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

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9 Radioactively contaminated land; Groundwater; Sulfide; Zero valent iron; HRC; MRC; EHC

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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 proprietary 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 phases²⁻⁵ 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

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

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

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

62 products, mainly Fe(II) minerals, can reduce Tc(VII) to poorly soluble Tc(IV) abiotically and
63 the H₂ may stimulate a number of beneficial microbial processes.^{29,30} These include: H₂
64 acting as an electron donor to generate additional Fe(II) by stimulating microbial Fe(III)
65 reduction^{31,32}; stimulating the production of sulfide via sulfate-reducing bacteria^{33,34} which
66 may precipitate Tc-sulfide minerals⁷; and potentially should elevated Tc(VII) concentrations
67 exist, H₂ may also stimulate enzymatic Tc(VII) reduction via hydrogenase enzymes.^{8,35–40}
68 Moreover, microbially-mediated Tc(VII) removal has been observed below the predicted
69 solubility threshold for TcO₂ precipitates, likely via sorption of Tc(IV) to sediment at these
70 very low concentrations.^{41,42}

71 Microbially-reduced sediments are known to be susceptible to oxidative remobilisation of
72 redox active radionuclides¹² and biogenic hydrous TcO₂ is partially reoxidised after exposure
73 to air, although it is less susceptible to reoxidation from the addition of nitrate (via the
74 formation of reactive nitrite).^{16,43,44} It is crucial to understand the factors that may affect the
75 longevity of microbially-precipitated Tc(IV) in the subsurface in order to tailor successful
76 long-term remediation strategies. As an alternative to stimulating the formation of short-chain
77 hydrous TcO₂ phases, the targeted formation of Tc-sulfides is of interest.^{6,7,26} Technetium
78 sulfides are poorly soluble and considered to be the solubility limiting phase for
79 technetium.^{45,46} Sulfide mineral phases can fix Tc(VII) through sorption and reductive
80 precipitation⁴⁶ and it has been suggested that TcS₂ is more resistant to oxidation compared to
81 TcO₂ under abiotic conditions.⁴⁷ Finally, the presence of Fe may play an important role in
82 limiting Tc(IV) reoxidation as Tc(IV) associated with Fe(III) is considered more resistant to
83 reoxidation than Tc(IV)O₂·nH₂O.^{3,48}

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

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

90 **MATERIALS AND METHODS**

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

98 **Initial testing of electron donors and Tc solubility:** Prior to conducting microbial Tc(VII)
99 reduction experiments, initial tests were performed to assess whether the microbial
100 community present in Sellafield sediments was able to use these electron donors to reduce
101 Fe(III). Microcosms were set up containing 3 g of a gravelly sand sediment collected from a
102 Sellafield site borehole (designed RB27 and fully described in past work)⁴⁹, 30 ml of sterile
103 artificial groundwater (containing the following in mM: K^+ 0.089; Na^+ 3.37; Ca^{2+} 1.69; Mg^{2+}
104 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-
105 release donor compound.⁵¹ The microcosm headspace was degassed with argon and the
106 bottles were incubated in the dark at room temperature. Additional tests were conducted to
107 determine whether 100 Bq ml^{-1} ($1.6 \mu\text{M}$) $^{99}\text{Tc(VII)}$ as pertechnetate was soluble in the
108 presence of the electron donors in sediment-free systems, by adding 0.15 g of electron donor
109 to 30 ml artificial groundwater under aerobic conditions in duplicate. An additional bottle
110 containing 3 g of sediment, 30 ml artificial groundwater and 0.15 g of EHC was sterilised by
111 autoclaving (120°C , 20 mins) and then spiked with Tc(VII) to assess the sorption of Tc to

112 EHC in a sterile sediment system. The sorption of technetium to sediment was examined
113 using bottles comprising of 3 g of Sellafield sediment, 30 ml artificial groundwater and
114 $^{99}\text{Tc(VII)}$ in triplicate.

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

123 Periodic geochemical monitoring was performed by withdrawal of an aliquot of sediment
124 slurry using a needle and syringe degassed with argon using aseptic technique. Iron(II) and
125 total bioavailable iron in sediment slurry were assessed via digestion in 0.5 N HCl or 0.25 N
126 hydroxylamine hydrochloride in 0.5 N HCl, then measurement of Fe(II) and total Fe using
127 the ferrozine assay.^{52,53} The supernatant was separated from the sediment by centrifugation
128 ($16,200 \text{ g}$, 5 minutes), and aqueous Tc was measured by liquid scintillation counting
129 (background counts in “unspiked” liquid scintillation fluid averaged ($n = 38$) $31.0 \pm 4.3 \text{ cpm}$,
130 which defined a minimal detectable ^{99}Tc concentration of 2.2 nM^{54}), nitrate, sulfate and
131 volatile fatty acids (VFAs) by ion chromatography (Dionex ICS 5000), and pH and Eh were
132 measured using calibrated electrodes. Gas samples were collected from the microcosm
133 headspace and analysed for the presence of methane and hydrogen via gas chromatography
134 flame ionisation detection (GC-FID).⁵⁵

135 **Molecular ecology:** To investigate the composition of the sediment microbial community,
136 DNA was extracted from sediment slurry samples using a PowerSoil DNA Isolation Kit (MO
137 BIO Laboratories INC, Carlsbad, CA, USA). Full details of the methodology are described
138 previously.^{49,56} Briefly, for archaeal polymerase chain reaction (PCR) amplification a
139 fragment of the 16S ribosomal RNA gene (approximately 940 base pairs) was amplified
140 using the primers 21F and 958R⁵⁷ and bacterial DNA was amplified using the universal 16S
141 rRNA primers 8F and 1492R.⁵⁸ The purity of the amplified products was determined by
142 electrophoresis in Tris-acetate-EDTA gel. PCR products were cleaned up, quantified, and
143 sequenced using a Roche 454 Life Sciences Junior System. Qiime 1.8.0⁵⁹ was used to
144 analyse the 454 pyrosequencing reads, the Ribosomal Database Project⁶⁰ was used for
145 taxonomic classification, and Blastn was used to identify the closest GenBank matches
146 (<http://blast.ncbi.nlm.nih.gov>).

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

Oxidative remobilisation of Tc(IV): To examine how the different electron donors influenced the oxidative remobilisation of Tc, a parallel set of microcosms were prepared and subsequently reoxidised. Sediment microcosms (5 g Sellafield sediment and 50 ml artificial groundwater) were prepared in triplicate with 0.25 g of HRC, MRC or EHC and 120 Bq ml⁻¹ (1.9 µM) ⁹⁹Tc. A positive control was prepared using a 5 mM acetate and 5 mM lactate electron donor mix. A sterile control for the EHC experiment was prepared by autoclaving (120°C, 20 minutes) prior to spiking with ⁹⁹Tc(VII). After 60 days, the fully reduced microcosms were reoxidised by decanting the sediment slurry into sterile 500 ml bottles and aerated daily by opening and shaking for 15 minutes. Sediment slurry was removed periodically for geochemical analysis as described previously. Additional experiments were carried out at higher Tc concentrations to investigate the oxidation state and speciation of technetium by XAS following reoxidation. These contained 0.7 g sediment, 10 ml artificial groundwater, 25 kBq ml⁻¹ (400 µM) TcO₄⁻ and 0.1 g HRC, MRC or EHC. After 139 days of anaerobic incubation, the bottles were opened to air within a 5 litre container and shaken gently on an orbital shaker.

RESULTS AND DISCUSSION

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

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

193 **Microbial reduction of Tc(VII) and biogeochemistry:** Sediment microcosms were
194 stimulated with the slow-release substrates to investigate their potential for technetium
195 remediation. Geochemical results showed that Fe(II) was produced almost immediately with
196 each slow-release amendment, indicating the rapid development of reducing conditions
197 (Figure 1). Corrosion of ZVI in the EHC system lead to the highest measured concentrations
198 of 0.5 N HCl extractable Fe(II). Tc(VII) was removed from solution almost immediately with
199 EHC, likely due to abiotic reduction by the Fe(II) to form poorly soluble Tc(IV) phases.^{28,62}
200 A lag of around four days was observed with the samples amended with HRC, MRC and the
201 acetate/lactate mix, with Tc(VII) removal associated with the onset of microbial Fe(III)-
202 reducing conditions and subsequent abiotic reduction via Fe(II), to form poorly soluble
203 Tc(IV) phases. This process has been observed in past experiments with representative
204 Sellafield sediments stimulated with simple electron donors¹⁹ and in a range of biotic and
205 abiotic systems containing Fe(II).^{4,16,48} Near-complete Tc removal was observed within 28
206 days in the experiments stimulated with MRC, EHC and the acetate/lactate mix, or after 90
207 days with HRC. The sterile controls containing HRC and MRC did not show significant
208 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 bacteria⁶⁵, 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 waste⁶⁶ 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 bacterium⁶⁸ 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 Bacteroidales 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

282 lower in these high level (20 kBq mL⁻¹, 320 µM) experiments compared to the low level (0.1
283 kBq mL⁻¹, 1.6 µM) experiments as observed previously¹³ and suggesting some inhibition of
284 bioreduction processes at elevated Tc concentrations (approximately 15 – 60 % of Tc was
285 removed within 120 days, see supporting information for further discussion).

286 Analysis of the XANES spectrum for the HRC sample, using linear combination fitting
287 between standards for Tc(VII) as pertechnetate and Tc(IV) as TcO₂⁷² indicated the sample
288 contained approximately 75% Tc(IV) and 25% Tc(VII) (Figure 2). The Tc(VII) observed is
289 most probably due to Tc(VII) in the pore-waters of the sample. Indeed, past workers have
290 observed similar levels (15 – 50 %) of Tc(VII) in XANES from partially oxidised
291 sediments.^{3,73} As the rate of reduction was slow in the HRC XAS experiments, a significant
292 amount of Tc(VII) would certainly be present in the aqueous phase associated with the moist
293 sediment pellet analysed using XAS. The EXAFS data were fitted assuming contributions of
294 75% Tc(IV) as hydrous TcO₂ and 25% Tc(VII) as pertechnetate and using relevant Tc(IV)
295 and Tc(VII) models from the literature.^{2,74,75} Here, a good fit was obtained for the first peak
296 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
297 Tc(IV) and Tc(VII) components of the sample (Figure 4, Table S4). The fit was further
298 improved by the addition of 1 Tc atom at 2.58 Å suggesting that the Tc(IV) may be present as
299 hydrous TcO₂.^{2,76} Given that not all the Tc had been removed from solution in this sample,
300 XANES analysis was attempted on the aqueous phase after 139 days (~30 ppm Tc). Linear
301 combination fitting of the resulting spectra between relevant Tc(IV) and Tc(VII) standards⁷²
302 found ~50% of Tc in the aqueous phase was present as Tc(IV) with ~50% as Tc(VII) (Figure
303 2). This suggested that a significant fraction of the aqueous phase was present as Tc(IV)
304 colloids or organic complexes, as reported in past studies.^{7,20,37,39} Clearly, the presence of a
305 significant component of colloidal or organic-complexed Tc(IV) in these systems is
306 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 structure⁷⁷ 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 model² 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

332 hydrous TcO_2 to surface Fe-O octahedra.^{3,4,43,48} In the current study, the EHC sediment fit
333 was slightly improved with a “short Fe” contribution with 1.6 Tc atoms at 2.55 Å and with a
334 physically realistic contribution of 0.5 Fe atoms at 2.56 Å (Figure S6, Table S5), suggesting
335 an association of the hydrous TcO_2 chains with Fe was possible in this Fe-rich system, similar
336 to past work.^{4,48} A good fit was obtained for the EHC with no sediment with six O atoms at
337 2.05 Å and two Tc atoms at 2.51 Å, again suggesting it was present in the form of short-chain
338 polymeric hydrous TcO_2 (Figure 4, Table S4).

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

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

the sulfide phases formed by microbial reduction, including TcS_2 , were recalcitrant to reoxidation under these conditions and also acted as redox “buffers”. Given that previous research has also demonstrated that Tc(IV) -sulfides are more resistant to oxidative remobilisation than Tc(IV) -oxides⁴⁷ the use of electron donors that favour sulfidic conditions such as MRC seem an appropriate bioremediation option for Tc-contaminated groundwaters, although the impacts of sulfidation on other groundwater contaminants would need to be considered further.

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

It is noteworthy that Tc(IV) in the form of hydrous Tc(IV)O_2 was generated by biostimulation with HRC, EHC and acetate and lactate¹⁹ but only the Tc(IV) from acetate/lactate biostimulation was reoxidised. This suggests that the redox buffering of the slow-release substrates may be responsible for protecting the Tc(IV) from reoxidation.

Parallel higher radioactivity samples were prepared for XAS analysis to investigate the speciation of Tc in these reoxidised systems. Again, the reaction rates in the higher Tc experiments (25 kBq ml^{-1} , 400 $\mu\text{M Tc}$) were lower than in the low level (0.1 kBq ml^{-1} Tc, 1.6 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 TcS₂ with a shift of the first peak to a shorter atomic distance (Figure S9). Fitting was informed by past work that suggested TcS₂ can reoxidise to Tc(IV)-oxides in air⁶ and by the short-chain hydrous TcO₂ model.² Linear combination fitting of the EXAFS data suggested a ~60% contribution from TcS₂ and ~40% contribution from short-chain hydrous TcO₂; shell-by-shell fitting using these contributions generated a good fit and suggested that partial oxidation of the TcS₂ to hydrous TcO₂ 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 TcO₂ 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.

60x43mm (300 x 300 DPI)

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

83x137mm (300 x 300 DPI)

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

60x42mm (300 x 300 DPI)

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.

42x21mm (300 x 300 DPI)

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).

211x561mm (300 x 300 DPI)

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 experiments¹), 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.peroxymchem.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__Beta proteobacteria;o__Methylophilales;f__Methylophilaceae	Methylophilaceae	Uncultured <i>Methylophilus</i> sp. clone Me60A10	GU472577.1	98 (98)	1049 (1108)	Sulfur cycle prokaryotes in low-sulfate lake. Similar results for aerobic methanotrophs, methylotrophs (<i>Methylotenera versatilis</i> strain 301, NR_074693.1, 96% similarity), methane consumption Arctic lakes
23	113	2.2	k__Bacteria;p__Proteobacteria;c__Beta proteobacteria;o__Burkholderiales;f__Comamonadaceae	Comamonadaceae	<i>Ideonella</i> 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__Beta proteobacteria;o__Burkholderiales;f__Comamonadaceae;g__Polaromonas;s__	Comamonadaceae	<i>Polaromonas</i> sp. H8N	KU586657.1	99 (99)	897 (910)	Arctic & Antarctic glacial surfaces. Similar results for anaerobic digester, landfill leachate, rhizosphere, river water
38	94	1.8	k__Bacteria;p__Nitrospirae;c__Nitrospirae;o__Nitrospirales;f__Nitrospiraceae;g__Nitrospira;s__	Nitrospiraceae	Uncultured <i>Nitrospira</i> 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 (<i>Nitrospira</i> sp. strain GC86, Y14644.1, 96% similarity)
40	89	1.7	k__Bacteria;p__Proteobacteria;c__Beta proteobacteria;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 digester
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	<i>Psychrosinus fermentans</i> strain FCF9	NR_115860.1	96 (96)	818 (848)	Lactate fermenting bacterium from Antarctic lake. Similar results for metal-reducing <i>Pelosinus</i> 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	<i>Flavobacterium</i> 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	<i>Pseudomonas</i> sp. type strain HMPB4	AM746975.1	99 (99)	870 (877)	Psychrotropic bacteria. Similar results for Antarctic / glacial / permafrost <i>P. mandelli</i> , humic degraders, elevated CO ₂ soils, <i>P. syringae</i> , <i>P. frederiksborgensis</i> (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 <i>P. mandelli</i> , humic degraders, elevated CO ₂ soils, <i>P. syringae</i> , <i>P. frederiksbergensis</i> (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 digester

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__MC G;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__	Methanomassiliicocaceae	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__MC G;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__MC G;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__	Methanomassiliicocaceae	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.

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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, $\alpha > 0.99$ 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 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.; Patrick, 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, $\alpha > 0.99$ 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 0.9. The asterisk denotes bond lengths that were fixed based on previous fits from the reduced phase.

References

Brookshaw, D. R.; Patrick, 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).

Technetium solubility with slow-release substrates or sediment

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 TcO₂ chain with Fe (above) and for EHC® no sediment fitted as crystalline TcO₂ (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 poisoning 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).