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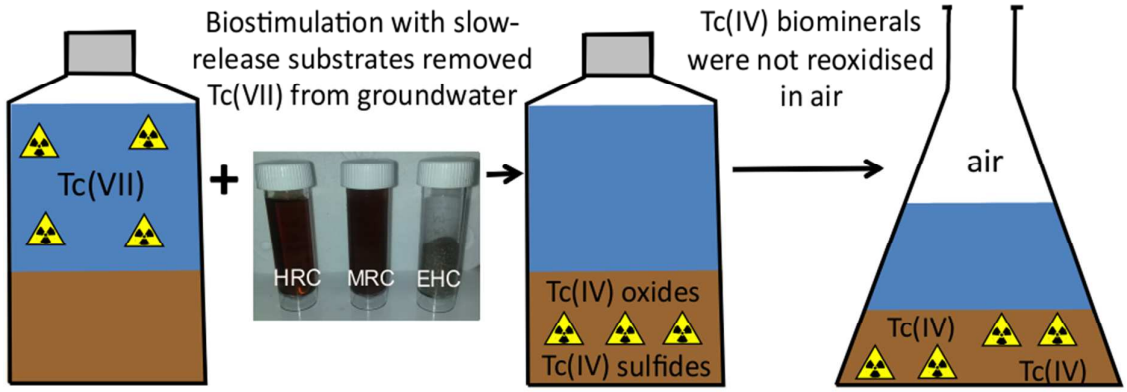

Radionuclides are present in groundwater at contaminated nuclear facilities with technetium-99 one of the most mobile radionuclides encountered. *In situ* bioremediation via the generation of microbially-reducing conditions has the potential to remove aqueous and mobile Tc(VII) from groundwater as insoluble Tc(IV). However, questions remain regarding the optimal methods of biostimulation and the stability of reduced Tc(IV) phases under oxic conditions. Here, we selected a range of slow-release electron donor / chemical reduction based substrates available for contaminated land treatment, and assessed their potential to stimulate the formation of recalcitrant Tc(IV) biominerals under conditions relevant to radioactively contaminated land. These included a slow-release poly-lactate substrate (HRC), a similar substrate with an additional organosulfur ester (MRC) and a substrate containing zero valent iron and plant matter (EHC). Results showed that Tc was removed from solution in the form of poorly soluble hydrous Tc(IV)-oxides or Tc(IV)-sulfides during the development of reducing conditions. Reoxidation experiments showed that these phases were largely resistant to oxidative remobilisation and were more resistant than Tc(IV) produced via biostimulation with an acetate/lactate electron donor mix in the sediments tested. The implications of the targeted formation of recalcitrant Tc(IV) phases using these proprietorial substrates *in situ* is discussed in the context of the long-term management of technetium at legacy nuclear sites.

INTRODUCTION

Technetium is a significant contaminant at legacy nuclear facilities, including Sellafield in the UK, the Hanford site in Washington, USA and Mayak, Russia.[1] In oxygenated environments Tc(VII) is soluble and mobile as the pertechnetate ion (TcO_4^-) but under reducing conditions it precipitates as Tc(IV) species including hydrous, short-chain TcO_2 phases[2–5] and under sulfidic conditions as TcS_2.[6,7] These reductive processes can be microbially mediated and are beneficial for treating radioactive contaminants in the

subsurface.[8–12] Previous studies have shown that the stimulation of sediment microbial communities by the addition of an electron donor can indirectly lead to the reduction of Tc(VII) to poorly soluble Tc(IV) phases, via reaction with biogenic Fe(II) or sulfide.[7,13–16] Moreover, iron(II)-containing minerals are able to reduce Tc(VII) to Tc(IV) abiotically[6,14,17] or in the case of biotite and chlorite, after the minerals have been primed by reaction with Fe(III)-reducing microorganisms.[4] Therefore stimulating the development of microbially-reducing conditions, in particular Fe(III) and sulfate reduction, shows potential for remediating Tc-contaminated groundwaters.

Most previous Tc bioremediation studies have used electron donors in the form of simple organics such as acetate or ethanol.[18–20] However, stimulating the subsurface with these may be unsuitable for radioactively contaminated land due to the likely need for repeated application of large volumes of liquid, which will be logistically challenging at a nuclear licensed site, and may potentially have deleterious effects on contaminant pathways and groundwater flow. In this situation, slow-release electron donors may be more appropriate; typically these are viscous liquids or fine grained solids that remain in the subsurface for longer periods whilst they slowly react (via corrosion or hydrolysis) or are biodegraded to gradually release electron donors to solution.[21–25]

In this study we investigated a range of slow-release substrates pertinent to radionuclide bioremediation including; a slow-release electron donor to stimulate anaerobic microbial metal reduction (HRC), a slow-release electron donor with the potential to stimulate sulfidation (MRC), and a slow-release substrate also containing zero-valent iron (ZVI) as a chemical reductant (EHC). ZVI has previously shown potential to remove Tc(VII) from solution[26,27] and can directly reduce Tc(VII) to poorly soluble Tc(IV).[28] Under anaerobic conditions ZVI corrodes to generate Fe(II) and hydrogen and therefore offers a powerful combination of reactants for Tc(VII) bioremediation; the Fe(0) and the associated corrosion

products, mainly Fe(II) minerals, can reduce Tc(VII) to poorly soluble Tc(IV) abiotically and the H_2 may stimulate a number of beneficial microbial processes.[29,30] These include: H_2 acting as an electron donor to generate additional Fe(II) by stimulating microbial Fe(III) reduction[31,32]; stimulating the production of sulfide via sulfate-reducing bacteria[33,34] which may precipitate Tc-sulfide minerals[7]; and potentially should elevated Tc(VII) concentrations exist, H_2 may also stimulate enzymatic Tc(VII) reduction via hydrogenase enzymes.[8,35–40] Moreover, microbially-mediated Tc(VII) removal has been observed below the predicted solubility threshold for TcO_2 precipitates, likely via sorption of Tc(IV) to sediment at these very low concentrations.[41,42]

Microbially-reduced sediments are known to be susceptible to oxidative remobilisation of redox active radionuclides[12] and biogenic hydrous TcO_2 is partially reoxidised after exposure to air, although it is less susceptible to reoxidation from the addition of nitrate (via the formation of reactive nitrite).[16,43,44] It is crucial to understand the factors that may affect the longevity of microbially-precipitated Tc(IV) in the subsurface in order to tailor successful long-term remediation strategies. As an alternative to stimulating the formation of short-chain hydrous TcO_2 phases, the targeted formation of Tc-sulfides is of interest.[6,7,26] Technetium sulfides are poorly soluble and considered to be the solubility limiting phase for technetium.[45,46] Sulfide mineral phases can fix Tc(VII) through sorption and reductive precipitation[46] and it has been suggested that TcS_2 is more resistant to oxidation compared to TcO_2 under abiotic conditions.[47] Finally, the presence of Fe may play an important role in limiting Tc(IV) reoxidation as Tc(IV) associated with Fe(III) is considered more resistant to reoxidation than $Tc(IV)O_2.nH_2O$.[3,48]

The goal of these experiments was to investigate the effectiveness of slow-release electron donors to remediate Tc(VII)-contaminated groundwater, in particular, to assess whether they could stimulate the *in situ* formation of recalcitrant Tc(IV) species including oxides and

sulfides. The results showed that each slow-release substrate stimulated the removal of Tc_{aq} from solution, and reoxidation experiments indicated that Tc was not remobilised under oxidising conditions.

MATERIALS AND METHODS

Proprietary substrates: The electron donors selected were the proprietary substances Hydrogen Release Compound (HRC), a glycerol poly-lactate compound, and Metals Release Compound (MRC), a glycerol poly-lactate compound containing an organo-sulfur ester, both supplied by Regenesis, and EHC, a mixture of ZVI and food-grade plant matter, supplied by Peroxychem (Table S1). These are designed to be slow-release substrates suitable for sustained stimulation of microbial activity *in situ,* while EHC also contains ZVI, a chemical reductant.

Initial testing of electron donors and Tc solubility: Prior to conducting microbial Tc(VII) reduction experiments, initial tests were performed to assess whether the microbial community present in Sellafield sediments was able to use these electron donors to reduce Fe(III). Microcosms were set up containing 3 g of a gravelly sand sediment collected from a Sellafield site borehole (designed RB27 and fully described in past work)[49], 30 ml of sterile artificial groundwater (containing the following in mM: K^+ 0.089; Na^+ 3.37; Ca^{2+} 1.69; Mg^{2+} 0.795; Cl^- 1.06; HCO_3^- 2.88; NO_3^- 0.332; CO_3^{2-} 1.69; SO_4^{2-} 0.39)[50] and 0.15 g of the slow-release donor compound.[51] The microcosm headspace was degassed with argon and the bottles were incubated in the dark at room temperature. Additional tests were conducted to determine whether 100 Bq ml^{-1} (1.6 μM) ^{99}Tc(VII) as pertechnetate was soluble in the presence of the electron donors in sediment-free systems, by adding 0.15 g of electron donor to 30 ml artificial groundwater under aerobic conditions in duplicate. An additional bottle containing 3 g of sediment, 30 ml artificial groundwater and 0.15 g of EHC was sterilised by autoclaving (120°C, 20 mins) and then spiked with Tc(VII) to assess the sorption of Tc to

EHC in a sterile sediment system. The sorption of technetium to sediment was examined using bottles comprising of 3 g of Sellafield sediment, 30 ml artificial groundwater and ^{99}Tc(VII) in triplicate.

Tc(VII) bioreduction and geochemistry: To investigate how the different electron donors performed in stimulating Tc(VII) bioreduction, sediment microcosms were set up in 120 ml glass serum bottles in triplicate containing 10 g of Sellafield sediment, 100 ml of artificial groundwater, 0.5 g of HRC, MRC or EHC and 100 Bq ml^{-1} (1.6 μM) Tc(VII). The bottles were crimp sealed, the headspace degassed with argon, and then incubated in the dark at room temperature. A positive control contained 5 mM acetate and 5 mM lactate as a simple electron donor mix. A negative control for each slow-release electron donor was sterilised by autoclaving on two occasions, 24 hours apart before spiking with Tc(VII).

Periodic geochemical monitoring was performed by withdrawal of an aliquot of sediment slurry using a needle and syringe degassed with argon using aseptic technique. Iron(II) and total bioavailable iron in sediment slurry were assessed via digestion in 0.5 N HCl or 0.25 N hydroxylamine hydrochloride in 0.5 N HCl, then measurement of Fe(II) and total Fe using the ferrozine assay.[52,53] The supernatant was separated from the sediment by centrifugation (16,200 *g*, 5 minutes), and aqueous Tc was measured by liquid scintillation counting (background counts in "unspiked" liquid scintillation fluid averaged (n = 38) 31.0 ± 4.3 cpm, which defined a minimal detectable ^{99}Tc concentration of 2.2 nM[54]), nitrate, sulfate and volatile fatty acids (VFAs) by ion chromatography (Dionex ICS 5000), and pH and Eh were measured using calibrated electrodes. Gas samples were collected from the microcosm headspace and analysed for the presence of methane and hydrogen via gas chromatography flame ionisation detection (GC-FID).[55]

Molecular ecology: To investigate the composition of the sediment microbial community, DNA was extracted from sediment slurry samples using a PowerSoil DNA Isolation Kit (MO BIO Laboratories INC, Carlsbad, CA, USA). Full details of the methodology are described previously.[49,56] Briefly, for archaeal polymerase chain reaction (PCR) amplification a fragment of the 16S ribosomal RNA gene (approximately 940 base pairs) was amplified using the primers 21F and 958R[57] and bacterial DNA was amplified using the universal 16S rRNA primers 8F and 1492R.[58] The purity of the amplified products was determined by electrophoresis in Tris-acetate-EDTA gel. PCR products were cleaned up, quantified, and sequenced using a Roche 454 Life Sciences Junior System. Qiime 1.8.0[59] was used to analyse the 454 pyrosequencing reads, the Ribosomal Database Project[60] was used for taxonomic classification, and Blastn was used to identify the closest GenBank matches (http://blast.ncbi.nlm.nih.gov).

Speciation of solid-phase technetium: Selected higher radioactivity microcosms were set up to investigate the oxidation state and speciation of technetium using X-ray absorption spectroscopy (XAS). These experiments contained 0.6 g sediment, 10 ml artificial groundwater, 0.1 g HRC, MRC or EHC and 20 kBq ml^{-1} (320 µM) ^{99}Tc(VII) as pertechnetate. The headspace was degassed with argon and the bottles incubated in the dark at room temperature. An additional bottle containing 0.7 g EHC with no sediment was set up to investigate abiotic interactions between the ZVI within the EHC and Tc(VII). After 67 days incubation (EHC microcosms) or 91 days (HRC and MRC), the concentrations of Fe(II)[52] and Tc_{aq} were measured, then the experiment sampled to yield approximately 0.1 g of moist sediment paste, mounted in a standard sample cell and stored at -80°C under argon prior to shipping to the Diamond Light Source, Harwell, UK for analysis. Samples were analysed using XAS on beamline B18 at liquid nitrogen temperature.[61] Tc K edge spectra were collected in fluorescence mode using either a 9 or 36 element Ge detector.

160 **Oxidative remobilisation of Tc(IV)**: To examine how the different electron donors
161 influenced the oxidative remobilisation of Tc, a parallel set of microcosms were prepared and
162 subsequently reoxidised. Sediment microcosms (5 g Sellafield sediment and 50 ml artificial
163 groundwater) were prepared in triplicate with 0.25 g of HRC, MRC or EHC and 120 Bq ml^{-1}
164 (1.9 μM) ^{99}Tc. A positive control was prepared using a 5 mM acetate and 5 mM lactate
165 electron donor mix. A sterile control for the EHC experiment was prepared by autoclaving
166 (120°C, 20 minutes) prior to spiking with $^{99}Tc(VII)$. After 60 days, the fully reduced
167 microcosms were reoxidised by decanting the sediment slurry into sterile 500 ml bottles and
168 aerated daily by opening and shaking for 15 minutes. Sediment slurry was removed
169 periodically for geochemical analysis as described previously. Additional experiments were
170 carried out at higher Tc concentrations to investigate the oxidation state and speciation of
171 technetium by XAS following reoxidation. These contained 0.7 g sediment, 10 ml artificial
172 groundwater, 25 kBq ml^{-1} (400 μM) TcO_4^- and 0.1 g HRC, MRC or EHC. After 139 days of
173 anaerobic incubation, the bottles were opened to air within a 5 litre container and shaken
174 gently on an orbital shaker.

175 **RESULTS AND DISCUSSION**

176 **Initial testing of electron donors and Tc solubility**: Addition of the three slow-release
177 substrates to sediment microcosms lead to the reduction of more than 50% of the 0.5 N
178 hydroxylamine-HCl extractable "bioavailable" iron within 8 days (Figure S1a), confirming
179 the suitability of these electron donors to stimulate anaerobic processes in Sellafield
180 sediment. The amount of "bioavailable" iron increased markedly in microcosms containing
181 EHC and the majority of this was present as Fe(II) (Figure S1a). This effect was not observed
182 in the systems without an additional iron source indicating that the ZVI in EHC was
183 corroding to generate bioavailable Fe(II).

In solubility tests, Tc(VII) at 100 Bq ml^{-1} (1.6 μM) remained soluble in artificial groundwater containing HRC and MRC and, as expected, did not sorb to Sellafield sediment (Figure S1b). The pH of the bottles containing HRC and MRC dropped to below 3 indicating that acidity had been generated, probably as lactic acid from the degradation of the substrates.[51] When both sediment and substrate were present the pH dropped from 7 to around 6.2 (Figure S1a) suggesting the buffering capacity of the sediment counteracted this effect. Tc was removed rapidly from solution in the presence of both EHC and EHC with sterile sediment, confirming that an abiotic reaction with Fe(0) or Fe(II) likely dominated in these systems.

Microbial reduction of Tc(VII) and biogeochemistry: Sediment microcosms were stimulated with the slow-release substrates to investigate their potential for technetium remediation. Geochemical results showed that Fe(II) was produced almost immediately with each slow-release amendment, indicating the rapid development of reducing conditions (Figure 1). Corrosion of ZVI in the EHC system lead to the highest measured concentrations of 0.5 N HCl extractable Fe(II). Tc(VII) was removed from solution almost immediately with EHC, likely due to abiotic reduction by the Fe(II) to form poorly soluble Tc(IV) phases.[28,62] A lag of around four days was observed with the samples amended with HRC, MRC and the acetate/lactate mix, with Tc(VII) removal associated with the onset of microbial Fe(III)-reducing conditions and subsequent abiotic reduction via Fe(II), to form poorly soluble Tc(IV) phases. This process has been observed in past experiments with representative Sellafield sediments stimulated with simple electron donors[19] and in a range of biotic and abiotic systems containing Fe(II).[4,16,48] Near-complete Tc removal was observed within 28 days in the experiments stimulated with MRC, EHC and the acetate/lactate mix, or after 90 days with HRC. The sterile controls containing HRC and MRC did not show significant removal of Tc(VII) from solution, nor did they produce Fe(II), confirming that these

processes were microbially-mediated (Figure 1). Reflecting the results of the initial Tc solubility experiments, Tc removal was also observed in the sterile control containing EHC, again likely due to abiotic ZVI-mediated reduction to poorly soluble Tc(IV) phases.[28,62]

X-ray absorption near edge structure (XANES) data from the experimental end points in higher activity systems showed that the solid-phase samples were dominated by Tc(IV), confirming reductive scavenging as the dominant mechanism for removal (Figure 2). Given the presence of substantial amounts of Fe(II) in these experiments, it is likely that the removal of Tc from solution occurred indirectly via microbial Fe(III)-reduction rather than direct enzymatic Tc(VII) reduction, while in the presence of EHC, abiotic ZVI-mediated reduction is likely to have been the dominant Tc_{aq} removal process.

Analysis of anions showed very rapid removal of nitrate from solution (Figure S2). Tc(VII) reduction generally occurred concurrently with Fe(II) production. Sulfate was fully removed from solution within 90 days with each amendment suggesting sulfidic conditions had developed. It is noteworthy that sulfate concentrations were higher in the MRC system due to the presence of sulfur in the slow-release donor, and that in this experiment Tc removal occurred at the same time as sulfate reduction (between days 4 and 14). Analysis of VFAs, monitored as proxies for organic electron donors, showed that a complex mix of organics was produced from each proprietary amendment (Figure S3). After 90 days, significant quantities of VFAs remained in solution with the HRC and MRC amendments, whilst the VFAs that had been produced in the EHC system were depleted by this point.

Gas was generated in each microbially-active experimental system, indicated by positive pressure observed on sampling. This occurred rapidly in the microcosms containing EHC, which were estimated to have produced more than 60 ml of gas during the 90 day experiment. Likewise, the HRC and MRC microcosms generated approximately 60 ml of gas

after 230 days incubation. Headspace analysis via GC-FID found that at these experimental end-points, the gas was nearly 100% methane. Furthermore, analysis of DNA extracted from the sediment microcosms confirmed the presence of archaea consistent with simulation of methanogenesis (Figure S4). Generating substantial volumes of gas is clearly undesirable for *in situ* bioremediation applications as it could potentially cause pore blocking and alter groundwater flow pathways.[63] Furthermore methane is considered to be a hazardous ground gas.[64] Although anecdotal evidence suggests that methane formation has not been observed in field applications of slow-release electron donors, this clearly warrants further work in the context of remediation of nuclear licensed sites.

Molecular ecology: Samples were taken from sediment microcosms on Day 0 and Day 90 (HRC, MRC and EHC) and DNA present was extracted and analysed to investigate changes in the composition of the microbial community after biostimulation with slow-release substrates (Table S2). 16S rRNA pyrosequencing showed that a diverse range of soil bacteria was present at Day 0 (***Figure 3, Table S3***), and 90 days post-biostimulation the microbial community had shifted towards species associated with anaerobic conditions, which can be linked to maintaining reducing conditions and consequently low concentrations of Tc_{aq} over prolonged time periods.

Following biostimulation with HRC, the microbial community at Day 90 was dominated by bacteria from the Mollicutes class of the Tenericutes phylum (51%), Firmicutes (22%) and Gammaproteobacteria (14%) (Figure 3). Mollicutes are mostly facultative anaerobic bacteria[65], the two most abundant OTUs (operational taxonomic units) were from Mollicutes and comprised 50% of the microbial community (Table S3). These results are consistent with the development of an anaerobic environment during breakdown of the slow-release HRC substrate.

The microbial community that developed after biostimulation with MRC was again consistent with a marked shift to anoxia after biostimulation, and was dominated by bacteria from Firmicutes (72%) and Gammaproteobacteria (21%) (Figure 3). Four of five most abundant OTUs were most closely related to "Bacterium Irt-JG1-53" (Table S3) isolated from uranium mine waste[66] and comprised 33% of the microbial community; these OTUs were assigned to *Ruminococcus*, a strictly anaerobic genus of heterotrophic bacteria.[67] Six OTUs were assigned to *Desulfosporosinus meridiei*, a known spore forming and sulfate-reducing bacterium[68] and previously detected in MRC-amended sediments.[69] *Desulfosporosinus meridiei* was undetected in the Day 0 sample, but by Day 90 comprised 2.1% of the microbial community, consistent with the stimulation of sulfate reduction.

Biostimulation with EHC lead to a bacterial community dominated by Bacteroidetes (62%) and Firmicutes (25%) (Figure 3), and an archaeal community dominated by Crenarchaeota (67%) and Euryarchaeota (33%). Most bacteria within the Bacteriodales are either facultative or strict anaerobes and commonly found in organic-rich anaerobic environments.[70,71] All five of the most abundant bacterial OTUs were assigned to Bacteroidales and were most closely related to uncultured bacteria from methane-rich or methanogenic environments (Table S3). All five of the most abundant archaeal OTUs were closely related to species associated with methanogenic environments (Table S3): two were assigned to the known methanogens Methanomassiliicoccaceae within the Euryarchaeota, while three were assigned to Crenarchaeota and closely related to uncultured species from methane-generating environments. This suggests that the microbial community was highly anaerobic and supports the biogenic origin of the methane produced after biostimulation of sediments with slow-release substrates.

Speciation of solid-phase technetium: Selected higher radioactivity solid samples were analysed using XAS to identify the speciation of Tc. The rate and extent of Tc removal were

lower in these high level (20 kBq ml^{-1}, 320 μM) experiments compared to the low level (0.1 kBq ml^{-1}, 1.6 μM) experiments as observed previously[13] and suggesting some inhibition of bioreduction processes at elevated Tc concentrations (approximately 15 – 60 % of Tc was removed within 120 days, see supporting information for further discussion).

Analysis of the XANES spectrum for the HRC sample, using linear combination fitting between standards for Tc(VII) as pertechnetate and Tc(IV) as TcO_2[72] indicated the sample contained approximately 75% Tc(IV) and 25% Tc(VII) (Figure 2). The Tc(VII) observed is most probably due to Tc(VII) in the pore-waters of the sample. Indeed, past workers have observed similar levels (15 – 50 %) of Tc(VII) in XANES from partially oxidised sediments.[3,73] As the rate of reduction was slow in the HRC XAS experiments, a significant amount of Tc(VII) would certainly be present in the aqueous phase associated with the moist sediment pellet analysed using XAS. The EXAFS data were fitted assuming contributions of 75% Tc(IV) as hydrous TcO_2 and 25% Tc(VII) as pertechnetate and using relevant Tc(IV) and Tc(VII) models from the literature.[2,74,75,] Here, a good fit was obtained for the first peak with 4.4 O atoms from Tc(IV)-O at 2.01 Å and 1 O from Tc(VII)-O at 1.65 Å reflecting the Tc(IV) and Tc(VII) components of the sample (Figure 4, Table S4). The fit was further improved by the addition of 1 Tc atom at 2.58 Å suggesting that the Tc(IV) may be present as hydrous TcO_2.[2,76] Given that not all the Tc had been removed from solution in this sample, XANES analysis was attempted on the aqueous phase after 139 days (~30 ppm Tc). Linear combination fitting of the resulting spectra between relevant Tc(IV) and Tc(VII) standards[72] found ~50% of Tc in the aqueous phase was present as Tc(IV) with ~50% as Tc(VII) (Figure 2). This suggested that a significant fraction of the aqueous phase was present as Tc(IV) colloids or organic complexes, as reported in past studies.[7,20,37,39] Clearly, the presence of a significant component of colloidal or organic-complexed Tc(IV) in these systems is potentially problematic and warrants further research.

While the edge position confirmed that Tc(IV) was precipitated following biostimulation with MRC, both the XANES and the EXAFS were markedly different to the TcO_2 standard and the other samples analysed (Figure 2, Figure S5) suggesting a different coordination environment in this sample. Inspection of the MRC Fourier transform showed the first shell was present at 2.36 Å (Figure 4, Figure S5) which is consistent with a TcS_2-like coordination environment[6,26] and therefore the TcS_2 crystal structure[77] was used to inform the fitting. A good fit was obtained with 6 S atoms at 2.36 Å and 2 Tc atoms at 2.78 Å (Table S4) confirming the formation of a Tc(IV)S_2 phase, likely stimulated by the additional sulfur present in MRC. The microbially-mediated formation of TcS_2 phases during stimulated sulfate reduction has been observed previously in both pure culture and sediment based systems[7,9] although hydrous TcO_2 phases are more commonly reported even in sulfate-rich marine environments.[13] In one recent study on sediment systems, partial Tc-sulfide formation was stimulated by indigenous microorganisms over several months when the system was enriched in sulfate highlighting the link between elevated sulfur concentrations, long incubations and TcS_2 formation.[7]

The edge position of Tc in both the EHC sediment, and the abiotic EHC no sediment systems confirmed that it was present as Tc(IV) (Figure 2). However, there were some modest differences between the EXAFS from these two samples, with a dampening of the oscillations around 9 and 11 $Å^{-1}$ in the EHC sediment system compared to the abiotic EHC spectrum, a greater height of the second peak in the Fourier transform of the EHC sediment spectrum, while the abiotic EHC spectrum showed some evidence for increased long range order to ~3.4 Å (Figure S5). The hydrous TcO_2 model[2] was used to fit these spectra. A good fit was obtained for the EHC sediment sample with six O atoms at 2.05 Å and 2.1 Tc atoms at 2.54 Å (Figure 4, Table S4), suggesting the Tc(IV) was present as short-chain polymeric hydrous TcO_2.[2,78] Recent work has highlighted the potential for attachment of short-chain

hydrous TcO_2 to surface Fe-O octahedra.[3,4,43,48] In the current study, the EHC sediment fit was slightly improved with a "short Fe" contribution with 1.6 Tc atoms at 2.55Å and with a physically realistic contribution of 0.5 Fe atoms at 2.56 Å (Figure S6, Table S5), suggesting an association of the hydrous TcO_2 chains with Fe was possible in this Fe-rich system, similar to past work.[4,48] A good fit was obtained for the EHC with no sediment with six O atoms at 2.05 Å and two Tc atoms at 2.51 Å, again suggesting it was present in the form of short-chain polymeric hydrous TcO_2 (Figure 4, Table S4).

Oxidative remobilisation of Tc(IV): Experiments were performed to investigate the potential for oxidative remobilisation of Tc(IV) in the treated sediments. These were designed to generate 'end member' highly oxidising conditions rather than simulate conditions that might be encountered in the subsurface. The results showed that the Tc(IV) formed with each of the slow-release amendments was recalcitrant to reoxidation with air, but this was not the case with the Tc(IV) produced after biostimulation with an acetate/lactate mix (Figure 5). Geochemical monitoring confirmed that oxidising conditions had been generated indicated by nitrate and sulfate in solution and 0.5N HCl-extractable Fe(II) being reoxidised to Fe(III) (Figure S7).

For the HRC and MRC amendments, the lack of Tc(IV) reoxidation might be due to the presence of excess electron donors in the form of long-lived VFAs (Figure S8) which could be metabolised by the indigenous microbial community, offering some redox buffering to protect the Tc(IV). This effect has been observed previously, where the presence of electron donor buffered U(IV) from oxidative remobilisation.[55] It is noteworthy that in these systems after the initial reoxidation of Fe(II) to Fe(III), it appeared that Fe(III)-reduction recommenced, confirming that these systems were poised close to anaerobic conditions due to the presence of residual electron donor. Furthermore, in the MRC system there was little sulfate released to solution until the later stages of reoxidation (Figure S7). This suggests that

357 the sulfide phases formed by microbial reduction, including TcS_2, were recalcitrant to
358 reoxidation under these conditions and also acted as redox "buffers". Given that previous
359 research has also demonstrated that Tc(IV)-sulfides are more resistant to oxidative
360 remobilisation than Tc(IV)-oxides[47] the use of electron donors that favour sulfidic conditions
361 such as MRC seem an appropriate bioremediation option for Tc-contaminated groundwaters,
362 although the impacts of sulfidation on other groundwater contaminants would need to be
363 considered further.

364 The sediments stimulated with EHC contained negligible amounts of VFAs after 14 days
365 of reoxidation. Likewise, less than 0.3 mM VFAs were detected in solution in the sterile
366 EHC system throughout the course of the reduction and reoxidation experiments. Therefore
367 in both cases it is unlikely that the presence of VFAs played a dominant role in protecting
368 Tc(IV) in these systems. The majority of the 0.5 N HCl extractable Fe(II) had been
369 reoxidised to Fe(III) in both the EHC and sterile EHC bottles by the end of reoxidation, but
370 despite this, the oxidative remobilisation of Tc(IV) was not observed. In this case, it is
371 possible that the unreacted ZVI or ZVI which had corroded to poorly leachable Fe(II)-bearing
372 phases such as magnetite may have buffered the system from reoxidation.[3,48,79]

373 It is noteworthy that Tc(IV) in the form of hydrous $Tc(IV)O_2$ was generated by
374 biostimulation with HRC, EHC and acetate and lactate[19] but only the Tc(IV) from
375 acetate/lactate biostimulation was reoxidised. This suggests that the redox buffering of the
376 slow-release substrates may be responsible for protecting the Tc(IV) from reoxidation.

377 Parallel higher radioactivity samples were prepared for XAS analysis to investigate the
378 speciation of Tc in these reoxidised systems. Again, the reaction rates in the higher Tc
379 experiments (25 kBq ml^{-1}, 400 μM Tc) were lower than in the low level (0.1 kBq ml^{-1} Tc, 1.6
380 μM) experiments. A reoxidation study was not performed on the high level HRC system

given the considerable amounts of Tc(IV) remaining in the aqueous phase (Figure 2). Some oxidative remobilisation of Tc(IV) in the high level MRC experiment occurred and a partially-reoxidised sample was collected for XAS analysis after 24 days of exposure to air when 63% of the Tc had remobilised to solution. The EXAFS data for this sample showed significant differences to the reduced TcS_2 with a shift of the first peak to a shorter atomic distance (Figure S9). Fitting was informed by past work that suggested TcS_2 can reoxidise to Tc(IV)-oxides in air[6] and by the short-chain hydrous TcO_2 model.[2] Linear combination fitting of the EXAFS data suggested a ~60% contribution from TcS_2 and ~40% contribution from short-chain hydrous TcO_2; shell-by-shell fitting using these contributions generated a good fit and suggested that partial oxidation of the TcS_2 to hydrous TcO_2 had occurred (Figure 6, Table S6).

Minimal amounts of Tc(IV) (approximately 6%) were reoxidised in the high level EHC sediment system after 89 days of exposure to air. XANES confirmed that Tc(IV) dominated in the reoxidised solid sample and EXAFS showed the reoxidised sample had a very similar coordination environment to the parallel reduced sample (Figure S9). Here, a good fit was obtained using the short-chain hydrous TcO_2 capped with Fe model[3,4,43,48] (Figure 6, Table S6). This is consistent with the iron-rich EHC environment and suggests that armouring of Tc(IV) by reoxidised Fe(III)[3,48] could be occurring, or incorporation of the Tc(IV) into secondary Fe phases[80–82], or that the system was redox buffered by residual ZVI.

ENVIRONMENTAL IMPLICATIONS

Tc(VII) is a problematic, mobile contaminant in groundwater at nuclear facilities. Here we showed that slow-release electron donors can stimulate microbially-reducing conditions to reduce Tc(VII) to Tc(IV), mostly via reduction mediated by reducing minerals. In addition, in the sterile EHC system we demonstrated that anaerobic corrosion of ZVI removed Tc(VII) from solution abiotically. However, colloidal or organic-complexed Tc(IV) was identified at

higher Tc concentrations in the HRC system; this would be undesirable in the subsurface due to the potential for increased radionuclide transport. The Tc in the systems treated with slow-release substrates was resistant to oxidative remobilisation, presumably due to redox buffering from residual organic electron donor in the HRC and MRC treatments and residual abiotic Fe(0) or non-acid leachable Fe(II) in the EHC systems. This suggests the Tc(IV) is likely to be stable over extended periods when slow-release substrates are used, and in contrast to Tc behaviour during bioreduction with simple electron donors such as acetate and lactate. This highlights the importance of redox buffering in maintaining low concentrations of Tc_{aq} for bioremediation at nuclear sites.

ASSOCIATED CONTENT

Supporting Information. Additional results including tables and figures showing additional geochemical and microbiological results are available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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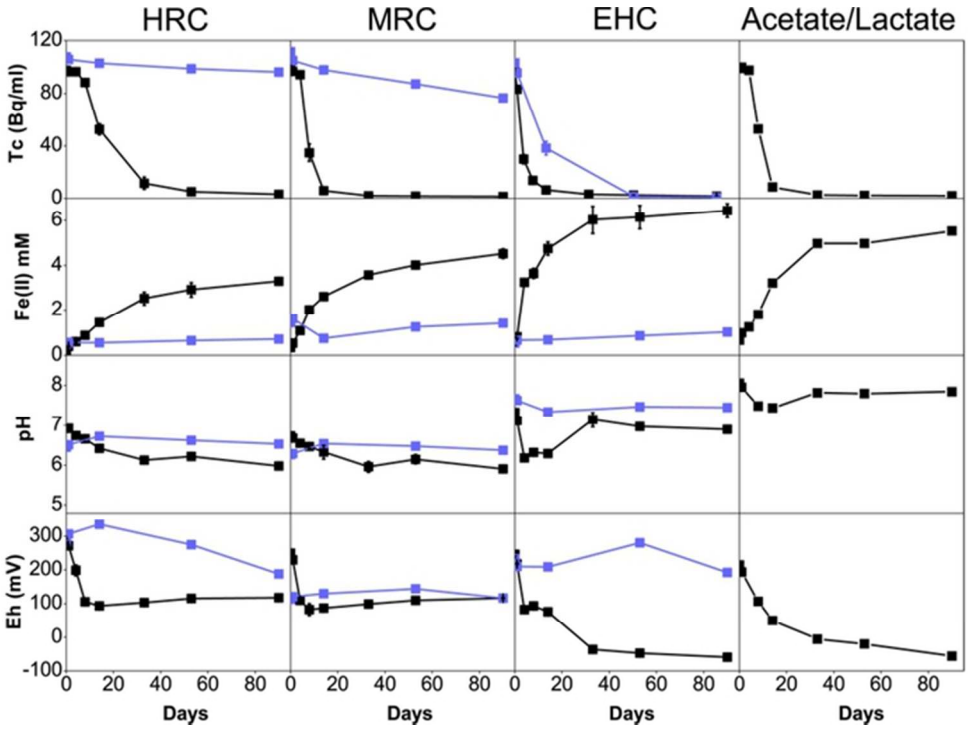

Figure 1. Results of microbial Tc(VII) reduction experiments. Black lines indicate biostimulated triplicate microcosms, blue lines are sterile controls. Points represent the average of three measurements, error bars +/- 1 SD. The acetate/lactate microcosm was a positive control.

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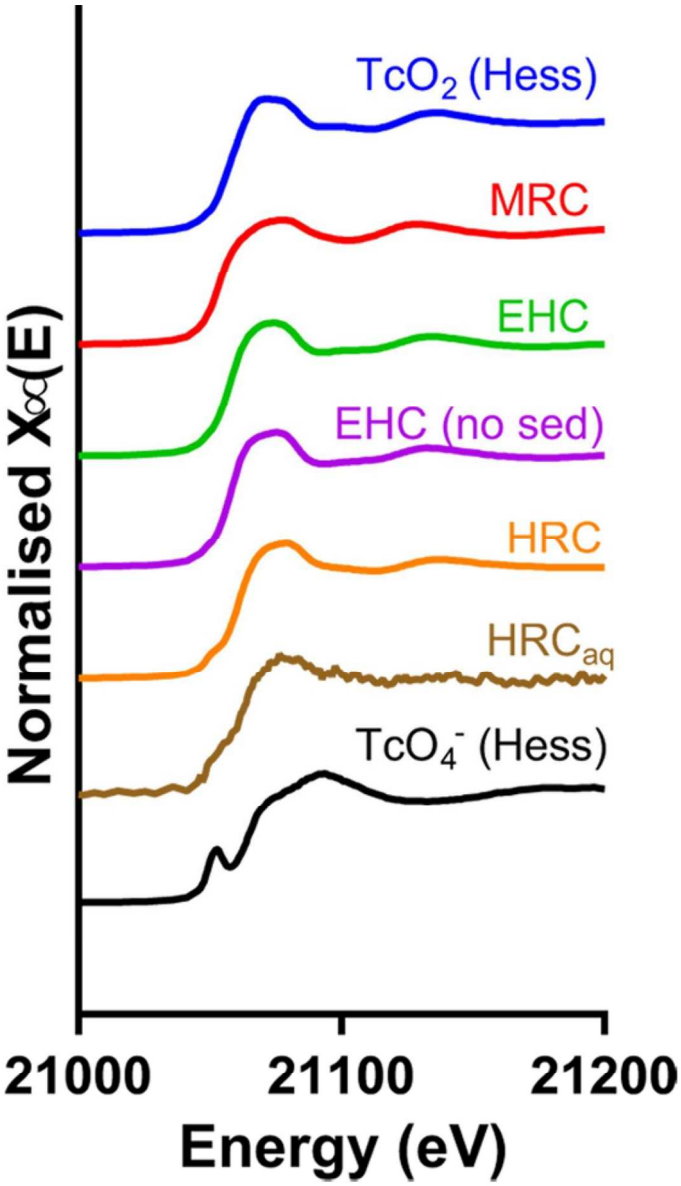

Figure 2. Solid phase XANES data showing reduction of Tc(VII) to Tc(IV) following amendment of sediments with slow-release substrates, or EHC with no sediment 'no sed' and aqueous phase XANES for the HRC amended sample. There is a clear but minor pre-edge Tc(VII) feature in the HRC amended sediment. The Tc(IV) and Tc(VII) standards are from Hess et al.72

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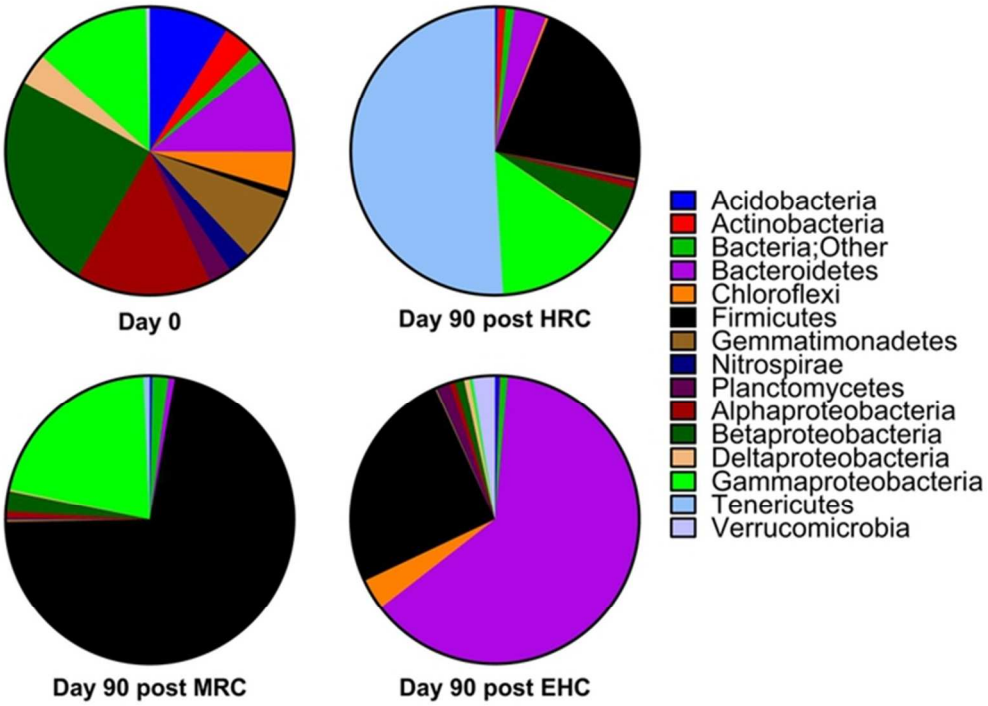

Figure 3. Bacterial phylogenetic diversity within Sellafield sediments after stimulation with HRC, MRC and EHC. Phyla/classes detected at greater than 1% of the bacterial community are illustrated.

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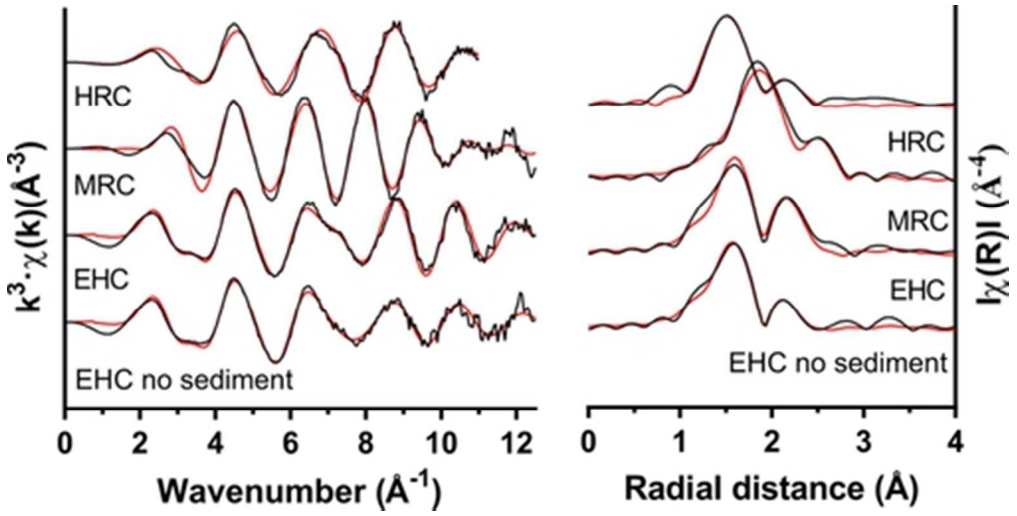

Figure 4. Non-phase shift corrected EXAFS data (black) and fits (red) for sediments biostimulated with HRC, MRC and EHC and for EHC with no sediment; fits are presented in Table S4.

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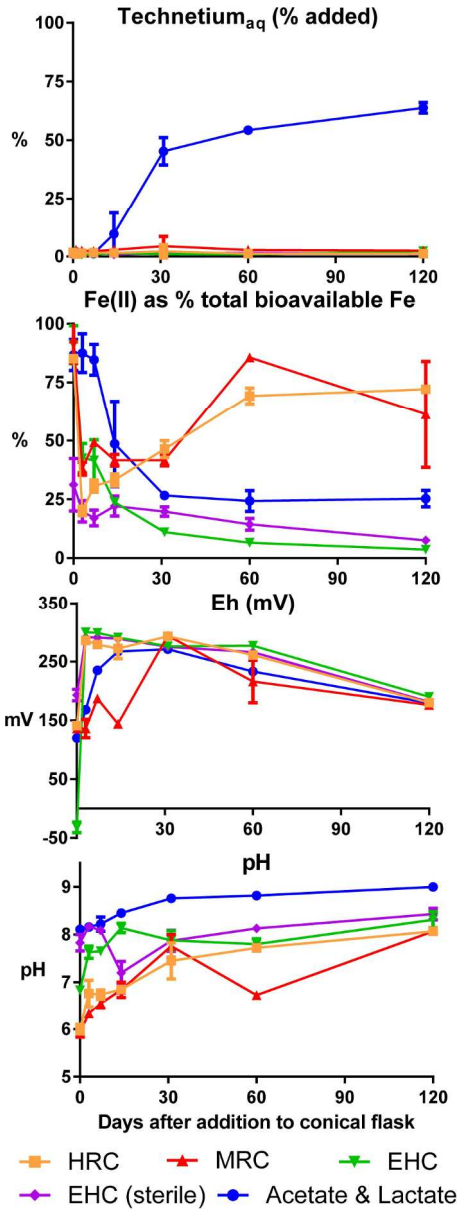

Figure 5. Reoxidation geochemistry for the low-level sediment microcosms. The Tc(IV) that had been formed by biostimulation with slow-release electron donors was recalcitrant to oxidative remobilisation, unlike with acetate and lactate (blue lines).

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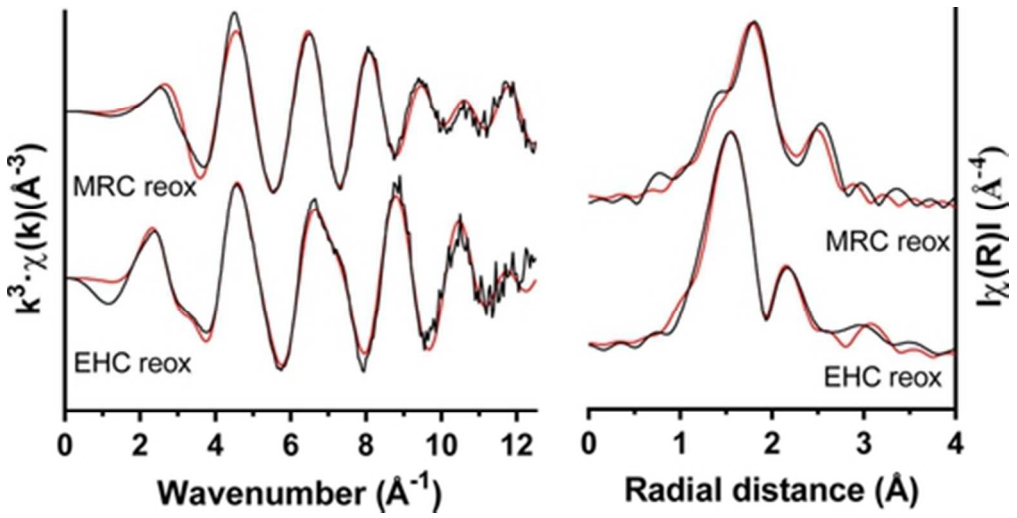

Figure 6 Non-phase shift corrected EXAFS data (black) and fits (red) for sediments biostimulated with MRC and EHC and then reoxidised in air. Fits are presented in Table S6.

42x21mm (300 x 300 DPI)

Long-term immobilization of technetium via bioremediation with slow-release substrates

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Discussion of Tc(VII) reduction rates at different Tc concentrations

In our low level experiments with HRC, MRC and EHC (0.1 kBq ml^{-1}, 1.6 µM Tc in the reduction experiments, 0.12 kBq ml^{-1}, 1.9 µM Tc in the reoxidation experiments[1]), almost all Tc(VII) was removed from solution within 90 days (Figure 1 main manuscript). Tc_{aq} removal was observed to be slower in the higher Tc(VII) concentration XAS experiments (20 kBq ml^{-1}, 320 µM in the reduction experiments, 25 kBq ml^{-1}, 400 µM in the reoxidation experiments), particularly in the high Tc HRC experiment where considerable quantities of Tc(VII) remained in solution after 139 days (Figure 2 main manuscript), but also to some extent in the time it took for Tc_{aq} removal in the high level MRC experiment. This effect has been observed previously e.g. by Burke et al. (2005). Possible explanations for this could be that: (a) Tc(VII) reduction was limited by the amount of bioavailable Fe(III) present, such as observed by Wildung et al. (2004); or (b) that the elevated Tc concentrations were toxic and so inhibited the activity of sediment bacteria.

(a) In terms of 0.5 N hydroxylamine-hydrochloride extractable "bioavailable" Fe(III) in the elevated Tc experiments, there was considerably less present in the HRC system at Day 139 compared to MRC and EHC (see table below), and certainly not enough to supply the electron demand to reduce 0.4 mM of Tc(VII) via a three electron transfer. Therefore it seems likely that Tc(VII) reduction may have been inhibited at elevated Tc(VII) concentrations due to limited amounts of microbial Fe(II) to drive the reduction.

Stoichiometry of Tc and Fe in the elevated Tc XAS reoxidation experiments at Day 139

Treatment	^{99}Tc(VII) added (mM)	^{99}Tc on solid if fully reduced (mM)	Fe(II) in slurry* (mM)	Fe(total bioavailable) in slurry (mM)	Fe(total bioavailable) in sediment (mM)	Fe(II) / Tc(VII)
HRC®	0.4	5.7	0.56	0.87	12	1.4
MRC®			2.1	2.2	32	5.3
EHC®			4.45	7.6	108	11

* Some of the Fe(II) will be present in the aqueous phase therefore it is not possible to estimate Fe(II) in sediment

Wildung et al. (2004) found that Fe(II)/Tc(VII) values greater than 4.3 were sufficient to reduce >80% of the Tc(VII) whereas most sediments with Fe(II)/Tc(VII) less than 1.1 reduced less than 20% Tc(VII). The Fe(II)/Tc(VII) in our HRC experiment was 1.4, which may be too low to drive Tc(VII) reduction.

(b) To further investigate whether the toxicity effects of high concentrations of Tc, an aliquot of sediment slurry (0.5 ml) was taken from each high level experiment after 139 days and added to 100 ml freshwater minimal medium with 20 mM nitrate or 20 mM ferrihydrite as the electron acceptors and 10 mM acetate as the electron donor. In this subsequent experiment the Tc concentrations was diluted to 125 Bq/ml. Results showed

[1] the different concentrations were due to a different stock solution being used

that the inoculum from each high level experiment was capable of both nitrate and Fe(III)-reduction in the minimal medium. This suggests that exposure to high levels of Tc did not sterilise the sediments, although it is possible the Tc(VII) may have inhibited the sediment microbial community to some extent.

Therefore in summary, the rate of Tc(VII) reduction in the HRC system may have been limited by the low amounts of Fe(II) to drive the reaction. In both the HRC and MRC systems microbial Fe(III)-reduction may have been slower due to toxicity effects from the high Tc concentrations. Tc(VII) reduction was not slower in the EHC system, which presumably was driven by chemical reduction at these higher Tc concentrations.

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Supporting Tables

Table S1. Details of slow-release electron donors. All are suitable for direct injection to the subsurface to treat contaminated groundwater; certain formulations of EHC® are also suitable for deployment in permeable reactive barriers

Slow-release donor	Details	Previously used for	Selected for use here because	Reference
Hydrogen Release Compound® (HRC®)	A glycerol tripoly-lactate compound that is designed to degrade slowly in groundwater to generate lactic acid over a prolonged time period. Lactic acid is then fermented by anaerobic microbes to release hydrogen which acts as an electron donor	Reductive dechlorination of chlorinated solvents	As a standard slow-release substrate that has widely been used in the subsurface. Potentially may stimulate the reductive precipitation of TcO_2, sulfate-reducing bacteria, or even direct Tc(VII) reduction via hydrogenases	www.regenesis.com
Metals Remediation Compound® (MRC®)	Similar to HRC® but also containing an organosulfur ester	Direct microbial reduction of Cr(VI) or indirect reduction via microbial generation of Fe(II) or sulfide	Potential to generate Tc(IV) sulfides	www.regenesis.com
EHC®	Mixture of micro-scale zero valent iron (~ 40 %) and food grade plant matter (~ 60%).	Chlorinated solvents	ZVI content means has the potential to stimulate Tc(VII) reduction in sediments containing low concentrations of bioavailable iron (such as Sellafield sandstone), can produce H_2 to stimulate microbial reduction	www.peroxychem.com

Table S2. Details of molecular ecology sequences

Sample	Number of reads	Number after quality control, chimera check & denoising	Number of identified OTUs	Shannon diversity at 4,366 reads
HRC® Day 0	7,557	5,236	820	8.57
HRC® Day 90	8,382	7,162	380	4.65
MRC® Day 90	5,526	4,482	353	6.08
EHC® Day 90	5,964	4,662	377	6.25
EHC® Day 90 Archaea	5,101	4,858	85	4.34

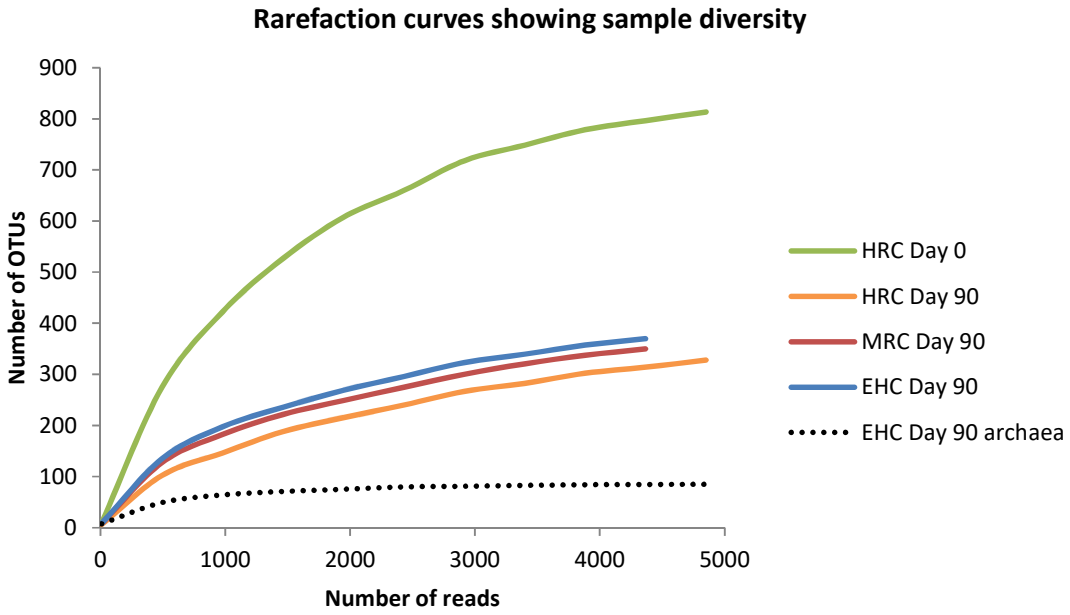

Table S3. Closest phylogenetic relatives of the five most abundant OTUs after biostimulation with slow-release electron donor substrates

OTU ID	No.	%	Classification assignment (consensus lineage)	Family	Closest phylogenetic relative				
					Name	Accession Number	% ID similarity (max)	Score (max)	Description
HRC® Day 0 bacteria									
25	156	3.0	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Methylophilales;f__Methylophilaceae	*Methylophilaceae*	Uncultured *Methylophilus* sp. clone Me60A10	GU472577.1	98 (98)	1049 (1108)	Sulfur cycle prokaryotes in low-sulfate lake. Similar results for aerobic methanotrophs, methylotrophs (*Methylotenera versatilis* strain 301, NR_074693.1, 96% similarity), methane consumption Arctic lakes
23	113	2.2	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae	*Comamonadaceae*	*Ideonella* sp. 201-F6	LC002525.1	99 (99)	955 (955)	PET degrading consortium. Similar results for elevated CO_2, weathered sandstone, rice paddy soils, biofilm reactor
31	95	1.8	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Polaromonas;s__	*Comamonadaceae*	*Polaromonas* sp. H8N	KU586657.1	99 (99)	897 (910)	Arctic & Antarctic glacial surfaces. Similar results for anaerobic digestor, landfill leachate, rhizosphere, river water
38	94	1.8	k__Bacteria;p__Nitrospirae;c__Nitrospira;o__Nitrospirales;f__Nitrospiraceae;g__Nitrospira;s__	*Nitrospiraceae*	Uncultured *Nitrospira* sp. clone Nsp1 16S	AY876621.1	99 (99)	782 (782)	Uncultured bacterium from study of nitrite oxidising community in grassland soils. Similar results for tufa, rhizosphere, methane emitting soils, permafrost, red mud, nitrite-oxidising bioreactor (*Nitrospira* sp. strain GC86, Y14644.1, 96% similarity)
40	89	1.7	k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__MND1;f__;g__;s__		Uncultured bacterium clone CL71 16S	KF247866.1	99 (99)	1099 (1099)	Uncultured bacterium from wetland sediments. Similar results for methane emitting soils, peat soil methanotrophs, river sediment, rhizosphere, rice paddy soils
HRC® Day 90 bacteria									
0	3062	42.8	k__Bacteria;p__Tenericutes;c__Mollicutes;o__RF39;f__;g__;s__		Uncultured bacterium clone D14R15C106	FM956796.1	99 (99)	969 (969)	Uncultured bacterium from rice field soil. Similar results for soil bacterial interactions with iron oxides, anaerobic digestor
97	478	6.7	k__Bacteria;p__Tenericutes;c__Mollicutes;o__RF39;f__;g__;s__		Uncultured bacterium clone D14R15C106	FM956796.1	99 (99)	1068 (1068)	Uncultured bacterium from rice field soil. Similar results for anaerobic digesters
16	209	2.9	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Pelosinus;s__	*Veillonellaceae*	*Psychrosinus fermentans* strain FCF9	NR_115860.1	96 (96)	818 (848)	Lactate fermenting bacterium from Antarctic lake. Similar results for metal-reducing *Pelosinus* UF01 (DQ295866.1, 95% similarity), a lactate utilising population under Fe(III)-reducing conditions in rice field soil, uranium bioreduction
8	197	2.8	k__Bacteria;p__Bacteroidetes;c__Flavobacteriia;o__Flavobacteriales;f__Flavobacteriaceae;g__Flavobacterium	*Flavobacteriaceae*	*Flavobacterium* sp. WB2.3-63	AM934649.1	98 (98)	708 (708)	Study of influence of aerobic heterotrophs on forest soil communities, also study of hard-water rivulet. Similar results for acetate amended subsurface, lake sediments
141	193	2.7	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__Pseudomonas;s__	*Pseudomonadaceae*	*Pseudomonas* sp. type strain HMPB4	AM746975.1	99 (99)	870 (877)	Psychrotropic bacteria. Similar results for Antarctic / glacial / permafrost *P. mandelli*, humic degraders, elevated CO_2 soils, *P. syringae*, *P. frederiksbergensis* (NR_117177.1 and NR_028906.1), coal gasification site

OTU ID	No.	%	Classification assignment (consensus lineage)	Family	Closest phylogenetic relative				
					Name	Accession Number	% ID similarity (max)	Score (max)	Description
MRC® Day 90 bacteria									
3	565	12.6	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminococcus;s	*Ruminococcaceae*	Bacterium Irt-JG1-53	AJ295665.1	99 (99)	1038 (1038)	Bacteria in uranium waste mining piles. Similar results from hot springs, anaerobic biocathodes, onion degrading bacteria, rhizosphere
6	449	10.0	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae	*Ruminococcaceae*	Bacterium Irt-JG1-53	AJ295664.1	94 (94)	825 (825)	Bacteria in uranium waste mining piles. Similar results from rhizosphere, onion degrading bacteria, dehalogenating enrichment
1292	251	5.6	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminococcus;s	*Ruminococcaceae*	Bacterium Irt-JG1-53	AJ295664.1	99 (99)	964 (964)	Bacteria in uranium waste mining piles. Similar results from hot springs, rhizosphere, dehalogenating enrichment
141	245	5.5	k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pseudomonadales;f__Pseudomonadaceae;g__Pseudomonas;s__	*Pseudomonadaceae*	*Pseudomonas* sp. type strain HMPB4	AM746975.1	99 (99)	870 (877)	Psychrotropic bacteria. Similar results for Antarctic / glacial / permafrost *P. mandelli*, humic degraders, elevated CO_2 soils, *P. syringae*, *P. frederiksbergensis* (NR_117177.1 and NR_028906.1), coal gasification site
613	227	5.1	k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminococcus;s__	*Ruminococcaceae*	Bacterium Irt-JG1-53	AJ295664.1	99 (99)	957 (957)	Bacteria in uranium waste mining piles. Similar results from rhizosphere, hyper-arid environment, hot springs, onion degrading bacteria
EHC® Day 90 bacteria									
5	736	15.8	k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__BA008;g__;s__	BA008	Uncultured bacterium clone t30d34L46	FM956231	98 (98)	650 (650)	Uncultured bacterium from study of syntrophic oxidation of propionate under methanogenic conditions in rice field soil. Similar results for rice paddy soils, soil bacterial interactions with iron oxides
9	306	6.6	k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__BA008;g__;s__	BA008	Uncultured Bacteroidetes bacterium clone BEMB12B-2H1	KJ955693.1	96 (97)	821 (834)	Uncultured bacterium from a hydrocarbon contaminated site. Similar results from pristine aquifer, bioremediation of hydrocarbon & chlorinated solvents, coal tar DNAPL, phenol contaminated aquifer
150	260	5.6	k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__BA008;g__;s__	BA008	Uncultured Bacteroidetes bacterium clone BEMB11B-2B1	KJ955674.1	97 (97)	805 (810)	Uncultured bacterium from a hydrocarbon contaminated site. Similar results from pristine aquifer, bioremediation of hydrocarbon & chlorinated solvents, fuel oil degrading sediments
10	202	4.3	k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__;g__;s__		Uncultured bacterium clone BProP7A06	LK024884.2	97 (98)	1103 (1126)	Uncultured bacterium from study of oxidation of ethanol, propionate and butyrate in methane emitting soil. Similar results from iron reducers from As contaminated paddy soil, petroleum contaminated sediments, biofilm reactor for wastewater treatment, PCB dechlorination
461	140	3.0	k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__BA008;g__;s__	BA008	Uncultured Bacteroidetes bacterium clone BEMB12B-2H1	KJ955693.1	98 (98)	829 (829)	Uncultured bacterium from a hydrocarbon contaminated site. Similar results from pristine aquifer, bioremediation of hydrocarbon & chlorinated solvents, coal tar DNAPL, anaerobic digestor

OTU ID	No.	%	Classification assignment (consensus lineage)	Family	Closest phylogenetic relative				
					Name	Accession Number	% ID similarity (max)	Score (max)	Description
EHC® Day 90 Archaea									
4	906	18.6	k__Archaea;p__Crenarchaeota;c__MCG;o__pGrfC26;f__;g__;s__		Uncultured archaeon clone L15	KJ424509.1	98 (98)	1076 (1085)	Uncultured archaeon from study of microbial methane formation in deep aquifers of coal sedimentary basin. Similar results to mining impacted sediments, acidic red soils, lake sediments. 95% similarity to anaerobic methanogenic archaeon ET1-8 (score 800)
1	817	16.8	k__Archaea;p__Euryarchaeota;c__Thermoplasmata;o__E2;f__[Methanomassiliicoccaceae];g__Methanomassiliicoccus;s__	*Methanomassiliicoccaceae*	Archaeon LL37A29	AJ745146.1	97 (97)	937 (1003)	Rice Cluster III archaea. Similar results to study of cultivating methanogens from deep aquifers, anaerobic filter system, anaerobic reactor, wastewater treatment, granular sludge. 94% similarity to *Methanomassiliicoccus luminyensis* strain B10 (score 976)
7	451	9.3	k__Archaea;p__Crenarchaeota;c__MCG;o__pGrfC26;f__;g__;s__		Uncultured crenarchaeote clone EP091-2.90	JF789765.1	96 (98)	892 (928)	Anaerobic metabolism in freshwater wetlands. Similar results for deep aquifers, sludge, uranium mine tailings, acid mine drainage system, deep aquifers of coal sedimentary basin. 95% similarity to anaerobic methanogenic archaeon ET1-10 (score 713)
26	409	8.4	k__Archaea;p__Crenarchaeota;c__MCG;o__pGrfC26;f__;g__;s__		Uncultured archaeon clone L15	KJ424509.1	97 (97)	874 (883)	Uncultured archaeon from study of microbial methane formation in deep aquifers of coal sedimentary basin. Similar results to mining impacted sediments, acidic red soils, lake sediments.
15	280	5.8	k__Archaea;p__Euryarchaeota;c__Thermoplasmata;o__E2;f__[Methanomassiliicoccaceae];g__Methanomassiliicoccus;s__	*Methanomassiliicoccaceae*	*Methanomassiliicoccus luminyensis* strain B10	NR_118098.1	96 (98)	937 (1002)	Methanogenic archaeon from human faeces. Similar results for lake sediment, anaerobic filter system, anaerobic sludge reactor, rice paddy field soils

Table S4. Details of EXAFS fit parameters for the technetium minerals formed after biostimulation with HRC®, MRC® and EHC® [a]

Sample	Path	Co-ordination number	Atomic distance (Å)	Debye-Waller factor σ^2 ($Å^2$)	Confidence level of adding shell (α) [b]	Energy shift ΔE_0 from calculated Fermi level (eV)	Reduced χ^2	R "goodness of fit factor"	Number of variables / number of independent points	k range	R range
HRC® [c]	O_1	1	1.65 (6)	0.010 (7)	0.92	0.05 ± 3.7	839	0.014	7 / 9.2	3 - 11	1.15 - 3.0
	O_2	4.4	2.01 (2)	0.005 (1)	-						
	Tc_1	1	2.58 (4)	0.010 (5)	1.00						
MRC® [c]	S	6	2.36 (2)	0.010 (1)	-	0.29 ± 1.7	1404	0.038	5 / 11.1	3 - 12.5	1.25 - 3.1
	Tc_1	2	2.78 (3)	0.009 (3)	0.94						
EHC® [d]	O	6	2.05 (1)	0.005 (1)	-	2.5 ± 1.1	243	0.016	6 / 14.0	3 - 12.5	1.15 - 3.5
	Tc_1	2.1	2.54 (1)	0.008 (2)	1.00						
	O-O MS	6	4.11 (2)	0.010 (2)	0.97						
EHC® [e] no sediment	O	6	2.05 (1)	0.007 (0.5)	-	2.4 ± 1.0	63.1	0.011	5 / 14.0	3 - 12.5	1.15 - 3.5
	Tc_1	2	2.51 (1)	0.013 (2)	0.98						
	O-O MS	6	4.13 (2)	0.014 (2)	1.00						

[a] Numbers in parentheses are the SD on the last decimal place. Additional shells were only included if they improved the fitting parameters with statistical significance. [b] f-test results, $\alpha > 0.99$ statistically improves the fit with 3 sigma confidence, $\alpha > 0.95$ with 2 sigma confidence, $\alpha > 0.68$ with 1 sigma confidence. Throughout the value of the amplitude factor (S02) was refined using known coordination numbers for the first shell (e.g. Tc-O, N=6) after (Brookshaw et al., 2015) [c] S02 fixed at 1.0. [d] S02 fixed at 0.8. [e] S02 was fixed at 0.9.

References

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Table S5. Alternative EXAFS fit parameters for the technetium minerals formed after biostimulation with EHC® [a]

Sample	Path	Co-ordination number	Atomic distance (Å)	Debye-Waller factor σ^2 ($Å^2$)	Confidence level of adding shell (α) [b]	Energy shift ΔE_0 from calculated Fermi level (eV)	Reduced χ^2	R "goodness of fit factor"	Number of variables / number of independent points	k range	R range
EHC® as short chain TcO_2 with Fe [c]	O	6	2.04 (1)	0.005 (1)	-	1.2 ± 1.4	224	0.011	7 / 14.0	3 - 12.5	1.15 – 3.5
	Tc_1	1.6	2.55 (1)	0.007 (2)	1.00						
	Fe	0.5	2.56 (6)	0.007 [e]	0.97						
	O-O MS	6	4.10 (2)	0.011(2)	0.99						
EHC® no sediment as crystalline TcO_2 [d]	O	6	2.05(1)	0.007(0.3)	-	1.6 ± 0.9	46.4	0.007	9 / 14.0	3 - 12.5	1.15 - 3.5
	Tc_1	2	2.51(1)	0.014(1)	1.0						
	Tc_2	2	3.34(4)	0.019(6)	0.95 [f]						
	Tc_3	4	3.84(6)	0.024(1)	0.95 [f]						
	O-O MS	6	4.12 (2)	0.014 (1)	0.99						

[a] Numbers in parentheses are the SD on the last decimal place. Additional shells were only included if they improved the fitting parameters with statistical significance. [b] f-test results, α > 0.99 statistically improves the fit with 3 sigma confidence, α > 0.95 with 2 sigma confidence, α > 0.68 with 1 sigma confidence. Throughout the value of the amplitude factor (S02) was refined using known coordination numbers for the first shell (e.g. Tc-O, N=6), after Brookshaw et al. (2015) [c] S02 fixed at 0.8. [d] S02 fixed at 0.9. [e] The Debye-Waller factor for Tc-Fe was fixed at 0.007, similar to Zachara et al. (2007). [f] f-test result for adding two additional shells of Tc atoms.

References

Brookshaw, D. R.; Pattrick, R. A. D.; Bots, P.; Law, G. T. W.; Lloyd, J. R.; Mosselmans, J. F. W.; Vaughan, D. J.; Dardenne, K.; Morris, K. Redox interactions of Tc(VII), U(VI), and Np(V) with microbially reduced biotite and chlorite. *Environ. Sci. Technol.* **2015**, *49* (22), 13139–13148.

Zachara, J. M.; Heald, S. M.; Jeon, B. H.; Kukkadapu, R. K.; Liu, C. X.; McKinley, J. P.; Dohnalkova, A. C.; Moore, D. A. Reduction of pertechnetate Tc(VII) by aqueous Fe(II) and the nature of solid phase redox products. *Geochim. Cosmochim. Acta* **2007**, *71*, 2137–2157.

Table S6. Details of EXAFS fit parameters for the technetium minerals formed following reoxidation of MRC® and EHC® treated sediments [a]

Sample	Path	Co-ordination number	Atomic distance (Å)	Debye-Waller factor σ^2 ($Å^2$)	Confidence level of adding shell (α) [b]	Energy shift ΔE_0 from calculated Fermi level (eV)	Reduced χ^2	R "goodness of fit factor"	Number of variables / number of independent points	k range	R range
MRC reoxidised[c]	S	3.7	2.34 (1)	0.009 (1)	-	-0.8 ± 1.4	687	0.017	6 / 12.4	3 – 13	1.15 – 3.15
	O	2.3	2.05 *	0.012 (5)	0.99						
	Tc	0.5	2.78 (2)	0.002(2)	1.00						
EHC reoxidised[d]	O	6	2.01 (1)	0.005 (0)	-	-0.1 ± 1.5	108.4	0.009	8 / 13.3	3 – 12	1.15 - 3.5
	Tc_1	1.2	2.54(1)	0.005 (2)	0.99						
	Fe_1	0.8	2.59 *	0.008 (6)	1.00						
	O-O MS	6	4.04(2)	0.011 (2)	0.99						
	Fe_2	0.8	3.51(5)	0.007 (6)	0.83						

[a] Numbers in parentheses are the SD on the last decimal place. Additional shells were only included if they improved the fitting parameters with statistical significance. [b] f-test results, α > 0.99 statistically improves the fit with 3 sigma confidence, α > 0.95 with 2 sigma confidence, α > 0.68 with 1 sigma confidence. Throughout the value of the amplitude factor (S02) was refined using known coordination numbers for the first shell (e.g. Tc-O, N=6), after Brookshaw et al. (2015) [c] S02 fixed at 1.0. [d] S02 fixed at 0.9. The asterisk denotes bond lengths that were fixed based on previous fits from the reduced phase.

References

Brookshaw, D. R.; Pattrick, R. A. D.; Bots, P.; Law, G. T. W.; Lloyd, J. R.; Mosselmans, J. F. W.; Vaughan, D. J.; Dardenne, K.; Morris, K. Redox interactions of Tc(VII), U(VI), and Np(V) with microbially reduced biotite and chlorite. *Environ. Sci. Technol.* **2015**, *49* (22), 13139–13148.

Supporting Figures

All geochemical monitoring figures show the average of three replicate measurements and error bars are +/- 1 standard deviation, unless otherwise stated

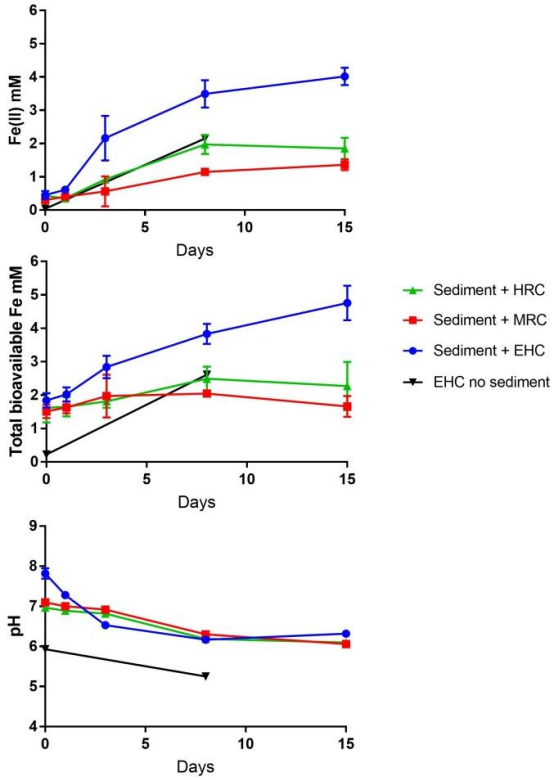

Figure S1a. Slow-release substrates stimulate Fe(III)-reduction in Sellafield sediments and EHC® without sediment was a single measurement and shows that EHC® corrodes abiotically to release Fe(II).

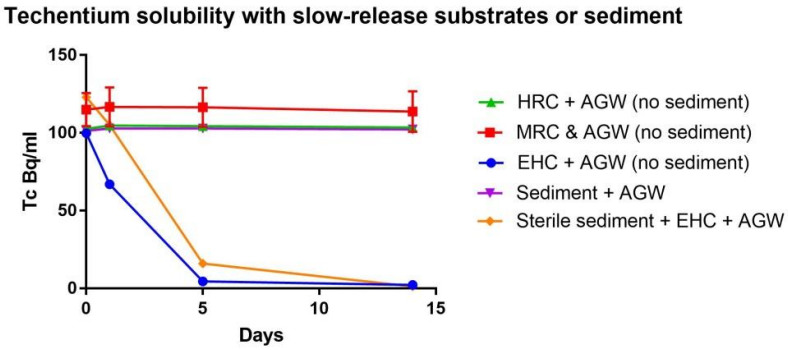

Figure S1b. Solubility tests with the proprietary electron donors. Technetium(VII) remains soluble in the presence of HRC® and MRC® and Sellafield sediment. Tc(VII) was removed from solution in the presence of EHC® (abiotically). Solubility tests were performed as duplicates, except the sterile sediment which was a single measurement.

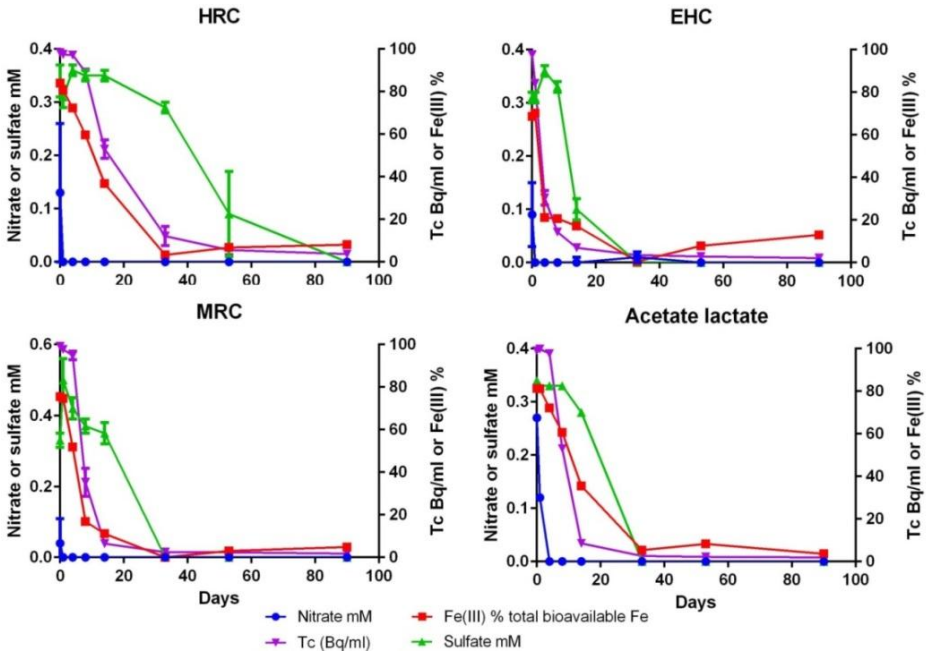

Figure S2. Redox cascade following biostimulation with different slow-release amendments. The acetate/lactate microcosm was a single positive control.

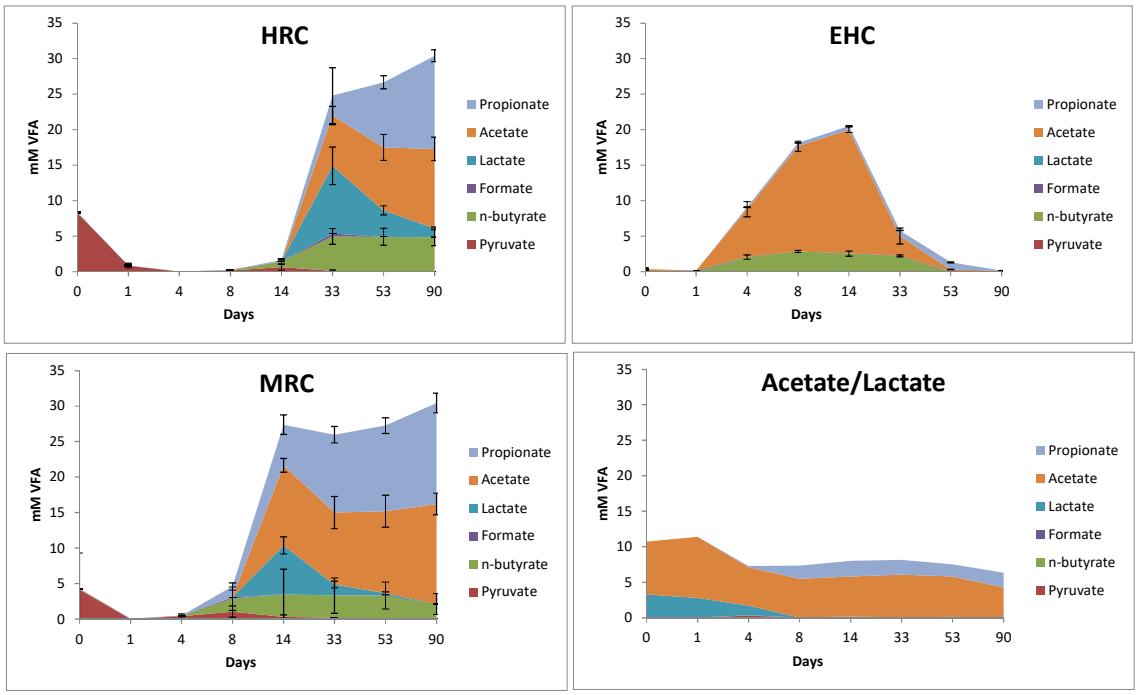

Figure S3. Production of volatile fatty acids following amendments with slow release donors. The acetate/lactate microcosm was a single positive control. Samples were diluted 100 times to ensure correct concentration range for the IC, and complex chromatographs were generated due to the complexity of the biodegradation of the proprietary substances. Data for sterile controls showed that trace VFAs (< 1 mM) were present in these samples.

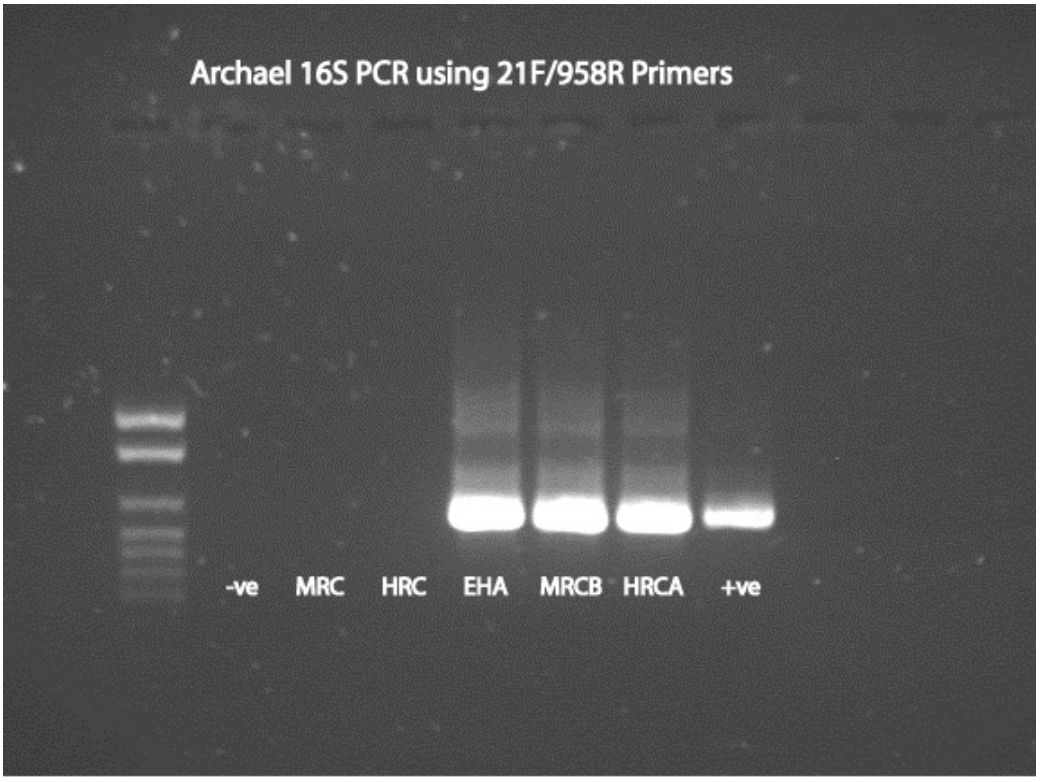

Figure S4. Archaeal PCR products for the MRC® and HRC® substrates, and for the sediment microcosms stimulated with EHC® 'EHA', MRC® 'MRCB' and HRC® 'HRCA' . Negative and positive controls were also included.

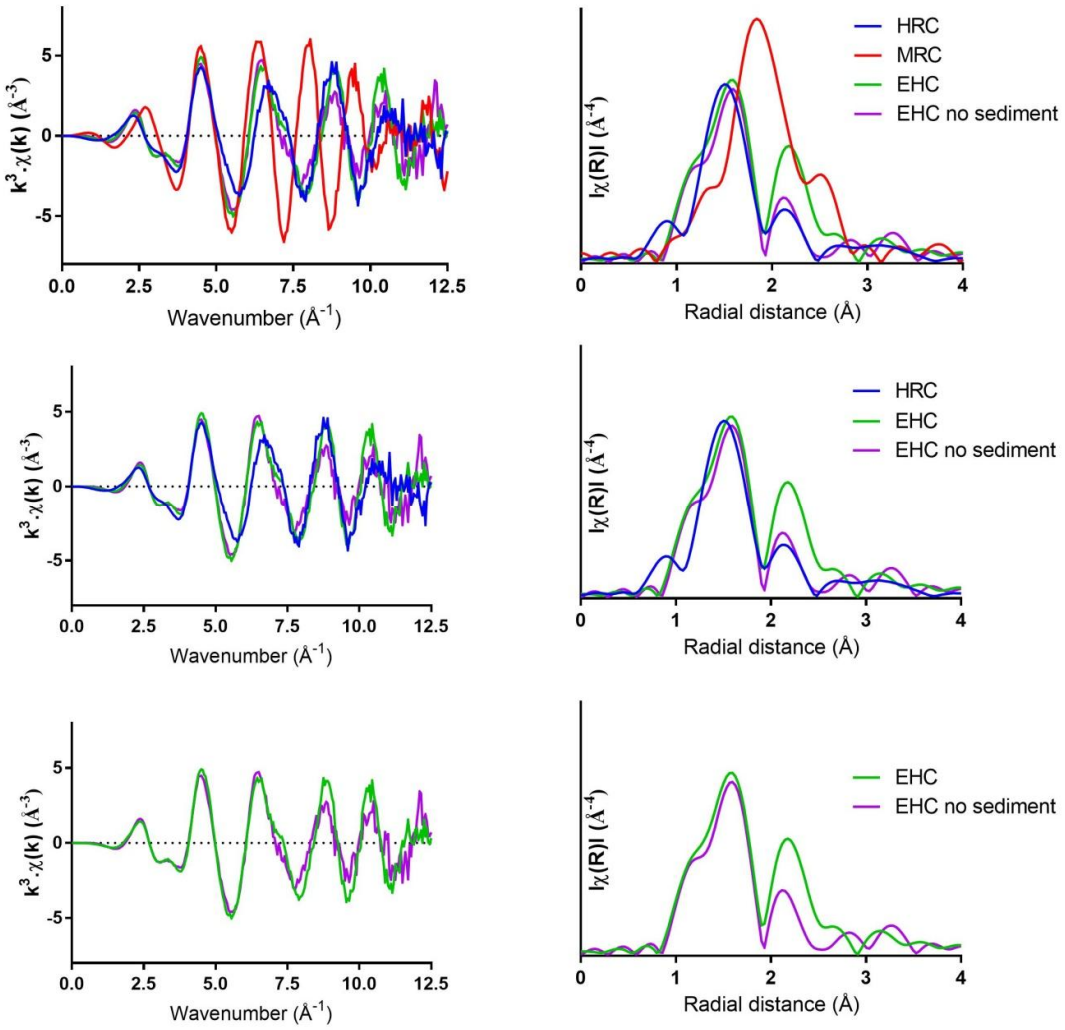

Figure S5. EXAFS data for all samples (top). The Tc(IV) formed by MRC® biostimulation was clearly different to the other samples; this was fitted as TcS_2. EXAFS data with MRC® removed for clarity (middle), and with HRC® removed to observe differences in the EHC samples (bottom). The spectra for HRC®, EHC® and EHC® no sediment were all fitted as variants of hydrous TcO_2.

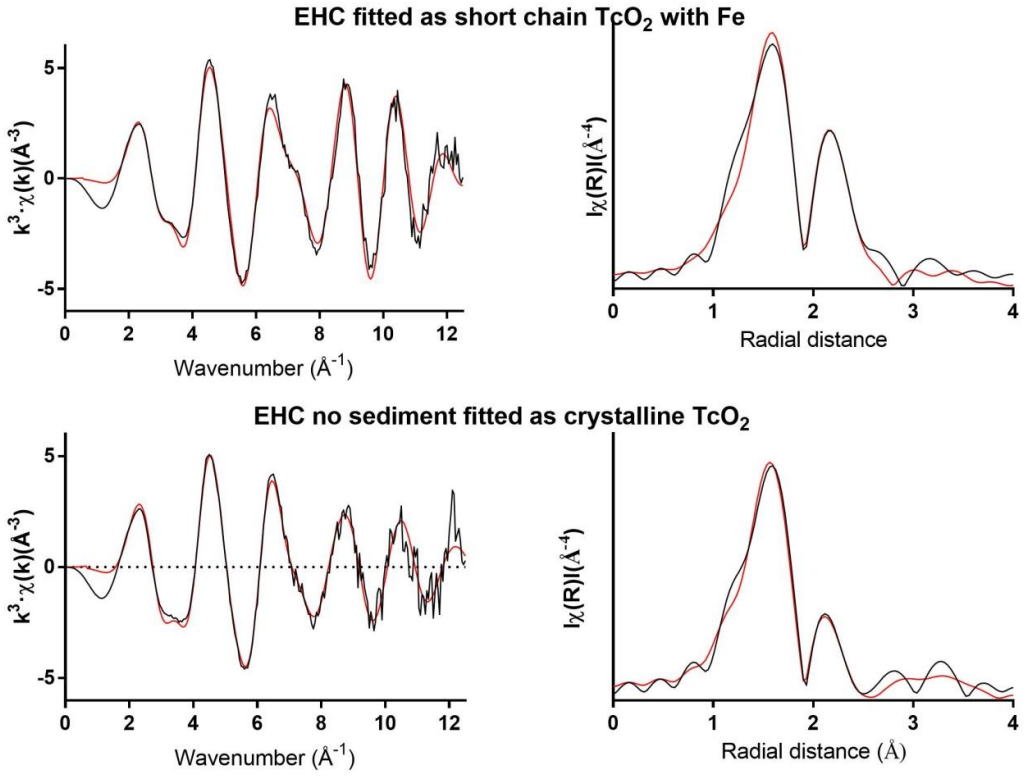

Figure S6. Non-phase shift corrected EXAFS data for sediments biostimulated with EHC® fitted as a short TcO_2 chain with Fe (above) and for EHC® no sediment fitted as crystalline TcO_2 (below).

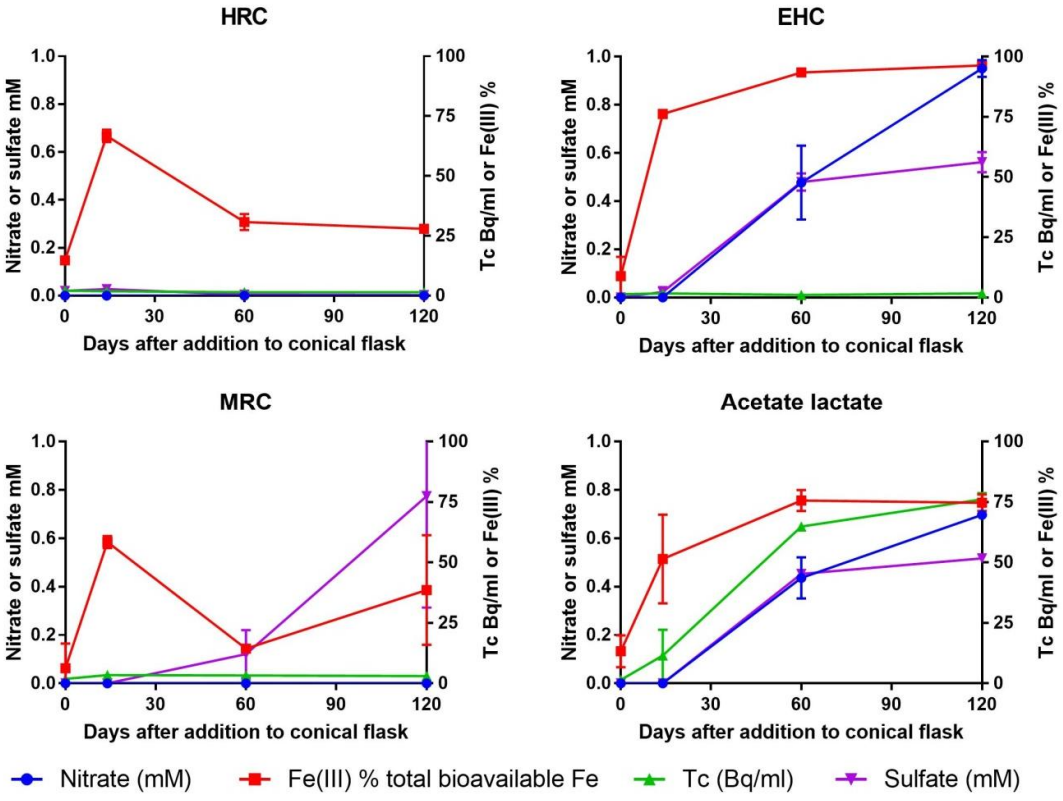

Figure S7. Oxidation of electron acceptors following reoxidation in air. Near-complete reoxidation of electron acceptors occurred in the experiments containing EHC and acetate-lactate, but no Tc(VII) was released to solution with EHC, perhaps due to armouring of Tc(IV) by reoxidised Fe(III), or due to redox buffering by residual ZVI. It is noteworthy that at the end of the experiment there was 18 mM of 0.5 N HCl-extractable total Fe in the EHC system compared to 2.6 mM in the acetate/lactate experiment. Incomplete reoxidation of electron acceptors was observed with HRC and MRC, likely due to the presence of residual slow-release electron donor poising the system close to anaerobic conditions which prevented oxidation to Fe(III) and nitrate.

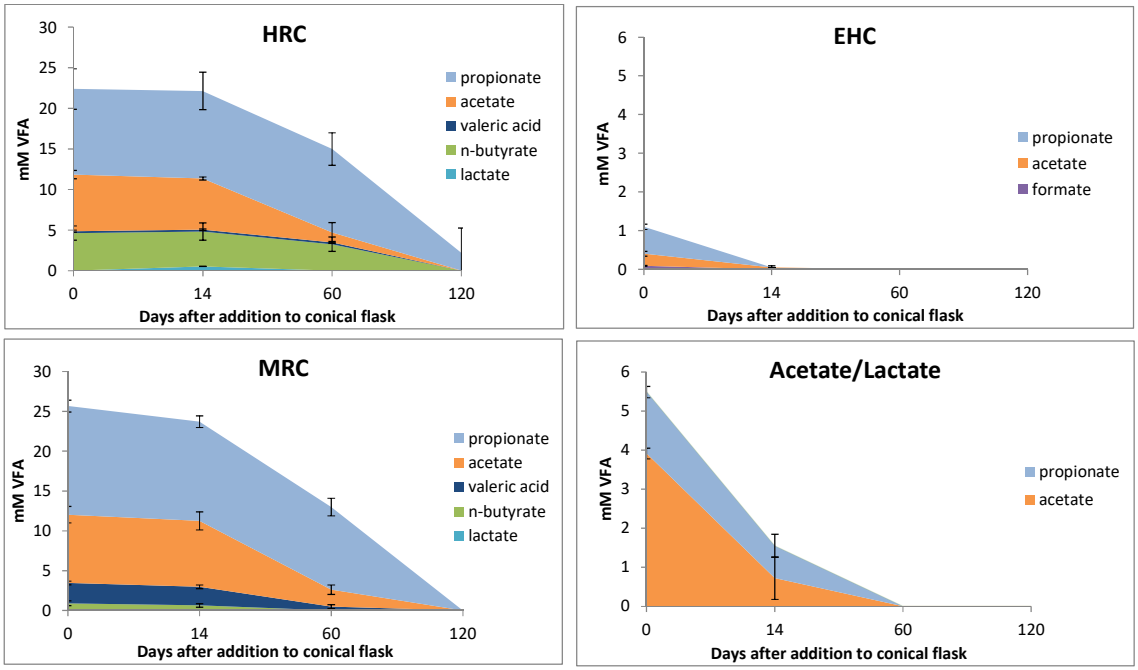

Figure S8. Changes in volatile fatty acids produced by biostimulation with slow release substrates and acetate/lactate during reoxidation conditions. Considerable quantities of VFAs remain present in the HRC and MRC systems even after 60 days exposure to highly oxidising conditions, which may offer a protective effect to Tc(IV) at lower concentrations. Note the differences in the y axis scales.

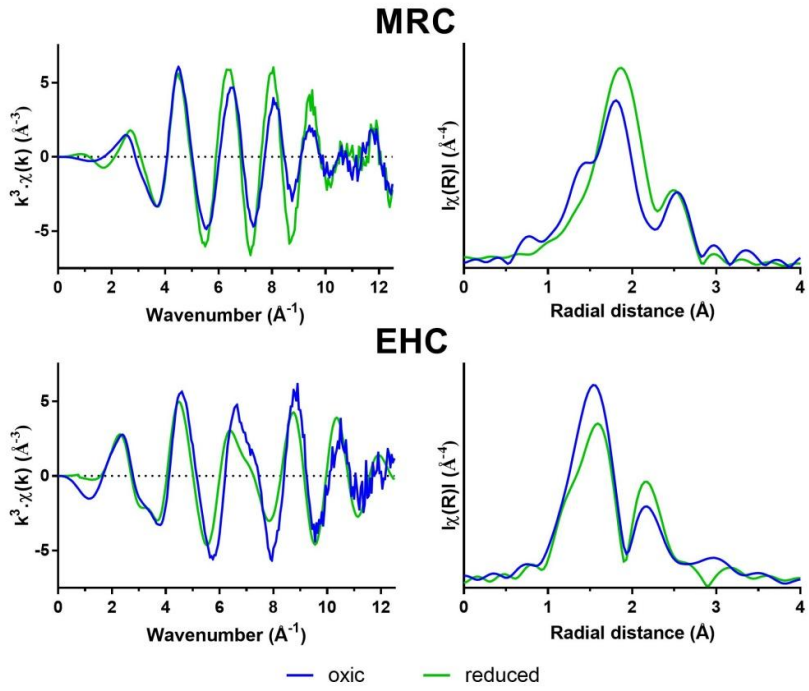

Figure S9. EXAFS of reoxidised samples (blue) plotted with reduced samples (green).