similar distribution of Fe between the sites as in stoichiometric magnetite. The

amount of Fe(II) compared to Fe(III) does, however, decrease with increasing Mn concentration and is lowest in the 2 at% Mn/8 at% Zn sample. EXAFS data in Fig. 4 and Table 2 shows good agreement with the XMCD data above, with the Fe cations occupying the T_d and O_h in similar ratios.

For the 4 at% Zn sample, the Fe L edge data is similar to the samples containing Mn as there is also an excess of cations (0.06) (Fig. 11). Fe cation distribution between the crystallographic sites is similar to that of stoichiometric magnetite but with a lower Fe(II)/Fe(III) ratio than standard magnetite a result of the reduction in the amount of Fe(II) in the structure.

Discussion

The metal reduction process: Previous work (Fredrickson et al., 2001; Parmar et al., 2001; Roh et al., 2001) on bacterial reduction of Ni-substituted or supplemented ferrihydrites has been undertaken using *S. oneidensis* CN32 or TOR-39 (a thermophile), between 1 and 2.5 mmol Ni(II) per L were used in cultures also containing 45 and 70 mmol Fe(III) per L (Roh et al., 2001). In these studies, incorporation of Ni into magnetite was inferred rather than directly measured and Parmar et al. (2001) noted that the major product was green rust with magnetite and vivianite as minor components. Roh et al. (2001) however were successful in incorporating 6 mM Co into 70 mM magnetite to produce a Co-ferrite, using Fe(III)-reducing bacteria.

In the current investigation the production of Ni, Co, Mn and Zn bioferrites has been confirmed and the production of a ferrite with >12at% Co, at room temperature using either *G. sulfurreducens* or *S. oneidensis* demonstrates that an end-member $CoFe_2O_4$ can be produced by this method. The type and concentration of the substituting metals altered the rate of reduction of ferrihydrite in the cultures. Oxidation of Mn(II) to Mn(III) and possibly Co(II) to Co(III) during the formation of Fe(III) oxides from M^{2+} species has been reported by (Cornell and Schwertmann, 2003), and the same oxidation process is likely to occur during the preparation of ferrihydrite in this study; Zn and Ni will have remained divalent. It is known that *G. sulfurreducens* and *S. oneidensis* are both able to reduce Co(III) and Mn(IV) (Caccavo Jr et al., 1994; Liu et al., 2002; Lovley et al., 2004; Myers and Nealson, 1988) and therefore this process is likely to have occurred coincident with the Fe(III)-reduction in the cultures. In cultures containing 1 at% Mn, a higher rate and final concentration of Fe(II) production was observed than in those containing 6 at% Mn, suggesting *G. sulfurreducens* preferentially reduced Mn. The rate of Fe(III) reduction in the low concentration Co and Ni cultures was similar to that in cultures that only contained Fe cations (data not shown) but reduced in those cultures containing a higher concentration of the substituting cations unless AQDS was present. Previous work on Ni-substituted hydrous ferric oxide (HFO) reduced by *S. oneidensis* CN32 has also suggested that co-precipitated Ni(II) inhibited bioreduction of HFO except when an electron shuttle was present (Fredrickson et al., 2001), as Ni has previously been found to retard the recrystallisation of amorphous Fe(III) oxyhydroxides by stabilising the oxide against dissolution and internal rearrangement which would allow alter the crystal structure (Cornell et al., 1992).

XMCD (L edge) and EXAFS (K edge): In this study two different XAS synchrotron techniques were used to examine the samples and these produced variable degrees of agreement. One reason for these discrepancies could be the different penetration depths at the two edges which could result in an increased contribution from surface atoms at the L -edge. The transition metal L

edges have an energy ca 800 eV and the X-rays only probe the top 4.5 nm of a surface, whereas the energy of the *K*-edges is ca 8 KeV and data is collected from the whole depth of a sample several millimetres thick. The spinel bio-nanoparticles produced are single nanocrystals with an approximate diameter of 25 nm and therefore information is gathered from ca 6000 nm³ of the total volume of a single particle (total ca 8100 nm³) particle or the ‘outer’ 74 % of the particle (assuming the particles are spherical and that the same number of electrons are emitted from each atomic layer). The favoured termination for magnetite crystals are the {111} surfaces and (Lovely et al., 2006) found from high-resolution electron microscopy that the {111} surfaces of magnetite are terminated by an excess of octahedral-coordinated Fe cations. Others suggest (111) terminates with either Fe-O_h or a layer of Fe-T_d or even with only oxygen cations (Jansen et al., 1994) and references therein). Other terminations, such as the (001) direction, end with either Fe-T_d or a combination of Fe-O_h and oxygen (Jansen et al., 1994) and references therein). Using Lovely et al.’s (2006) data 1931 metal atoms would be at the surface of a 25nm particle compared to a total of 334,371 atoms (0.6 % of the total). The 1931 surface atoms will account for 0.6 % and 0.8 % of the atoms measured by the *K* and *L* edge, respectively.

The differences between the *L*-edge and *K*-edge results are shown in Table 5, where the number of cations that each of the techniques assigns to the two crystallographic sites are compared. From Table 5 it can be seen that it is the *K*-edge data that generally give values for total T_d and O_h site occupancy that differ from the expected 33% T_d; 66% O_h found in stoichiometric magnetite, particularly for samples containing Ni where the percentage of cations in T_d coordination varies between 39-49 %. In samples containing Mn, the two different techniques agree to within 2 % and for samples containing Co, the discrepancies between the techniques are small but in the opposite sense to those for the Ni ferrites. The indications are, therefore, that EXAFS is a relatively crude method of determining T_d/O_h site occupancies compared to XMCD unless it is presumed that these nanoparticles have very different ordering to their natural and synthetic counterparts. However, there is no evidence from this study for this. One caveat to this is illustrated by the assignment to Ni(II) T_d where it is impossible to identify or eliminate a small component in the *L* edge and XMCD spectra, whereas the EXAFS clearly identifies its presence.

Substitutional chemistry of the bio-ferrites: Most natural and synthetic Co-ferrites are not 100% inverted as some Co(II) occupies the T_d sites. For instance, the thermal histories during the formation/annealing of synthetic ferrites affects Co site occupancy, and quenching from temperatures of 1200°C can lead to up to 24% of total Co to occupy T_d (Na et al., 1993). Annealing at 1200°C for 5 hours then slowly cooling at 5°C/hour, however, leads to ordering and a fully inverted Co(II)_{O_h} spinel (Na et al., 1993). Both the EXAFS and the Fe XMCD (indirectly) provide evidence of the presence of Co(II) T_d in the bio-ferrites although there is no evidence in these bio-spinels for the presence of Co(III) T_d which has been invoked in previous studies to maintain charge neutrality (De Guire et al., 1989; Na et al., 1993), but is not required for these bio-ferrites which balance without the need for Co(III). The Ni EXAFS also indicate the presence of Ni(II) T_d. Therefore the Co and Ni bio-ferrites are also not fully inverted, showing the same ‘disorder’ as high temperature, quickly cooled inorganic ferrites. Although the growth mechanism of bio-magnetite nanoparticles is not understood, the availability of Fe(II) is a major control (Hansel et al., 2003) and at ambient temperature it is unlikely that a fully ordered bio-ferrite phase would be produced.

In the 4 at% Zn sample, the Zn cations show a clear preference for the T_d sites and previous studies using neutron scattering, muon-spin-rotation/relaxation experiments, Mossbauer measurements and X-ray diffraction have all shown this affinity, with between 0 and 19% inversion recorded (O'Neill, 1992; Schiessl et al., 1996; Waseda et al., 1995). EXAFS data suggest an inversion at this upper limit in these bio-spinels, in agreement with this previous work.

There is no consensus on the distribution and oxidation state of Mn cations in spinels in the literature, and these can be dependent on preparation conditions such as temperature and 'atmosphere', as well as particle size effects (Jeyadevan et al., 2000). (O'Handley, 2000) found that Mn(II) and Mn(III) have a very strong preference for tetrahedral and octahedral sites, respectively but as Fe(III) also has a strong preference for T_d coordination it competes with Mn(II) for the T_d sites. The degree of inversion for these bioferrites is less than for the Co and Ni samples, but there is still a significant amount of Fe(III) in T_d coordination. The major change in Fe cation site occupancy, according to XMCD, between these two samples is a reduction in the amount of Fe(II) O_h showing that in O_h coordination the Mn is preferentially replacing Fe(II). Additionally, as the amount of Mn increases from 1.5 to 6 at%, some of the Mn is not completely reduced to Mn(II) but remains as Mn(III), most likely in O_h sites.

For the sample containing 2 at% Mn/8 at% Zn the data are complicated by two cation substitutions. In these compositions Mn(II), Zn(II) and Fe(III) all have a preference for the T_d sites. (note that ferrites of this chemistry are found on a large scale in the ore deposits at Franklin Furnace, USA, and Långban, Sweden (Pearce et al., 2006)). The order of preference determined by (O'Handley, 2000) for T_d coordination was Zn(II)>Mn(II)>Fe(III). This pattern is also seen in these bio-ferrites as EXAFS indicates 80% of the Zn is T_d coordinated and 36-60% Mn (depending on whether the L or K edge data is used) and 30-32% of the Fe. Similar site occupancy data for ZnMn-ferrites has been found by (Calvin et al., 2002) who, using EXAFS analyses, found all Zn cations to be T_d coordinated and the Mn distributed so that more than half was O_h coordinated. Using the Mn and Fe site distribution data from fitting the L edge XAS and Zn EXAFS, the total cation site occupancies are $n(T_d) = 1.17$ and $n(O_h) = 1.91$, giving an apparent $T_d:O_h$ ratio of 0.63 compared to magnetite of 0.50. Even allowing for the likely maximum errors in site allocation, this represents a T_d excess although charge balance is maintained by the preferential replacement of Fe $d^6 O_h$ cations by the Zn(II) and Mn(II). In the spinel structure only $\frac{1}{4}$ of the T_d and $\frac{1}{2}$ of the O_h sites are occupied so there are plenty of empty T_d sites to be filled. It would therefore appear that it is energetically favourable to have an excess of these T_d -phyllitic elements in a slightly distorted structure, than a conventional stoichiometry; this may be more easily accommodated in nanoparticles than larger crystalline matrices. This effect is not present in the samples which only contain one of either Mn or Zn; the two together, both which have such a strong T_d preference, have altered the stoichiometry of the spinel.

The bond lengths in the biogenic Fe_3O_4 samples (Table 2) are close to published data for stoichiometric magnetite (Finger et al., 1986). Comparison with published spinel M-O bond lengths in the literature (see Table 6 in (Henderson et al., 2007; Hill et al., 1979) reveals the Fe-O bond lengths in both T_d and O_h sites in the biogenic ferrites to be similar to those in inorganic ferrites in most cases. However significant discrepancies are seen in the Co and Ni samples where the Fe-O bond length in tetrahedral coordination is ca 0.1 Å lower and 0.1 Å higher, respectively. In addition the data show that the Co-O and Ni-O distances for both the T_d and O_h sites in these biogenic

spinel are slightly shorter than in the inorganic samples, with the discrepancy greater for the T_d sites. The data for the Zn-O bonds are comparable to published data, identical for O_h sites and 0.02 Å larger in T_d sites. Mn-O bonds are slightly longer in T_d sites than published data by 0.03-0.06 Å and slightly shorter for O_h sites. The sample containing both Mn and Zn has comparable Mn-O bond distances but the Zn-O bond lengths are shorter than those in both published data and the biospinel containing only Zn, especially on the O_h site-perhaps as this site is distorted by the three different elements. The data indicate some small scale distortion of the substituted bio-spinels compared with synthetic and mineral samples which, given their mode and temperature of formation and large surface area, is not surprising.

The calculation of stoichiometries and cation vacancies (Table 3) in the biospinels gives a negative value for the pure bio ferrites, demonstrating an excess of cations in the Fe(II) d^6 O_h site (Coker et al., 2007) but an overall charge balance maintained by lower Fe(III) contents (Table 3). The Ni-containing bio-spinels are cation deficit but in this case an excess of Fe(III) O_h provides charge balance; the same is true of the low Co sample. The Mn, Zn and MnZn samples all have a cation excess but this can be explained by excess in $M(II)$ T_d and again the charge balance is maintained by Fe(III) > 66%. It would seem that the stoichiometry, vacancies and charge balance in these particles are interrelated and that the charge balance is an overriding control. This may well reflect the formation of these particles by redox processes involving biologically driven electron transfer in which the system would be continually stabilising electron charge.

This study has shown that substituted bioferrites can be produced by metal-reducing bacteria and the chemistry of the biospinels can be controlled by manipulating the chemistry of the starting materials. The site chemistry of the nano-bioferrites produced is similar to inorganic spinels. The challenge is now to identify the magnetic and chemical properties of these potentially useful bionanominerals, and develop fully optimised bioprocess technologies to produce suitable quantities of the materials for specific applications.

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Table 4.1. Table of atomic % of Fe(III)-oxyhydroxides containing additional transition metal cations.

Table 4.2. Parameters obtained from fitting the EXAFS data for Fe, Co, Ni, Mn and Zn K-edges of biogenic ferrite samples and standards

Table 4.3. Fe site occupancies and cation vacancies for biogenic ferrites and standards, calculated from XMCD. The spectra were fitted to the three main spectral features in the Fe L_3 peaks only and the ratios were calculated to the number of Fe atoms per unit spinel formula on a 4 oxygen basis.

Table 4.4. Parameters obtained from fitting the Mn XAS L-edge of biogenic ferrite samples.

Table 4.5. Comparison of data obtained for T_d and O_h site occupancies by fitting Fe, Ni, Co, Mn and Zn K- and L-edges of biogenic ferrites.

Figure 4.1. The calculated Fe $L_{2,3}$ XMCD spectra for the three different Fe sites in magnetite together with the total spectrum.

Figure 4.2. Fe(II) concentration variation with time for reduction of Fe(III) oxyhydroxides to form magnetite by Fe(III)-reducing bacteria. **A**, 2 at% Co (diamonds), 12 at% Co (squares); **B**, 2 at% Ni (diamonds), 6.5 at% Ni (squares); **C**, 4 at % Zn (diamonds), 16 at% Zn (squares); **D**, 1 at% Mn (diamonds), 6 at% Mn (squares); **E**, as A; **F**, as B. **A-D**, Fe(III) reduction by *G. sulfurreducens*, **E-F**, Fe(III) reduction by *S. oneidensis*. Each culture contained an electron donor and the starting oxyhydroxide with additions as follows; controls containing no cells (dotted lines), cultures containing cells (dashed lines), cultures containing cells and AQDS (solid lines).

Figure 4.3. superSTEM images showing spinel nanoparticles from samples (a) 6.5 at% Ni (HAADF images) & (b) 12 at% Co (HAADF image with a BF image inset showing streams atoms) produced by *G. sulfurreducens*.

Figure 4.4. Co, Ni, Mn and Zn K edges. **A, C, E, G** are the EXAFS spectra and **B, D, F, H** are the corresponding radial distribution functions for each of the K-edges mentioned for labelled samples. The best fit (dotted line) of the experimental data (solid line) was calculated using the parameters in Table 2. *G. sulfurreducens* (**GS**); *S. oneidensis* MR-1 (**SO**).

Figure 4.5. Fe K-edge EXAFS (left panel) and corresponding radial distribution functions (right panel) for each labelled sample. *G. sulfurreducens* (**GS**); *S. oneidensis* MR-1 (**SO**). The best fit (dotted line) of the experimental data (solid line) was calculated using the parameters in Table 2.

Figure 4.6. Experimental Ni $L_{2,3}$ -edge isotropic XAS and corresponding XMCD spectrum for sample 6.5 at% Ni supplemented with *G. sulfurreducens*. (solid lines) with calculated best-fit spectra describing (A) Ni(II) $d^8 O_h$ and (B) Ni(II) $d^8 T_d$, using a crystal field atomic multiplet model (van der Laan and Kirkman, 1992; van der Laan and Thole, 1991) where the calculations use a crystal field splitting of $10Dq = 1.2$ (dotted lines) and $10Dq = -0.6$ eV, respectively. The T_d site gives only a weak XMCD.

Figure 4.7. Experimental Fe L -edge XMCD spectra (solid lines) for samples produced using samples 2 and 6.5 at % Ni produced by either *G. sulfurreducens* (GS) or *S. oneidensis* MR-1 (SMR1) and for the standard Ni-ferrite. Experimental spectra are overlaid by best fit calculated spectra (dotted lines) based on the three theoretical site components shown in Fig. 1.

Figure 4.8. (A) Experimental Co $L_{2,3}$ -edge isotropic XAS and corresponding XMCD spectrum for 12 at% Co sample produced by *G. sulfurreducens*. (solid lines) with corresponding spectra for a thin film standard of CoFe_2O_4 (dashed line) containing only Co(II) O_h shows a good fit with the biogenic Co-ferrite (van der Laan et al., 2007). (B) Calculated XAS and XMCD spectra describing Co(II) $d^7 \text{O}_h$ (solid lines) and T_d (dashed lines) using a crystal field atomic multiplet model (van der Laan and Kirkman, 1992; van der Laan and Thole, 1991) where the calculation used a crystal field splitting of $10Dq = 1.2$ and -0.6 respectively.

Figure 4.9. Experimental Fe $L_{2,3}$ -edge XMCD spectra (solid lines) for samples 2 and 12 at% Co produced by *G. sulfurreducens* (GS) or *S. oneidensis* MR-1 (SMR1) and for the standard Co-ferrite. Experimental spectra are overlaid by best fit calculated spectra (dotted lines) based on the three theoretical site components shown in Fig. 1 with quantitative analyses shown in Table 3.

Figure 4.10. Experimental Mn $L_{2,3}$ edge spectra for samples 1.5 and 6 at% Mn and 2at% Mn/8 at% Zn produced by *G. sulfurreducens* (solid lines) overlaid by the best-fit calculated spectra (dotted lines) for Mn(II) and Mn(III) in either O_h or T_d coordination in a spinel with $10Dq$ values of 1.0 and -0.6 eV, respectively (shown above experimental and best-fit data)

Figure 4.11. Experimental Fe $L_{2,3}$ -edge XMCD spectra (solid lines) for samples produced using *G. sulfurreducens* and 1.5 and 6 at% Mn, 4 at% Zn and 2at% Mn/8 at% Zn. Experimental spectra are overlaid by best fit calculated spectra (dotted lines) based on the three theoretical site components shown in Fig. 1 with quantitative analyses shown in Table 3.

782 **Table 1.**

Hydroxide sample	Fe at %	Ni at %	Co at %	Mn at %	Zn at %
Ni-1	50	2	-	-	-
Ni-2	46	6.5	-	-	-
Co-1	50	-	2	-	-
Co-2	40	-	12	-	-
Mn-1	51	-	-	1.5	-
Mn-2	46	-	-	6	-
Zn-1	48	-	-	-	4
Zn-2	36	-	-	-	16
MnZn-1	43	-	-	2	8

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Table 2

Hydroxide Sample	Edge	Tetrahedral site		2σ ² (Å ²)	Octahedral site		2σ ² (Å ²)	Edge	Tetrahedral site		2σ ² (Å ²)	Octahedral site		2σ ² (Å ²)
		%	r/A		%	r/A			%	r/A		%	r/A	
Geobacter sulfurreducens														
Fe ₃ O ₄	Fe K	35.0	1.82	0.043	65.0	2.04	0.025	-	-	-		-	-	-
2at% Co	Fe K	27.5	1.78	0.080	73.3	1.98	0.024	Co K	45.0	1.90	0.008	55.0	2.08	0.004
12at% Co	Fe K	30.0	1.83	0.079	70.0	1.98	0.023	Co K	36.0	1.92	0.005	64.0	2.09	0.004
2at% Ni	Fe K	50.0	1.99	0.018	50.0	1.99	0.055	Ni K	20.0	1.84	0.004	80.0	2.03	0.002
6.5at% Ni	Fe K	45.0	1.98	0.026	56.7	2.01	0.042	Ni K	17.5	1.90	0.009	81.7	2.05	0.006
1.5at% Mn	Fe K	30.0	1.85	0.070	68.3	2.03	0.029	Mn K	67.5	2.07	0.002	33.3	2.16	0.005
6at% Mn	Fe K	32.5	1.84	0.057	66.7	2.02	0.029	Mn K	60.0	2.10	0.015	40.0	2.15	0.005
4at% Zn	Fe K	35.0	1.84	0.016	66.7	2.06	0.017	Zn K	80.0	1.99	0.012	20.0	2.12	0.003
2at%Mn/ 8at%Zn	Fe K	30.0	1.88	0.040	68.3	2.01	0.018	Mn K	60.0	2.10	0.005	40.0	2.21	0.002
								Zn K	80.0	1.94	0.011	20.0	2.03	0.017
Shewanella oneidensis														
2at% Co	Fe K	30.0	1.88	0.080	68.3	1.99	0.024	Co K	37.5	1.90	0.009	61.7	2.04	0.005
12at% Co	Fe K	25.0	1.84	0.080	73.3	1.98	0.020	Co K	35.0	1.92	0.004	65.0	2.08	0.004
2at% Ni	Fe K	40.0	1.97	0.013	60.0	2.03	0.040	Ni K	20.0	1.88	0.002	80.0	2.04	0.004
6.5at% Ni	Fe K	45.0	1.97	0.012	55.0	2.02	0.037	Ni K	20.0	1.88	0.004	80.0	2.05	0.006
Chemically synthesized														
Fe ₃ O ₄	Fe K	35.0	1.88	0.009	65.0	2.02	0.029	-	-	-		-	-	-
CoO	-	-	-	-	-	-	-	Co K	-	-	-	100.0	2.13	0.012
CoFe ₂ O ₄	Fe K	40.0	1.95	0.010	61.7	1.93	0.043	Co K	50.0	1.90	0.029	48.3	2.02	0.002
NiO	-	-	-	-	-	-	-	Ni K	-	-	-	100.0	2.08	0.017
NiFe ₂ O ₄	Fe K	45.0	1.92	0.013	55.0	1.95	0.037	Ni K	12.5	1.87	0.017	86.7	2.03	0.007

Table 3

Hydroxide Sample	d ⁶ O _h	d ⁵ T _d	d ⁵ O _h	Total Fe	Cation total	Vacancy	Fe(II)/Fe(III)	% T _d Fe	% O _h Fe
<i>Geobacter sulfurreducens</i>									
Fe ₃ O ₄	1.13	0.98	0.93	3.04	3.04	-0.04	0.59	32	68
2at% Co	0.68	1.02	1.13	2.82	2.93	0.07	0.32	36	64
12at% Co	0.40	0.92	1.03	2.35	3.02	-0.02	0.20	39	61
2at% Ni	0.58	1.03	1.17	2.78	2.90	0.10	0.27	37	63
6.5at% Ni	0.47	0.97	1.16	2.59	2.94	0.06	0.22	37	63
1.5at% Mn	1.08	0.96	0.94	2.98	3.06	-0.05	0.57	32	68
6at% Mn	0.84	0.95	0.92	2.71	3.06	-0.07	0.45	35	65
4at% Zn	0.93	0.91	1.09	2.93	3.06	-0.06	0.47	31	69
2at%Mn/ 8at%Zn	0.73	0.81	1.02	2.56	3.08	-0.08	0.40	32	68
<i>Shewanella oneidensis</i>									
Fe ₃ O ₄	1.03	0.99	0.98	3.01	3.01	-0.01	0.52	33	67
2at% Co	0.63	1.03	1.15	2.80	2.92	0.08	0.29	36	63
12at% Co	0.42	0.89	1.04	2.35	3.03	-0.03	0.21	38	62
2at% Ni	0.69	0.94	1.19	2.82	2.94	0.06	0.50	33	67
6.5at% Ni	0.59	0.91	1.13	2.63	2.98	0.02	0.53	35	66
Chemically synthesized									
Fe ₃ O ₄	0.70	1.03	1.17	2.90	2.90	0.10	0.32	35	65
CoFe ₂ O ₄	0.21	0.87	1.00	2.08	3.07	-0.07	0.11	42	58
NiFe ₂ O ₄	0.18	0.91	0.98	2.07	3.06	-0.06	0.09	44	56

Table 4

Hydroxide sample	Tetrahedral site		Octahedral site		% T _d	% O _h
	d ⁴	d ⁵	d ⁴	d ⁵		
1.5at% Mn	0	46	0	54	46	54
6at% Mn	0	45	9	46	45	55
2at%Mn/ 8at%Zn	0	36	36	28	36	64

Table 5

SAMPLE		IRON (at %)		DOPANT (at %)		TOTAL (at %)		TOTAL (%)	
		T _d	O _h	T _d	O _h	T _d	O _h	T _d	O _h
<i>Geobacter sulfurreducens</i>									
2at% Co	K-edge	13.7	36.6	0.9	1.1	14.7	37.8	28.0	72.0
	L-edge	18.0	32.0	0.2	1.8	18.2	33.8	35.0	65.0
12at% Co	K-edge	12.0	27.9	4.4	7.8	16.3	35.7	31.4	68.6
	L-edge	15.7	24.2	1.2	10.9	16.9	35.1	32.5	67.5
2at% Ni	K-edge	24.9	24.9	0.4	1.7	25.3	26.7	48.7	51.3
	L-edge	18.4	31.4	0.2	2.0	18.7	33.3	35.9	64.1
6.5at% Ni	K-edge	20.5	25.8	1.1	5.3	21.6	31.1	41.0	59.0
	L-edge	17.0	28.5	0.6	5.8	17.6	34.4	33.9	66.1
1.5at% Mn	K-edge	15.2	34.6	0.9	0.4	16.1	35.1	31.5	68.5
	L-edge	16.3	34.3	0.6	0.7	16.9	35.1	32.6	67.4
6at% Mn	K-edge	15.0	30.8	3.5	2.3	18.5	33.1	35.9	64.1
	L-edge	16.2	30.0	2.6	3.2	18.8	33.2	36.2	63.8
<i>Shewanella oneidensis</i>									
2at% Co	K-edge	15.0	34.1	0.8	1.3	15.8	35.4	30.8	69.2
	L-edge	18.3	31.6	0.2	1.8	18.5	33.5	35.6	64.4
12at% Co	K-edge	10.0	29.2	4.3	7.9	14.2	37.1	27.7	72.3
	L-edge	15.1	24.7	1.2	10.9	16.4	35.6	31.5	68.5
2at% Ni	K-edge	19.9	29.9	0.4	1.7	20.4	31.6	39.2	60.8
	L-edge	16.6	33.2	0.2	2.0	16.8	35.2	32.4	67.6
6.5at% Ni	K-edge	20.5	25.0	1.3	5.2	21.8	30.2	41.9	58.1
	L-edge	15.7	29.8	0.6	5.8	16.4	35.6	31.5	68.5

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7

Figure 8

Figure 9

Figure 10

Figure 11