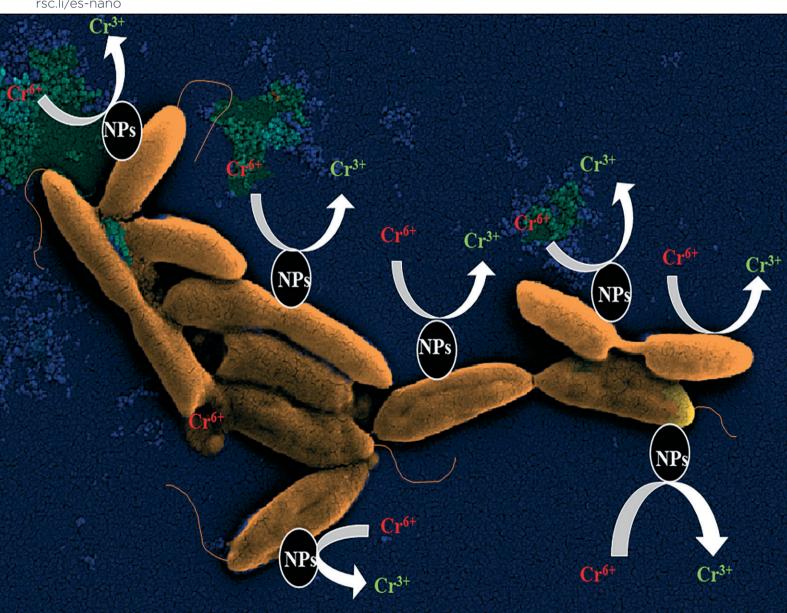
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Protection of Shewanella oneidensis MR-1 by manganese ferrite nanoparticles during chromate bio-reduction†

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Shewanella oneidensis (S. oneidensis) MR-1 is a metal-reducing bacterium that can bio-reduce the carcinogenic hexavalent chromium (Cr^{6+}) to a less toxic trivalent form (Cr^{3+}) . The bacteriocidal effect of Cr^{6+} challenges the above bio-reduction process. This work aims to illustrate the protective role of manganese ferrite nanoparticles (Mn_{0.2}Fe_{2.8}O₄ NPs) to S. oneidensis MR-1 bacteria during the bio-reduction of Cr⁶⁺. Nanostructures were characterised by transmission electron microscopy (TEM) and X-ray diffraction (XRD). The interaction between S. oneidensis MR-1, Cr⁶⁺ and Mn_{0.2}Fe_{2.8}O₄ NPs was monitored by X-ray photoelectron spectroscopy (XPS), which helped to unravel the oxidation states of Cr. The XPS analysis provided key insights into the oxidation states of Mn and Fe, confirming the redox interactions facilitating Cr⁶⁺ reduction. Mn_{0.2}Fe_{2.8}O₄ NPs boosted the detoxification of the removed Cr⁶⁺ by 2.1 and 1.4 times compared to using S. oneidensis MR-1 alone and NPs alone, respectively. Scanning electron microscopy (SEM) imaging evaluated the changes in the morphology of bacterial cells. After exposure to Cr⁶⁺, S. oneidensis MR-1 cells revealed their inability to produce nanofibers, which are electrically conductive bacterial appendages. Yet, Mn_{0.2}Fe_{2.8}O₄ NPs provoked the formation of bacterial nanofibers. These findings highlight the potential of $Mn_{0.2}Fe_{2.8}O_4$ NPs for enhancing the bioremediation of Cr^{6+} contaminated environments.

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Environmental significance

Carcinogenic hexavalent chromium leaks from industrial sites due to improper wastewater treatment into surface and groundwater, exposing flora and fauna to danger. The metal-reducing bacterium, Shewanella oneidensis MR-1, can reduce Cr⁶⁺ into less toxic Cr³⁺; bacteria lose their viability during treatment due to the toxicity of Cr⁶⁺. The novelty of this work is the discovery of a protective role of Mn-ferrite nanoparticles to S. oneidensis MR-1 bacteria during Cr⁶⁺ bio-reduction. We show that Mn_{0.2}Fe_{2.8}O₄ NPs induced bacterial cell elongation and promoted nanofiber formation. Such morphological changes improve bacterial cell viability in response to the sub-lethal dose of Cr⁶⁺ and enhance their detoxification capability. Our findings provide a promising application of using nano-Mn_{0.2}Fe_{2.8}O₄ in the bioremediation of Cr⁶⁺-contaminated environments.

1. Introduction

Contamination of air, soil and water with heavy metals is hazardous to human health and the environment due to their toxicity, even at low concentrations¹ as they are nonbiodegradable materials.² The Agency for Toxic Substances and Disease Registry (ATSDR) ranked chromium (Cr) the 17th on the substance priority list among many heavy metals.³

Cr mainly occurs in two valence states: hexavalent (Cr⁶⁺) and trivalent (Cr3+). Human exposure to Cr6+ can cause liver damage, pulmonary congestion, oedema, skin irritation, ulcer formation,4 neurotoxicity,5 and carcinogenesis.6 U.S. Environmental Protection Agency (EPA) and WHO guidelines reported a permissible limit of Cr⁶⁺ in drinking water of 50 ppb. According to the EU drinking water directive, the regulation limit for the total Cr will be 25 μ g L⁻¹ by 12 January 2036.8 Since Cr3+ has low mobility, limited bioabsorptivity, and lower toxicity than Cr6+,9 Cr6+ should be reduced to Cr3+ for its safe removal.10

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Bio-reduction of Cr⁶⁺ is a cost-effective and environmentally friendly method, attracting widespread interest. 11 Some bacteria can reduce metals, acting as terminal electron acceptors under anaerobic conditions. 12 So, metal-reducing bacteria can be used for the biotic reduction of heavy metals for detoxification purposes. Such a natural process is applicable for the biological reduction of the carcinogenic Cr⁶⁺ into less toxic Cr³⁺ form.¹³

Shewanella oneidensis MR-1 is a model metal-reducing bacteria for detoxifying Cr⁶⁺. ¹⁴⁻¹⁸ S. oneidensis MR-1 can employ as a terminal electron acceptor under anaerobic conditions. 14,15,19 The biosafety of S. oneidensis MR-1 is an essential criterion for selecting bioremediation biological agents. In contrast, Pseudomonas aeruginosa bacteria can be used for Cr⁶⁺ removal but are not preferred for bioremediation because they cause diseases in humans and animals.^{20,21} Yet, the lethal effect of Cr⁶⁺ on the microbes during their respiration limited the bioremediation of Cr⁶⁺.²²

Mn_{0.2}Fe_{2.8}O₄ NPs showed a higher adsorption capacity for Cr⁶⁺ than Fe₂O₃ NPs and other tested Mn_xFe_{3-x}O₄ NPs.²³ This chemical structure improved the bacterial viability and microbial detoxification of Cr⁶⁺.²³ The adsorption of Cr⁶⁺ can limit the availability of the toxic cations to cells, which could improve their viability and bio-reduction efficiency.

Herein, to the best of our knowledge, we showed for the first time the protective role of the Mn_{0.2}Fe_{2.8}O₄ NPs to S. oneidensis MR-1 during the bio-reduction of Cr⁶⁺. Raie et al.²³ primarily investigated the adsorption and bio-removal of Cr⁶⁺ using Mn_{0.2}Fe_{2.8}O₄ NPs and S. oneidensis MR-1, respectively.

This article builds upon findings by Raie et al., 23 and elucidates the reduction process of Cr⁶⁺ using XPS. In addition, this work presents bacterial imaging to visualise morphological changes in response to Cr⁶⁺ and NPs, providing deeper insights into the mechanism of Cr⁶⁺ reduction.

XPS revealed the possible reduction of Cr⁶⁺ to Cr³⁺ due to its interaction with Mn_{0.2}Fe_{2.8}O₄ NPs. This allowed us to confirm the redox-based interaction among Cr6+ and Mn_{0.2}-Fe2.8O4 NPs. In addition, SEM showed the morphological change response of S. oneidensis MR-1 as a coping strategy in response to the toxic Cr6+ in the presence of Mn_{0.2}Fe_{2.8}O₄ NPs. This article will advance the treatment of Cr⁶⁺ by demonstrating its removal, unravelling its reduction mechanism and the biological implications, thereby contributing novel insights and practical advancements to nanobiotechnology and environmental applications.

2. Materials and methods

2.1 NPs preparation and characterisation

Mn_{0.2}Fe_{2.8}O₄ NPs were prepared by an adapted polyol solvothermal synthetic process^{24,25} at 250 °C as described in our recent work.²³ In 20 mL of tetraethylene glycol (TEG), 0.3 M of iron(III) acetylacetonate (2.1 g) and 0.1 M manganese(II) acetylacetonate (0.5 g) were added. The mixture was added into a 45 mL Teflon-lined stainless-steel autoclave after being homogenised by vortex and sonication to be placed in an oven (Memmert, model UFP400) and heated within 30 min up to 250

°C for a 6 h hold at that temperature. In polvol synthesis, metal precursors are reduced by TEG, which acts as a high-temperature capping agent, solvent, and reductant. The formed metal nuclei grow and controllably coalesce together to produce the desired particles.^{26,27} The produced black dispersion underwent characterisation and functionalisation by tri-sodium citrate via ligand exchange.²³ A JEOL JEM 1200-EX microscope operating at an acceleration voltage of 120 kV was employed to investigate the shape and size of the produced particles. The polydispersity index (PDI) is the ratio between the standard deviation and the mean nanoparticle diameter. To determine the crystal phase and the average crystallite size, we used XRD (PANalytical XPERT PRO MPD) coupled with Co K_{α} radiation source ($\lambda = 1.789 \text{ Å}$) and an X'Celerator detector operated at 40 kV and 40 mA. An Optima 3100 XL Perkin Elmer Inductively Coupled Plasma Atomic Emission (ICP-AES) spectrometer was employed to determine the chemical composition of Mn_xFe_{3-x}O₄ particles. To quantify the iron content of the functionalised NPs dispersed in water, a colorimetric phenanthroline method was applied for the aciddigested NPs using a spectrophotometer (SpectraMax M2e, Molecular Devices, UK).

2.2 Sources for bacteria of interest

A freeze-dried culture of S. oneidensis MR-1 (LMG 19005) was purchased from BCCM/LMG bacteria collection.

2.3 Viability of S. oneidensis MR-1 to Mn_{0.2}Fe_{2.8}O₄ NPs

The impact of Mn_{0.2}Fe_{2.8}O₄ NPs on the viability of the S. oneidensis MR-1 was assessed using Guava easyCyte® flow cytometer (Merck, UK) following a protocol previously utilised by Raie et al.,23 under anaerobic conditions overnight. A homogeneous bacterial cell suspension (10 µL with OD measured at $\lambda = 600$ nm equal to 0.1) was added to 80 μ L of M9 minimal salts (×2) medium, containing 20 mM sodium lactate as a sole electron source, 5 mL L⁻¹ each of vitamins and minerals and pH was adjusted to 7.2 by 10 mM 4-(2hydroxyethyl)-1-piperazineethanesulfonic acid buffer. 23,28 Sodium fumarate (20 mM) was used as a terminal electron acceptor. 23,28 Mn_{0,2}Fe_{2,8}O₄ NPs (10 μL) were added to the mixture. The tested concentrations of NPs ranged from 1-60 mg mL⁻¹ with an approximate total Fe content from 0.7 $mg mL^{-1}$ to 40.6 $mg mL^{-1}$.

2.4 The exposure of S. oneidensis MR-1 to Cr⁶⁺ and Mn_{0.2}-Fe_{2.8}O₄ NPs

S. oneidensis MR-1 was exposed to Cr⁶⁺ and Mn_{0.2}Fe_{2.8}O₄ NPs individually and also in a combined way overnight, in conditions similar to that mentioned in Section 2.3. Cr⁶⁺ (as a terminal electron acceptor) and Mn_{0.2}Fe_{2.8}O₄ NPs were added to this medium with concentrations of 50 mg L-1 (sublethal dose, as reported by Raie et al.)23 and 1 mg mL-1, respectively.23

2.5 Analysis of oxidation state of Cr^{6+} , Mn^{x+} , and Fe^{y+}

The oxidation states of Mn and Fe in Mn_{0.2}Fe_{2.8}O₄ NPs and Cr were investigated after being incubated together or separately with S. oneidensis MR-1 by XPS; a Kratos Analytical AXIS Ultra DLD system with aluminium X-ray source (λ_{Ka} = 1486.6 eV) was used, operated under ultra-high vacuum conditions (10⁻⁹ torr). The experimental curves were best fitted by combining Gaussian (70%) and Lorentzian (30%) distributions, while background subtraction was performed using the Shirley equation. A normalised peak area of each element is calculated by dividing its area by the sensitivity factor.29 To determine the redox interaction between Cr6+ and Mn_{0.2}Fe_{2.8}O₄ NPs, we compared the normalised peak areas of Mn²⁺ to Mn³⁺, Fe²⁺ to Fe³⁺ and Cr³⁺ to Cr⁶⁺ in the high-resolution Mn 2p, Fe 2p and Cr 2p spectra, respectively, while only the ratios between that peak areas of Cr³⁺ to Cr⁶⁺ were analysed in the case of applying bacterial cells. The relative fold increase in Cr6+ bio-reduction was calculated by its equivalent atomic fraction to the reference values.

2.6 Imaging bacteria by SEM

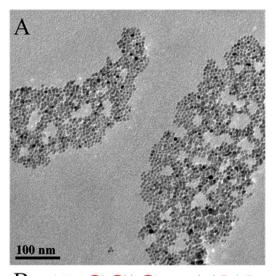
To acquire SEM images, 50 µL from the untreated or Mn_{0.2}-Fe2.8O4 NPs treated S. oneidensis MR-1 bacteria cell suspension were deposited on a microscope cover glass (Fisher, UK). The samples were imaged using Philips XL30 FEG SEM (FEI, Eindhoven, Netherlands), which operates at an accelerating voltage of 5 keV. Cell fixation was performed using glutaraldehyde (2.5% v/v in 0.01 M PBS) for 30 min at room temperature. Samples were washed three times in phosphate-buffered saline (PBS, 0.01 M) and dehydrated for 5 min in ethanol aqueous solutions. The concentrations of ethanol aqueous solutions were 10% v/v, 30% v/v, 50% v/v, 70% v/v, 90% v/v, 100% v/v, sequentially. A double-sided carbon tape (Agar Scientific, UK) was used to attach the glass slide with the SEM specimens onto aluminium stubs. Samples were then sputter-coated with gold-palladium at 20 mA and 1.25 kV for 90 s (Palaron E5000 sputter coater).

3. Results and discussion

3.1 Characterisation of NPs

3.1.1 Morphology of NPs. Regarding the obtained spherical Mn_xFe_{3-x}O₄ NPs (Fig. 1A), our results agree with Raie et al., 23 Vamvakidis et al.,25 and García-Soriano et al.,30 who used the polyol solvothermal technique for producing spherical Mn_xFe_{3-x}- O_4 NPs. 23,25,30 The mean size of $Mn_xFe_{3-x}O_4$ NPs is 7.4 \pm 1.3 nm. The PDI is 0.18, which indicates a relatively narrow size distribution.31 Similarities in spherical shape and small size range (approximately 7-9 nm) are attributed to the specific procedure where sole polyols were used to prepare the NPs. 23,25,30

3.1.2 Crystal structure of NPs. Powder XRD patterns for the prepared Mn_xFe_{3-x}O₄ NPs recorded at room temperature are illustrated in Fig. 1B. All the diffraction peaks show the presence of the face-centred cubic (FCC) crystal structure, while no impurity phase was observed. So, the formation of



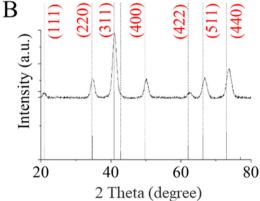


Fig. 1 Mn_{0.2}Fe_{2.8}O₄ NPs prepared at 250 °C for 6 h: (A) TEM images and (B) XRD patterns, and XRD reference for MnFe₂O₄ (PDF card no 00-010-0319).

Mn_xFe_{3-x}O₄ NPs was obtained through a facile polyol solvothermal process with reaction times of 6 h.

3.1.3 Elemental analysis of Mn_xFe_{3-x}O₄ NPs. The formed $Mn_xFe_{3-x}O_4$ NPs have a low Mn content (x = 0.2), based on ICP-AES results. Etemadi & Plieger, 32 Oberdick et al., 33 and Raie et al. 23 reported similar results of low Mn doping levels because Mn(acac)₂ is more thermally stable than Fe(acac)₃.³⁴

3.2 Interaction of Cr⁺⁶ with Mn_{0.2}Fe_{2.8}O₄ NPs

 $Mn_{0.2}Fe_{2.8}O_4$ NPs adsorbed 16.8 ± 1.6 mg g⁻¹ (around 61%) of Cr⁶⁺.23 The possible reduction of the adsorbed Cr⁶⁺ by Mn_{0.2}-Fe_{2.8}O₄ NPs was explored here by studying the oxidation state of Mn, Fe, and Cr of the adsorbent and adsorbate by XPS, as shown in Fig. 2A.

3.2.1 Oxidation state of Mn in Mn_{0.2}Fe_{2.8}O₄ NPs after Cr⁶⁺ adsorption. In Fig. 2B, the position of binding energy (BE) for Mn 2p was slightly shifted from 640.45 eV²³ to higher BE (641.80 eV), which could be attributed to the possible oxidation of Mn2+ into Mn3+ upon interacting with Cr6+. The dissolved Mn³⁺ could generate manganese oxide (MnO_x), which provides more adsorption sites for Cr⁶⁺ removal.³⁵

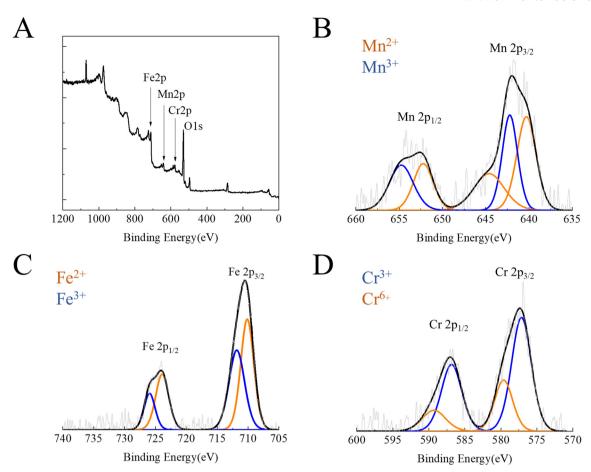


Fig. 2 Mn_{0.2}Fe_{2.8}O₄ NPs treated by Cr⁶⁺: (A) wide scan XPS spectrum, and high-resolution XPS spectra of (B) Mn 2p, (C) Fe 2p, and (D) Cr 2p.

In Mn_{0.2}Fe_{2.8}O₄ NPs,²³ Mn 2p peak, in Fig. 2B, was fitted by 5 contributions at 640.3 eV, 642.2 eV, 644.61 eV, 652.2 eV and 654.8 eV. Mn 2p3/2 was deconvoluted into 640.35 eV and 642.25 eV peaks, representing Mn²⁺ and Mn³⁺, respectively, as shown in Fig. 2B. The peak of Mn 2p_{1/2} was fitted into two contributions of Mn²⁺ and Mn³⁺ at 652.15 eV and 654.6 eV, respectively. 36-39 The fifth small satellite peak at 645.2 eV was assigned to Mn²⁺ of MnO.³⁸ Since stoichiometric MnFe₂O₄ can be expressed as MnO-Fe₂O₃, this pointed to the formation of Mn_xFe_{3-x}O₄ NPs.

3.2.2 Oxidation state of Fe after Cr6+ interaction with Mn_{0.2}Fe_{2.8}O₄ NPs. After Cr⁶⁺ adsorption on Mn_{0.2}Fe_{2.8}O₄ NPs, XPS (in Fig. 2C) showed the position of BE for Fe 2p at 711 eV. A peak of Fe 2p_{3/2} was spotted at 710.75 eV, and the asymmetric peaks are situated at 723.9 eV, attributed to $2p_{1/2}$. The observed signals at these BE positions probably correspond to the formation of iron oxide phase, i.e., hematite or maghemite phase. 41 Unlike untreated Mn_{0.2}Fe_{2.8}-O₄ NPs, ²³ Fe 2p missed the satellite peak at 718 eV as shown in Fig. 2C, which was due to the presence of Fe₃O₄.⁴⁰ The ratio between Mn and Fe was doubled from 0.24 to 0.44 (as was reported by XPS and range based on elemental analysis by ICP in our recent work) compared to untreated Mn_{0.2}Fe_{2.8}-O₄ NPs, ²³ which can be ascribed to the release of iron in the medium.

3.2.3 Reduction of Cr⁶⁺ by Mn_{0.2}Fe_{2.8}O₄. In Fig. 2D, XPS spectra of Cr 2p showed two different peaks, corresponding to the Cr 2p_{3/2} (576.0 eV-578.0 eV) and Cr 2p_{1/2} (585.0 eV-587.0 eV) orbits. After fitting peaks with the use of the Gauss-Lorentz algorithm, two peaks arised with the BE of 577 eV relating to $\operatorname{Cr}^{3+} 2p_{3/2}$ and 586 eV belonging to $\operatorname{Cr}^{3+} 2p_{1/2}$, 42,43 which mainly corresponds to the precipitation of insoluble Cr3+ species, Cr(OH)₃ and Cr₂O₃. The adsorbed [CrO₄]²⁻ on NPs³⁷ explained the presence of peaks at BE of 579.6 eV and 589 eV, representing Cr^{6+} $2p_{3/2}$ and $2p_{1/2}$, respectively.⁴³ The ratio of $[Cr^{3+}]/[Cr^{6+}]$ was estimated to be equal to 2.56. Our results point out a significant finding: the interaction between Cr6+ and Mn_{0.2}Fe_{2.8}O₄ NPs involved a redox reaction in addition to what was stated in our recent work regarding adsorption.²³ Raie et al. reported that the oxidation state of Mn in Mn_{0.2}Fe_{2.8}O₄ was mainly Mn²⁺ with a minor fraction of Mn⁴⁺, and that of Fe was a mixture of Fe²⁺ and Fe3+.23 In the present study, the possible oxidation of Mn2+ to Mn³⁺ and Fe²⁺ to Fe³⁺, besides the iron release, is due to the redox reaction between Mn_{0.2}Fe_{2.8}O₄ NPs and Cr⁶⁺. The absence of Mn4+ XPS related peak after interaction with Cr6+ was attributed to the ability of Fe2+ to reduce Mn4+, yielding Fe3+ and Mn²⁺.44 In addition to being a stabilising agent, citrate can act as a chelating agent 45 and as a reductant for Cr6+,46 due to its ability to donate electrons through ligand-metal electron transfer. 46 Mn²⁺ catalyses the reduction reaction. 47

3.3 Mn_{0.2}Fe_{2.8}O₄ NP-assisted bacterial respiration of Cr⁶⁺

oneidensis MR-1 can respire Cr⁶⁺ under anaerobic conditions. ⁴⁸⁻⁵⁰ The adsorption of Cr^{6+} (9 ± 1.5 mg g⁻¹, *i.e.* 30 ± 0.5% of removal) by Mn_{0.2}Fe_{2.8}O₄ NPs supported microbial survival in media supplemented by the tested S. oneidensis MR-1.23 The mechanism of bio-removal of Cr6+ can be attributed to the respiration of Cr⁶⁺ into Cr³⁺ (ref. 48–50) or bio-sorption^{51,52} by bacterial cells. Examining the oxidation state of Cr element via XPS analysis determines the interaction between Cr⁶⁺, Mn_{0.2}-Fe_{2.8}O₄ NPs and S. oneidensis MR-1, as illustrated in Fig. 3, which positively related to the enhanced Cr⁶⁺ bio-reduction by 2.7-3.6 fold.²³ The reported significant drop in the XPS revealed the presence of peaks related to both Cr⁶⁺ and Cr³⁺ after exposing S. oneidensis MR-1 to Cr⁶⁺.

Peaks of Cr 2p XPS were observed at BE 576.7 eV and 585.9 eV, which were related to Cr3+, while peaks at 579.2 eV and 588.6 eV were assigned to Cr⁶⁺, as presented in Fig. 3A. S. oneidensis MR-1 can reduce Cr⁶⁺ into Cr³⁺, as confirmed by our XPS results in Fig. 3A and supported by the literature. 48,53

Our findings reveal an extracellular interaction between Cr⁶⁺ and S. oneidensis MR-1 bacteria. A portion of Cr6+ was reduced to Cr³⁺, resulting in a [Cr³⁺]/[Cr⁶⁺] ratio of 1.7, while the remaining 41% of Cr⁶⁺ is adsorbed on the bacterial cell surface. The extracellular reduction of Cr⁶⁺ can occur via direct contact of Cr6+ with the metal-reducing protein complex on the cell surface and nanofiber. Also, S. oneidensis MR-1 can produce electron shuttles to promote mediated electron transfer between the cell and Cr⁶⁺. S. oneidensis MR-1 can uptake Cr⁶⁺ to be reduced inside the cell to Cr3+, but our results could not confirm the intracellular reduction of Cr6+ due to the depth limitation of XPS (7-10 nm).

Our XPS results revealed peaks related to both Cr⁶⁺ and Cr³⁺ after being incubated with S. oneidensis MR-1 in the presence of Mn_{0.2}Fe_{2.8}O₄ NPs. Peaks of Cr 2p XPS observed at BE 576.7 eV and 585.9 eV denote the presence of Cr³⁺. Cr⁶⁺ is represented by one peak at 579.2 eV,38 as illustrated in Fig. 3B. Similar results were reported due to using Cr6+ as a terminal electron acceptor during the respiration process of S. oneidensis MR-1. 48,53 The ratio between extracellular Cr³⁺ and Cr⁶⁺ was equal to 3.5. Bacteria can reduce Fe3+ to Fe2+, and biogenic Fe2+ can detoxify Cr6+ to Cr³⁺. 54,55 The affinity of MnFe₂O₄ NPs to proteins on the bacterial outer membrane can improve the contact area between a single bacterium and Cr⁶⁺ as an external electron acceptor. ^{56–59}

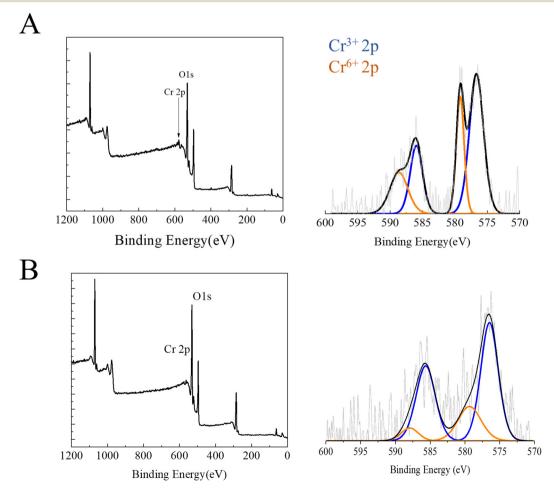


Fig. 3 Wide scan and high-resolution XPS spectra of (A) Cr 2p treated by S. oneidensis MR-1 alone, (B) Cr 2p after incubation of S. oneidensis MR-1 and Mn_{0.2}Fe_{2.8}O₄ NPs.

In this work, the presence of both S. oneidensis MR-1 and Mn_{0.2}Fe_{2.8}O₄ NPs removed Cr⁶⁺ 1.37 times more than using the NPs alone. Some possible scenarios could explain how NPs enhanced the bio-reduction of S. oneidensis MR-1 from Cr⁶⁺ to Cr³⁺. By adsorption, NPs can bridge the bacterial cell and Cr⁶⁺ to promote electron transfer. Cr⁶⁺ is adsorbed onto the MnFe₂O₄ NPs via partial chemisorption^{60,61} and partial physisorption.³⁷ The Mn in MnFe₂O₄ can interact via ionic bonding with the O atoms of HCrO₄₋/CrO₄²⁻, facilitating Cr⁶⁺ adsorption. 60,61 Mn²⁺ can reduce Cr⁶⁺ to Cr³⁺ and be oxidised to Mn³⁺. The disproportionation of oxidised Mn3+ produced Mn2+, causing Mn²⁺ to continue participating in the Cr⁶⁺ reduction. Cr³⁺ is deposited on the MnFe₂O₄ surface as Cr(OH)₃ colloids.^{60,61}

The limited availability of adsorbed Cr⁶⁺ improved the efficiency of microbial respiration, 48,54 as was indicated by our results. Since MnFe₂O₄ NPs have electrochemical properties, ^{59,62} metal oxides can link S. oneidensis MR-1 with Cr⁶⁺ to promote direct electron transfer and act as an electron mediator from the cell to Cr⁶⁺, a terminal electron acceptor.⁶³

In addition, NPs can act as physical shields for bacterial surfaces from Cr⁶⁺, which could reduce the direct damage to bacteria caused by heavy metals. Encapsulating S. loihica by biochar reported that it could avoid the lethal effect of Cr⁶⁺.63 In addition, Mn_{0.2}Fe_{2.8}O₄ NPs can sustain bacterial viability, as shown in Fig. S1† and supported by the literature. 64 The Mn content in the chemical structure of Mn_{0.2}Fe_{2.8}O₄ NPs improved the anti-oxidant activity of NPs and, in turn, cell viability.65 Substituting Fe2+ by Mn2+ in Mn0.2Fe2.8O4 NPs decreased the lethal effect of Fe²⁺ on bacterial viability. This explains how Mn_{0.2}Fe_{2.8}O₄ NPs improved the viability of S. oneidensis MR-1 under the sub-lethal concentration of Cr⁶⁺ by 3.3 times.²³

3.4 Boosting the bacterial tolerance to Cr⁶⁺ by Mn_{0.2}Fe_{2.8}O₄ NPs

SEM imaging monitored the alterations in the morphology of bacterial cells following the bio-reduction.

3.4.1 Morphology of untreated bacterial cells. The untreated tested S. oneidensis MR-1 demonstrated their viability under anaerobic redox conditions, as shown in Fig. 4A. In the absence of both Cr⁶⁺ and Mn_{0.2}Fe_{2.8}O₄ NPs, bacterial cells of S. oneidensis MR-1 were observed as rod-shaped with smooth surfaces as commonly described. 22,49,63,66-68 The formation of the division ring (Z-ring) at the division site at the mid-cell on the bacteria was an indicator of cell division, as depicted in Fig. 4A. Parker et al.22 reported that the delay in separating daughter cells could be ascribed to the minimum availability of nutrients in the media.22 The presence of bacterial nanofibers as extensions of the outer membrane and periplasm (Fig. 4A) was demonstrated to be increased under oxygen-limited conditions.66 Nanofibers were reported to have the multiheme cytochromes responsible for the extracellular electron transport pathway for linking the respiratory chain of bacteria to an external electron acceptor.66 Electrons are transferred along nanofibers of S. oneidensis MR-1 between the close cytochromes via an electron-hopping mechanism. 67,68

3.4.2 Rupture of S. oneidensis MR-1 cells in response to a sub-lethal dose of Cr⁶⁺. The impact of exposure of S. oneidensis MR-1 to Cr6+ was observed on the rupture on one pole of a cell, as shown in Fig. 4B. A shrunken surface and crack formation in bacteria cells were also observed after the reaction with Cr⁶⁺. ^{22,49,69} As in the case of untreated cells, attempts of cell division were still observed for cells exposed to Cr⁶⁺, as demonstrated in Fig. 4B. The presence of cell division septa was an indicator for the initial phase of cell division of S. oneidensis cells.²² SEM images of S. oneidensis MR-1 revealed the inability to produce nanofibers after exposure to Cr⁶⁺. The variation in the length of cells exposed to Cr3+ is presented in Fig. 4B. Bacterial cells modified their shape as a coping strategy for tolerating the stress induced by Cr⁶⁺, 55,63

3.4.3 Cellular compatibility of Mn_{0.2}Fe_{2.8}O₄ NPs. Fig. S1[†] shows the biocompatibility of different concentrations of Mn_{0.2}Fe_{2.8}O₄ NPs towards S. oneidensis MR-1. Our findings were supported by Desai et al., who reported that MnFe₂O₄ NPs showed no antimicrobial activity against some pathogenic bacteria. 70 Shewanella can survive upon exposure to 50 mg mL⁻¹ of magnetite (Fe₃O₄) with approximately 36.2 mg mL⁻¹ of total iron content under anaerobic conditions. Such tolerance to high iron concentrations was due to the cellular attachment to magnetite for Fe3+ acquisition.71 The tolerance of S. oneidensis MR-1 to such concentrations of Mn_{0.2}Fe_{2.8}O₄ NPs could be attributed to the presence of Mn²⁺ in the chemical structure of NPs, which improved the antioxidant activity, cell viability, and ability to respire metal.65 In addition, the Mn2+ content in Mn0.2Fe2.8O4 NPs lowered Fe²⁺ concentration, which could decrease the lethal effect of Fe²⁺ on the viability of the tested bacterial strain. The presence of Fe3+ in Mn_{0.2}Fe_{2.8}O₄ NPs²³ has less toxicity than Fe²⁺ under physiological conditions.⁷² The resistance of S. oneidensis MR-1 to Fe²⁺ depends on the ClpXP protease complex, which removes the mis-metallated protein. ClpX is an unfoldase, and ClpP is a peptidase that degrades damaged or misfolded proteins.73

The capability of Shewanella to produce nanofibers in the presence of Mn_{0.2}Fe_{2.8}O₄ NPs is shown in Fig. 4C. The poles of Shewanella cells were reported to be attractive to the metal oxide/hydroxides under both aerobic and anaerobic conditions,74,75 which explains the polar rupture of some cells in Fig. 4C.

3.4.4 Enhanced tolerance of Shewanella to Cr⁶⁺ by Mn_{0.2}-Fe_{2.8}O₄ NPs. In the presence of Mn_{0.2}Fe_{2.8}O₄ NPs, the surface of the treated S. oneidensis MR-1 cells by Cr6+ retained a smooth surface but with an elongated morphology (see Fig. 4D). Such stretching in the shapes of cells was observed by S. loihica PV-4 in response to Cr⁶⁺ in a mixture containing biochar and $\alpha\text{-Fe}_2O_3$ together. 63 The morphological changes observed in the bacteria are adaptive strategies for coping with environmental stresses like the presence of toxic Cr⁶⁺. Inhibiting cell division while maintaining cell growth leads to increased cell length76 and boosts the extracellular electron transfer by S. oneidensis MR-1.77

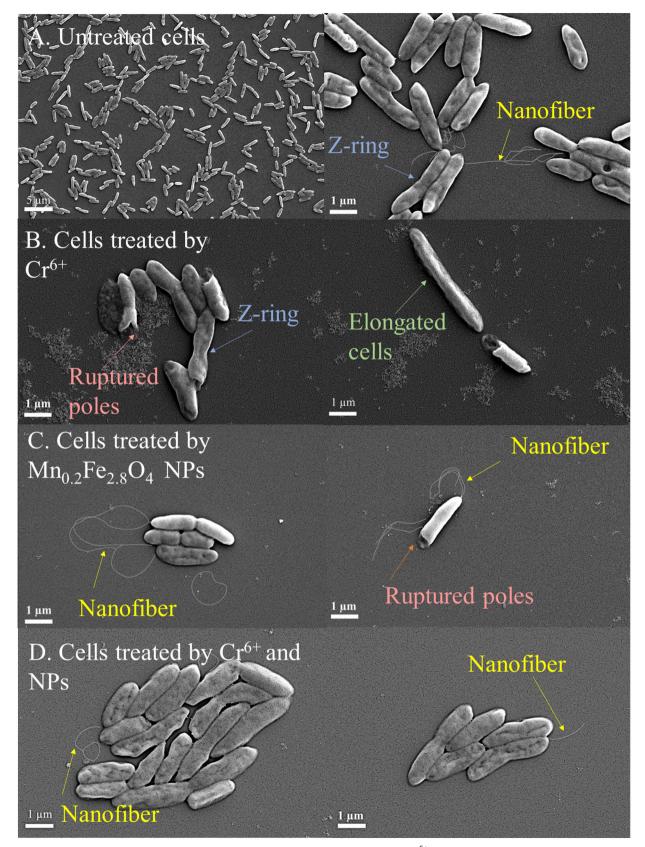


Fig. 4 SEM micrographs of (A) untreated S. oneidensis MR-1 cells, (B & C) treated cells by Cr⁶⁺ alone and NPs alone, respectively, and (D) treated cells by both Cr6+ and NPs.

Mn_{0.2}Fe_{2.8}O₄ NPs provoked the formation of bacterial nanofiber in the presence of Cr⁶⁺, as depicted in Fig. 4D. Nanofibers are extensions of the outer membrane and periplasm, which are the extracellular electron transport components.66 Nanofibers are important for long-range extracellular electron transfer. 53,66 The ability of NPs to regenerate bacterial nanofiber production agrees with the findings reported by Yu et al. 53 Such observation in response to the interaction between Mn_xFe_{3-x}O₄ NPs and cells was confirmed in the present work by electron microscopy.

So, Fig. 5 summarises the protective role of Mn_{0.2}Fe_{2.8}O₄ NPs to S. oneidensis MR-1 bacterial cells during Cr⁶⁺. The use of Mn_{0.2}Fe_{2.8}O₄ NPs improved the viability of S. oneidensis MR-1 under a sub-lethal concentration of Cr⁶⁺ by 3.3 times, as shown in our previous report.23 Employing Mn_{0.2}Fe_{2.8}O₄ NPs as the adsorbent can limit the availability of Cr⁶⁺ to S. oneidensis MR-1, boosting the tolerance to Cr⁶⁺. 18,69 The positive adsorptive effect of NPs on Cr6+ concerning the viability of bacteria has been reported in the presence of goethite, humic acid,³⁴ and ferric oxyhydroxide mediators. 18,69 As reported in our recent investigation, 23 Cr6+ was adsorbed on Mn_{0.2}Fe_{2.8}O₄ NPs following the Langmuir adsorption isotherm model. Based on this model, the adsorption and desorption rates should be equal. Adsorption is the separation of molecules from the aqueous solution by being attached to the surface of the adsorbent. The desorption is inversely related to adsorption processes, where adsorbates are transferred from the adsorbed state to bulk solution.⁷⁸ This possible continuous adsorption-desorption rate of Cr⁶⁺ can sustain a release of Cr⁶⁺ from the surface of NPs, which makes the exposure of cells to Cr6+ occur at a gradual rate.

Furthermore, Mn_{0.2}Fe_{2.8}O₄ NPs reduce Cr⁶⁺ into Cr³⁺, as shown in Fig. 3B. Cr3+ is less toxic than Cr6+ towards S. oneidensis MR-1.22 Bacterial cells exposed to Cr3+ experienced viability loss but maintained some enzymatic activity and cellular integrity, 22 which explains the morphological response of S. oneidensis MR-1 to Cr⁶⁺ in the presence of Mn_{0.2}Fe_{2.8}O₄ NPs, as shown in Fig. 4B.

4. Conclusion

This study describes a possible protecting role of manganese ferrite nanoparticles (Mn_{0.2}Fe_{2.8}O₄ NPs) to Shewanella oneidensis (S. oneidensis) MR-1 during hexavalent chromium (Cr⁶⁺) bioreduction. Mn_{0.2}Fe_{2.8}O₄ NPs can reduce the highly toxic Cr⁶⁺ to less toxic Cr3+. Under anaerobic conditions, we found that Mn_{0.2}Fe_{2.8}O₄ NPs induced the elongation of the bacterial cells and promoted the formation of nanofibers. Such morphological change could improve the viability of S. oneidensis MR-1 cells in response to the sub-lethal dose of Cr⁶⁺ and, in turn, enhance

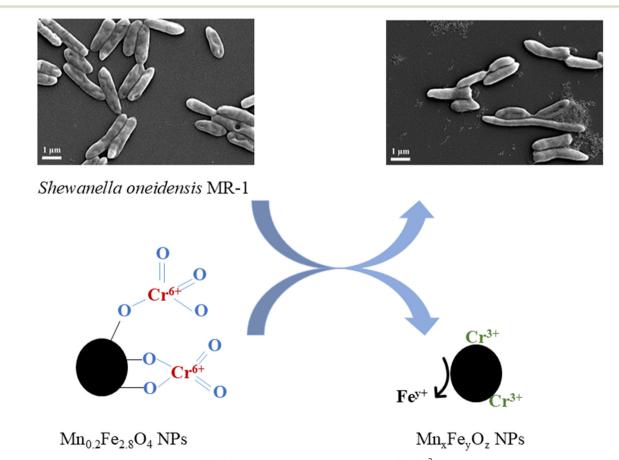


Fig. 5 Illustration of the protective role of Mn_{0.2}Fe_{2.8}O₄ NPs to S. oneidensis MR-1 during [CrO₄]²⁻ bio-reduction.

their detoxification ability. Integrating both S. oneidensis MR-1 and Mn_{0.2}Fe_{2.8}O₄ NPs enhanced Cr⁶⁺ detoxification by 2.1-fold compared to S. oneidensis MR-1 alone and 1.4-fold compared to NPs alone. Therefore, the present article provides evidence of Cr⁶⁺ bio-reduction and the bacterial response to Cr⁶⁺ and Mn_{0.2}-Fe_{2.8}O₄ NPs. This study will open a venue for applying nanotechnology in the bio-remediation of highly contaminated sites by heavy metals.

Data availability

The data within this study is included in either the main article or ESI† figures.

Author contributions

N. T. K. T. and L. C. devised and coordinated the project and provided resources. D. S. R. designed and did most of the experiments and wrote the manuscript. I. T. assisted in particle synthesis and data analysis. N. T. K. T. and S. M. provided expertise, revised the manuscript and helped to acquire funding. E. D. carried out XPS characterisation, processed data and corrected the manuscript. A. M. did a part of the characterisation and edited the manuscript.

Conflicts of interest

The authors declare no competing financial interest.

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