



The fate of technetium in reduced estuarine sediments: Combining direct and indirect analyses

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ABSTRACT

Technetium-99 is an important fission product in radioactive wastes. As Tc(VII)O_4^- , Tc is highly mobile in oxic environments but, under reducing conditions, Tc becomes strongly associated with sediments as hydrous Tc(IV)O_2 like phases. In order to further examine the behaviour of Tc over a range of concentrations in estuarine sediments, anoxic incubation experiments were combined with a range of direct (transmission electron microscopy and gamma camera imaging) and indirect (incubation experiments and chemical extractions) experimental techniques. When TcO_4^- was incubated in sediment microcosms at micro-molar ($10^{-6} \text{ mol L}^{-1}$) concentrations, >99% TcO_4^- was removed from solution over the course of 36 days in systems undergoing active microbial Fe(III)-reduction. By contrast, when spiked into pre-reduced estuarine sediments that were predominantly Fe(III)-reducing (incubated for 60 days) or SO_4^{2-} -reducing (incubated for 270 days), >99% TcO_4^- was removed from solution in under 10 min in both microbially active and heat sterilised systems. Chemical extraction techniques showed that $70 \pm 3\%$ of Tc bound to sediments was remobilised when sediments were exposed to the first strong oxidant (H_2O_2) in the extraction scheme. At higher Tc concentrations ($\sim 0.05 \text{ mol kg}^{-1}$ of sediment) scanning transmission electron microscopy, combined with energy dispersive X-ray mapping, was used to examine the associations of Tc in sediments. At these concentrations, Tc was localised and co-associated with nanometre size Fe(II)-rich particles, consistent with the hypothesis that removal of Tc may be controlled by reduction of Tc(VII) to Tc(IV) by biogenic Fe(II) in sediments. In addition, gamma camera imaging with the γ -emitting $^{99\text{m}}\text{TcO}_4^-$ (half-life 6 h) at pico-molar ($10^{-12} \text{ mol L}^{-1}$) concentrations, was used to visualise the interaction of Tc in sediments at very low concentrations. Here, over the course of 24 h the scavenging of Tc to SO_4^{2-} -reducing sediments was observed. As the Tc concentrations used in the $^{99\text{m}}\text{Tc}$ experiments were below the solubility limits for hydrous Tc(IV)O_2 (ca. $10^{-9} \text{ mol L}^{-1}$ at pH 7–9), sorption of Tc(IV) species is likely to be a significant control on Tc behaviour in these sediments even at very low concentrations. Overall, the results of this study show that multiple approaches are essential to understanding Tc speciation in complex heterogeneous sediments over the wide range of concentrations relevant to contaminated natural and engineered environments.

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1. Introduction

During the 1980s and early 1990s, the β -emitting radionuclide ^{99}Tc was one of the most radiologically significant radionuclides

released via authorized discharges from the Sellafield Reprocessing Facility into UK coastal environments (Nelson, 2004). As the pertechnetate ion Tc(VII)O_4^- , Tc is highly mobile under oxic conditions, and the Sellafield Tc-signature has been successfully used as an oceanographic tracer in the North Sea (Brown et al., 1999). Under anoxic conditions, Tc is reduced and retained in sediments as hydrous- Tc(IV)O_2 like phases (Fredrickson et al., 2004; Maes et al., 2004; Wildung et al., 2004; Burke et al., 2005; McBeth et al., 2007; Morris et al., 2008; Begg et al., 2008). During anaerobic microbial metabolism in sediments, Tc(VII) may be reduced and removed

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from solution by either direct enzymatic reduction, or by abiotic transfer of electrons from reduced species such as Fe(II) (Lloyd and Macaskie, 1996; Lloyd et al., 2000). Direct enzymatic reduction of Tc has been observed, but microbial reduction was only observed with H₂ as the electron donor, suggesting that the hydrogenase enzyme was responsible for electron transfer to Tc(VII) in subsurface bacteria (De Luca et al., 2001; Lloyd et al., 2000), as noted in the enteric bacterium *Escherichia coli* (Lloyd et al., 1997). Indeed, several recent studies have reported that active ingrowth of biogenic, sediment-associated Fe(II)_(s) caused Tc-reduction in sediments (Lloyd et al., 2000; Wildung et al., 2004; Fredrickson et al., 2004; Burke et al., 2005; McBeth et al., 2007; Begg et al., 2008), although, in Fe-poor sediments, enzymatic reduction may be important (Wildung et al., 2004). This interaction with reducing sediments has led to Tc being retained in coastal, river and estuarine sediments over several decades (Aarkrog et al., 1997; Morris et al., 2000; Standring et al., 2002; Keith-Roach et al., 2003; Leonard et al., 2004; McCubbin et al., 2006; Finegan et al., 2009).

Estuaries are important interfacial environments, and zones of intense human activity. Technetium discharge to surface waters and transport into estuarine environments will lead to interaction with estuarine sediments. As a result it is vital to understand the long-term fate of Tc in mineralogical and biologically complex estuarine sediments. It is difficult to obtain a mechanistic understanding of Tc in these complex sediments *in situ*. In contrast, microcosm experiments provide a better control over environmental variables, and here, both direct and indirect techniques can be used to study the biogeochemistry of Tc. Indirect techniques, such as monitoring Tc in solution during sediment incubations (Bird and Schwartz, 1997; Abdelouas et al., 2002, 2005; Istok et al., 2004; Burke et al., 2005; Begg et al., 2007; McBeth et al., 2007; Li and Krumholz, 2008) and sequential extractions (Abdelouas et al., 2005; Keith-Roach et al., 2003; Standring et al., 2002) can be used to quantify Tc removal kinetics and the resistance of sediment-bound Tc to remobilisation. In contrast, direct techniques such as X-ray absorption spectroscopy (XAS) (Burke et al., 2005; Fredrickson et al., 2004; Maes et al., 2004; McBeth et al., 2007; Wildung et al., 2004; Morris et al., 2008; Begg et al., 2008; Perytyzhko et al., 2008) and electron microscopy (Fredrickson et al., 2004; Lear et al., 2009), can be used to provide complementary information such as the bound valence state, local chemical environment, and images of Tc localization. In this study, results are presented from both indirect and direct methodologies that have been used to study the fate of Tc in Humber Estuary sediments, and critically assess the limitations that exist when interpreting such data.

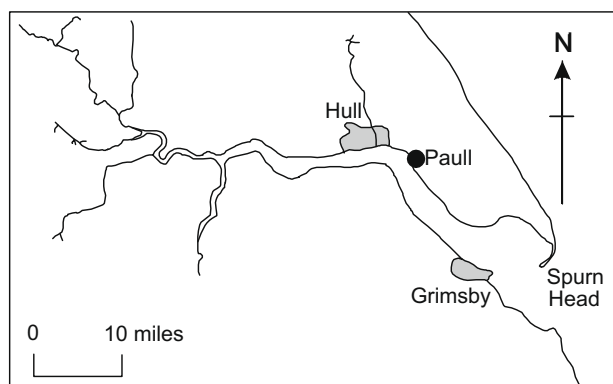


Fig. 1. Location map of the Humber Estuary showing the Paull sampling site.

2. Methods and materials

2.1. Sediment and water sampling

A brackish water site (Fig. 1 – 53°43'11 N, 0° 14'10 W), within the Humber Estuary, NE England, UK, was chosen for use in this study as fine-grained, oxidised estuarine sediments and river waters are readily available. Surface sediments from the Paull mudflat were sampled in August 2002 and care was taken to collect only the top few millimetres of sediment. Sediments were transferred directly into sterile polythene containers, transported back to the laboratory and stored in the dark at 4 °C. Experiments were started within 4 days of sample collection and sediment manipulation was kept to a minimum prior to incubation. In addition, a river water sample was taken adjacent to the sampling site to create sediment slurries used in microcosm experiments. At the time of collection the sediment and water temperature was 13–14 °C. Sediments consisted of quartz, calcite, dolomite, kaolinite, clinocllore, muscovite, albite and orthoclase; contained 1.7% total inorganic C and 2.3% total organic C and had a major element composition as follows: SiO₂ 52.0%, TiO₂ 0.92%, Al₂O₃ 13.0%, Fe₂O₃ 6.1%, Mn₃O₄ 0.15%, MgO 2.5%, CaO 6.7%, Na₂O 2.4%, K₂O 2.4%, P₂O₅ 0.36%, Cr₂O₃ 0.02%, LOI at 1025 °C 14.6% (Burke et al., 2005). River water had a salinity of 15.5‰, pH 7.8, Eh +124 ± 11 mV and NO₃⁻, Fe, SO₄²⁻ and Cl⁻ concentrations of 137 ± 10 μmol L⁻¹, 0.7 ± 0.8 μmol L⁻¹, 17.2 ± 1.3 mmol L⁻¹, and 221 ± 8 mmol L⁻¹, respectively (Burke et al., 2005).

2.2. Reduction microcosm experiments

Microcosms containing 5 g sediment (dry weight) and 25 mL river water were prepared in 30 mL glass serum bottles (Wheaton Scientific), sealed with butyl rubber stoppers (Bellco Glass Inc.) and Al crimps. A series of repeat microcosms were then spiked with Tc(VII) (as ammonium pertechnetate, UK Atomic Energy Authority, Harwell) to a final concentration of either 1.5 or 5 μmol L⁻¹ and incubated anaerobically in the dark at 10 °C to develop microbially induced anoxia. Sterile control series were also established by autoclaving (3 times for 20 min at 120 °C). Microcosms were sampled periodically over a 36 day period and in triplicate to provide time-series data. Sampling was done in an anaerobic cabinet (95% N₂, 5% H₂, Coy Laboratory Products Inc.). At each time-point aqueous samples were collected, filtered (<0.2 μm, Sarstedt AG and Co.) and a range of redox indicators (Eh, NO₃⁻, Fe²⁺, SO₄²⁻, HS⁻), pH and Tc were determined immediately or after storage at –20 °C. Additionally, in the sediment slurry, 0.5 M HCl extractable Fe(II) was also determined (Lovley and Phillips, 1986).

Separate microcosms were also incubated for 60 or 270 days, the microcosm sediments were then assigned descriptions as either Fe(III)-reducing (60 days) or SO₄²⁻-reducing (270 days) sediments, respectively, based on the prevalent geochemical conditions observed in microcosms at these times (see Table 1 for geochemical data). Technetium was then spiked into both pre-reduced microbially active, and sterile (120 °C, 20 min) sediments by addition of ammonium pertechnetate (final concentration, 1.5 μmol L⁻¹) to 15 mL sediment slurry (solid solution ratio = 100 g L⁻¹) in a 20 mL syringe under N₂ gas. Following capping and rapid manual mixing, pore waters were separated from sediments by filtration (<0.2 μm, Sarstedt AG and Co.) from 20 s to 20 min.

2.3. Geochemical methods

Technetium-99 activity in aqueous samples was determined by liquid scintillation counting (LSC) on a Packard Tri-Carb 2100TR. A 1 mL aliquot of filtered porewater was transferred to a scintillation

Table 1

Typical range of values for geochemical indicators obtained during incubation experiments with 1.5 $\mu\text{mol L}^{-1}$ ammonium pertechnetate and Humber Estuary sediments.

Sediment experiment	Eh (mV)	pH	Fe (aq) (μM)	% Ext. Fe as Fe(II)	HS ⁻ (μM)	NO ₃ ⁻ (μM)	SO ₄ ²⁻ (mM)
Surface sediment (day 0)	+50 to +164	7.6–7.8	0.7–1.2	17–39	n.d.	119–247	14.0–19.1
Fe(III)-reducing (day 60)	–112 to –243	7.5–7.8	199–644	52–71	n.d.–0.1	n.d.	13.4–19.0
SO ₄ ²⁻ -reducing (day 270)	–260 to –347	7.5–7.7	520–599	96–100	0.5–6.0	n.d.	n.d.–1.2

vial with 15 mL EcoscintTM A. Samples were then stored in darkness for 24 h prior to counting for 100 min. This geometry produced a counting efficiency of ca. 75% and a detection limit of $0.36 \pm 0.03 \text{ Bq mL}^{-1}$. In addition to total Tc analysis in solution, selective solvent extraction of TcO₄⁻ into an organic phase consisting of tetraphenylarsonium (TPA) chloride in chloroform (Tribalat and Beydon, 1953; Wildung et al., 2000) was performed at selected time-points. The activity of TcO₄⁻ extracted into the organic phase was compared with total Tc activity in the aqueous solution assessed on a parallel sample to determine the amount of TcO₄⁻ in solution. Total HS⁻ and Fe were measured spectrochemically (Cline, 1969; Viollier et al., 2000) on a Cecil CE 3021 at 400 nm and 562 nm respectively, and SO₄²⁻, Cl⁻ and NO₃⁻ were determined by ion chromatography on a Dionex DX100 fitted with an AS14 analytical column. Iron(II) associated with solids was determined after extraction in 0.5 M HCl and reaction with Ferrozine (Lovley and Phillips, 1986). Standards were regularly used to check method quality and calibrations normally gave R² values of 0.99 or better. Eh and pH were measured using an Orion 420a bench-top meter, a Pt ORP electrode (VWR International Ltd.) and a calibrated double junction Gelglass pH electrode (VWR International Ltd.).

2.4. Chemical extractions

Sequential extraction was used to examine the fate of Tc in the microcosm experiments using the technique developed by Tessier et al. (1979), adapted for use in anoxic sediments (Kersten and Förstner, 1987; Wallmann et al., 1993) and applied to analysis of sediments naturally radiolabeled with Tc (Keith-Roach et al., 2003). The sediment–aqueous extractant ratio was maintained at 1 g:10 mL throughout and extractions were carried out in sealed Teflon tubes with continual shaking (150 rpm) on an orbital shaker. All samples were handled in an anaerobic cabinet (95% N₂:5% H₂), and all reagents were prepared with deoxygenated deionised water until the H₂O₂ leaching stage. A full list of reagents and handling procedures are given in Table 2. Total Tc was determined on leachates as detailed above, with quench monitoring to compensate for the effect of different matrices on counting efficiency.

An additional alkaline extraction process was also carried out on selected radiolabeled sediment samples. Alkaline extraction causes the dispersal of humic organic matter (Macleod and Semple, 2000; Séby et al., 1997), but is not strongly oxidising or reducing and does not reoxidise redox active trace metal contaminants such as Cr (James et al., 1995; Vitale et al., 1997). After aqueous sample extraction, 1 g of anoxic sediment was leached for 24 h in 10 mL deoxygenated 2 mol L⁻¹ NaOH. The residual solids were then leached in 70% HNO₃ as for the final stage in the Tessier scheme. In both the Tessier and alkaline extractions, >90% of total Tc was recovered in the extracts.

2.5. Transmission electron microscopy

Energy dispersive X-ray spectroscopy (EDX) has an average detection limit of ca. 0.5% (w/w) and sample preparation thus involved spiking ca. 0.25 mL of a slurry (400 mg sediment per mL) of SO₄²⁻-reducing Humber Estuary sediment with ca. 300 kBq

(0.5 mg) ammonium pertechnetate. After two days incubation in the dark at 21 °C, the reacted sediment was prepared for imaging by washing with 1.0 mL of 2.5% glutaraldehyde solution in 0.1 M phosphate buffer (2 h) followed by washing with 1.0 mL of 0.1 M phosphate buffer and dehydrating with 1.0 mL ethanol. Approximately 5 mg of sediment was then added to 1.5 mL Spurr's resin (Spurr, 1969) in a separate tube and, after polymerisation (24 h at 70 °C), thin sections of resin embedded sediment were cut using a microtome and placed on Cu support grids (Agar Scientific, UK) for TEM analysis. All transfers were carried out under Ar gas, and all solutions were deoxygenated prior to use by bubbling with N₂ for 20 min. At each transfer the sediment was centrifuged for 5 min at 10,000 rpm and the supernatant was removed and discarded.

The specimens were examined using a Philips/FEI CM200 field emission gun TEM fitted with a scanning (STEM) unit and an ultra thin window energy dispersive X-ray detector (Oxford Instruments ISIS EDX). Parallel electron energy loss spectra (EELS) were collected using a Gatan Imaging Filter (GIF 200) with the microscope operated in diffraction mode (image coupled), using an approximate 0.18 μm diameter selected area aperture. EEL spectra were acquired with an energy resolution of 0.8 eV and with collection and convergence semi-angles of 6 mrad and 1 mrad, respectively.

2.6. Gamma camera imaging

Two microcosm bottles containing either oxic surface sediment or pre-reduced SO₄²⁻-reducing estuarine sediments (ca. 10 g dry weight) and 100 mL river water were spiked with 10 MBq (final concentration $\sim 5 \times 10^{-12} \text{ mol L}^{-1}$) of the γ -emitting ^{99m}Tc (half-life 6 h, 140.5 keV) and shaken. Following spiking, gamma camera images were then taken over a 24 h period using a medical gamma camera (GE Millennium MPR/MPS Digital CES Single Detector Gamma Camera S8003YA with eNTEGRA NM workstation collection software, GE Medical Systems, Milwaukee, WI, USA) at the Department of Nuclear Medicine, Manchester Royal Infirmary. The camera was fitted with a high resolution collimator (2 mm) and accuracy was $\sim 0.5 \text{ cm}$. Images produced are composites of 5 min data acquisitions.

3. Results and discussion

3.1. Fate of Tc: incubation experiments

Past work has shown that when oxic estuarine sediments are anaerobically incubated with Tc(VII), Tc removal was microbially mediated and occurred over several tens of days (Burke et al., 2005; Fig. 2a). During the 36 day incubation period, pH varied between 7.6 and 8.1 with no discernable trend. Chloride concentrations also only varied slightly between 218 and 230 mmol L⁻¹ indicating that no major change in ionic strength occurred. Eh, however, reduced from $+124 \pm 11 \text{ mV}$ to $-140 \pm 22 \text{ mV}$ indicating a significant change in redox conditions. Solvent extraction of solution phase samples with TPA indicated that during the development of anoxia, any Tc in the aqueous phase was predominantly Tc(VII). Removal of Tc occurred after NO₃⁻ was removed from solution during active Fe(II) ingrowth in sediments indicated by 0.5 M

Table 2
Sequential extraction scheme (Tessier et al., 1979), adapted for use with anoxic samples Kersten and Förstner, 1987; Wallmann et al., 1993) and for use with Tc-radiolabeled sediments (Keith-Roach et al., 2003).

Fraction	Target phase	Leachate composition	Leach time (h)
Porewater	Soluble metals	Supernatant	
Exchangeable	Weakly sorbed exchangeable metals	1 mol L ⁻¹ magnesium chloride (pH 7)	2
Carbonate	Metals adsorbed or bound to carbonates	1 mol L ⁻¹ sodium acetate, adjusted to pH 5 with acetic acid	5
Reducible	Metals bound to Fe–Mn-oxyhydroxides	0.1 mol L ⁻¹ ammonium oxalate, adjusted to pH 3 with HCl	12
Oxidisable	Metals bound to sulfides or organic matter	30% H ₂ O ₂ , adjusted to pH 2–3 with HNO ₃ , heated (80 °C) until dryness. Then leached with 1 mol L ⁻¹ ammonium acetate in 6% (v/v) HNO ₃ (pH 2)	24/6
Residual	Metals held within the mineral matrix	70% HNO ₃ heated (80 °C) until dryness. Then leached with 1 mol L ⁻¹ ammonium acetate in 6% (v/v) HNO ₃ (pH 2)	48/6

HCl extraction, before any Fe was detected in the aqueous phase, and also before any SO₄²⁻ was removed from solution. This is consistent with studies by other workers (e.g. Abdelouas et al., 2005; Begg et al., 2007; Fredrickson et al., 2004; Wildung et al., 2004; McBeth et al., 2007; Li and Krumholz, 2008) and suggests that Tc removal is mediated via reduction of soluble Tc(VII) to poorly soluble Tc(IV), either via enzymatic pathways or abiotic reaction with Fe(II)_(s) generated by dissimilatory Fe(III)-reduction.

To further investigate the mechanism of Tc removal, short time scale experiments (<1 h) were performed to examine the rate of Tc(VII) scavenging by pre-reduced microbially active Fe(III)- and SO₄²⁻-reducing sediments. Sterilised pre-reduced Fe(III)- and SO₄²⁻-reducing sediments were also reacted with Tc(VII), to assess whether purely abiotic reduction governed Tc removal. In both microbially active and sterile experiments, Tc removal from the aqueous phase occurred rapidly over a period of 10 min (Fig. 2b and c). This rapid removal indicates that in these pre-reduced systems, Tc is abiotically reduced, presumably by reaction with Fe(II) (or possibly by sulfide, in SO₄²⁻-reducing sediments).

To examine the behaviour of Tc in this study compared to others, it is useful to examine distribution coefficients between Tc in the solid and liquid phase (K_d values), where

$$K_d = \frac{[\text{solid (Bq kg}^{-1}\text{)}]}{[\text{liquid (Bq L}^{-1}\text{)}]} \quad (1)$$

Distribution coefficients are operationally defined values and thus will be strictly relevant only to the system of study. Nevertheless K_d values can be used to indicate the extent of radionuclide sorption occurring in different systems. Thus, K_d values were calculated for progressively anoxic Fe(III)-reducing and SO₄²⁻-reducing sediments after 36 and 181 days incubation, respectively (Table 3) as well as for rapid reaction with pre-reduced sediments. The K_d values calculated for both progressively reducing and rapidly reacted experiments were similar, with K_d values for Fe(III)-reducing sediments and SO₄²⁻-reducing sediments at ca. 10³ and ca. 10⁴ L kg⁻¹, respectively (Table 3). These are consistent with reported K_d values for Tc-radiolabeled, fine-grained, organic-rich, anoxic sediments, where K_d values of ca. 10⁴ L kg⁻¹ have been found in anoxic lake (Bird and Schwartz, 1997), fjord (Keith-Roach et al., 2003) and fluvial (Standing et al., 2002) sediments. In Tc-radiolabeled coastal sediments from the Irish Sea, which are reported to be Fe(III)-, but not SO₄²⁻-reducing (McCubbin et al., 2006), K_d values of ca. 10³ L kg⁻¹ have been reported (McCubbin et al., 2006), consistent with the calculated K_d values for Fe(III)-reducing sediments studied here.

Overall, the progressive anoxia microcosms demonstrated that Tc removal is microbially mediated, occurs over several tens of days, and is concomitant with Fe(II) ingrowth to sediments. Addi-

tionally, experiments with pre-reduced sediments showed remarkably fast (sub 20 min) removal of >99% of Tc(VII), indicating that abiotic removal of Tc by reaction with Fe(II) was possible in these systems. In these short term experiments, there was little discernible difference in Tc removal rates between Fe(III)- and SO₄²⁻-reducing sediments, and between microbially active and sterile sediments. Furthermore, an empirical measure of the extent of removal, K_d , shows these experiments to be remarkably consistent with studies from a range of sedimentary environments. It is impossible, however, to directly ascertain the chemical form or mineral interactions of the insoluble Tc bound to these sediments.

3.2. Fate of Tc: chemical extractions

A sequential extraction technique was used to assess the associations of sediment-bound Tc during progressive anoxia experiments under both Fe(III)- and SO₄²⁻-reducing conditions (Fig. 3). The descriptive names for the different chemical leaches described in the adapted, anoxic Tessier scheme are applied here (Table 2; Tessier et al., 1979; Keith-Roach et al., 2003) for ease of reference. Chemical extractions have well known limitations in that they do not generally access well defined mineral fractions; rather, they provide operationally defined data on associations of metals in sediments, and over-interpretation of these results must be avoided. The limitations of sequential extraction techniques are discussed in detail by Keith-Roach et al. (2003).

In sequential extractions performed on both Fe(III)- and SO₄²⁻-reducing sediments, the results were very similar with 70 ± 3% of bound Tc found in the oxidisable fraction, and 21 ± 3% found in the reducible fraction even though the Fe(III)- and SO₄²⁻-reducing sediment have very different geochemistries (Table 1). These results are consistent with other sequential extractions on Tc-labelled sediments and using similar chemical leaches where the dominant proportion of Tc was found in the oxidisable fraction (e.g. 60–66%, Abdelouas et al., 2005; 72–89%, Keith-Roach et al., 2003; 80–85%, Standing et al., 2002). In the scheme applied here, the H₂O₂ oxidisable fraction, where most of the Tc was recovered, is considered to leach metals primarily from organic matter and non-acid volatile sulfide minerals such as FeS₂ (Keith-Roach et al., 2003; Tessier et al., 1979; Wallmann et al., 1993). Despite the suggestion from pure culture studies that biogenic sulfide is a potent reductant for Tc(VII) (Lloyd et al., 1998), there is no evidence linking SO₄²⁻-reduction to Tc-reduction and removal in Humber Estuary sediments (Burke et al., 2005). Extraction results could, therefore, be interpreted as suggesting that reduced Tc may be incorporated into sedimentary organic matter, consistent with the conclusions of other workers (Abdelouas et al., 2005; Keith-Roach et al., 2003; Maes et al., 2004; Standing et al., 2002). To further investigate the associations of Tc and organic

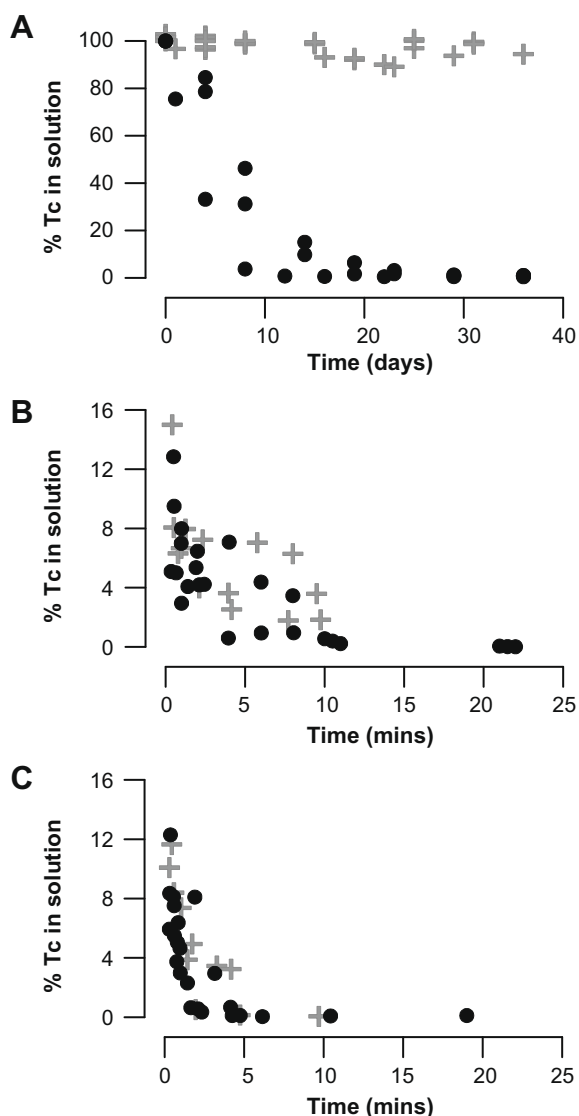


Fig. 2. Incubation of 1.5 or 5.0 $\mu\text{mol L}^{-1}$ ammonium pertechnetate solution with Humber Estuary sediments from: (A) the oxidised surface layer, which becomes Fe(III)-reducing when incubated for 36 days (60–65% Fe(II)); (B) pre-reduced, surface sediments which had been incubated until Fe(III)-reducing conditions (60–65% Fe(II)) were established; and (C) with pre-reduced, SO_4^{2-} -reducing sediments sampled from 20 to 25 cm depth below surface (95–100% Fe(II)). Experiments with active microbial populations (●) and after sterilisation by heat treatment (+) are shown.

matter alkaline extraction of Fe(III)-reducing sediments was performed (Fig. 4). Here, only 28–30% Tc was leachable in 2 M NaOH after 24 h and significantly less than was observed in the oxidisable fraction in the Tessier scheme. Alkaline extraction in 2 M NaOH is reported to leach sedimentary organic matter (MacLeod and Semple, 2000; S by et al., 1997), and is not expected to oxidise Tc(IV). Furthermore, the potential formation of soluble anionic phases such as Tc(IV)O(OH)_3^- at high pH (Warwick et al., 2007) lead to added complications when interpreting these data. Nonetheless, there is disagreement between the alkaline leach data suggesting up to ~30% association of Tc(IV) with organic matter phases, and the anoxic Tessier extraction results which suggest that in the absence of a strong association with sulfides, Tc is predominantly (~70%) associated with organic matter. Overall, these results highlight the difficulties inherent when interpreting extraction data; indeed, the majority of Tc being extracted into the

Table 3

Range of partition coefficients (K_d) calculated for Humber Estuary sediments incubated with 1.5 or 5.0 $\mu\text{mol L}^{-1}$ ammonium pertechnetate solutions compared to values reported for other anoxic sediments. (a) Standing et al. (2002), (b) Keith-Roach et al. (2003), (c) Bird and Schwartz (1997) and (d) McCubbin et al. (2006).

Sediment experiment (incubation time)	$K_d \pm 1\sigma$ (L kg^{-1})
Humber Estuary surface sediments (36 days, Fe(III)-reducing)	1166 \pm 110 4302 \pm 470
Humber Estuary surface sediments (181 days, SO_4^{2-} -reducing)	13,370 \pm 1200
Humber Estuary, Fe(III)-reducing (10 min)	1500 \pm 700
Humber Estuary, SO_4^{2-} -reducing (10 min)	8400 \pm 2800
Mayak river sediments, Russia ^a (<i>in situ</i>)	14,500 \pm 630
	14,900 \pm 670
Mariager Fjord sediments, Norway ^b (<i>in situ</i>)	30,000
Shield lake sediments, Canada ^c (6 days)	9900 \pm 590
Irish Sea sediments, ^d (<i>in situ</i>)	1900

oxidisable Tessier fraction may simply reflect that H_2O_2 , which is known to oxidise Tc(IV) to Tc(VII) (German et al., 2003) simply causes Tc reoxidation and remobilisation.

3.3. Fate of Tc: spectroscopic analysis

Detailed results of Tc XAS data collected from Tc-radiolabeled Humber Estuary sediments in progressively bioreduced, Fe(III)- and SO_4^{2-} -reducing microcosm experiments have been published previously (Burke et al., 2005; Morris et al., 2008). Briefly, for

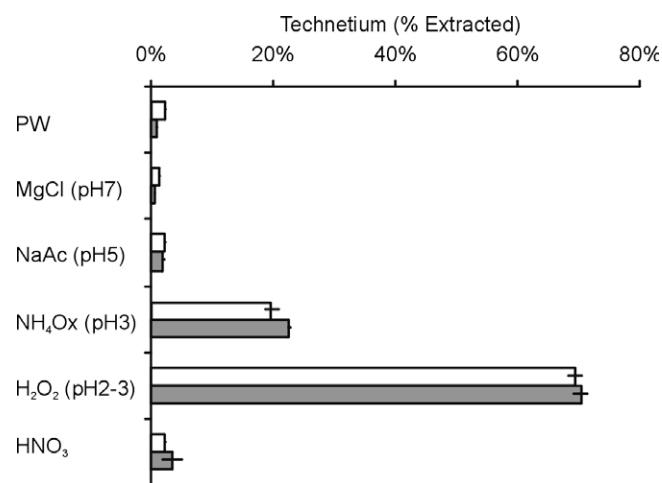


Fig. 3. Sequential extraction data for Tc extracted from Fe(III)- and SO_4^{2-} -reducing Humber Estuary sediments (light and dark bars respectively). PW = aqueous Tc present prior to extraction. Error bars are the range of duplicate results.

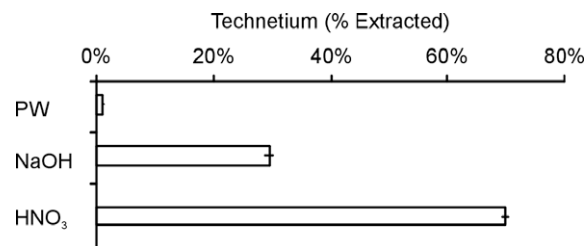


Fig. 4. Alkaline extraction data for Tc after 36 days incubation. PW = aqueous Tc present prior to extraction. Error bars are the range of duplicate results.

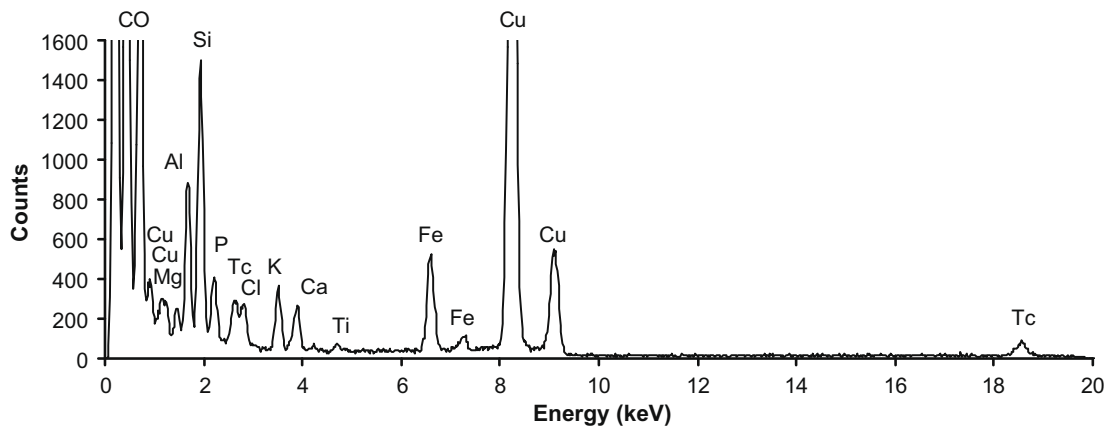


Fig. 5. Energy dispersive X-ray spectrum of the whole region shown in Fig. 6. Cu and C peaks are present due to stray X-rays emitted by the TEM grid and films, respectively, all other major peaks in the spectrum have been identified and labelled. Tc $L\alpha$ X-rays at 2.42 keV are indistinguishable from S $K\alpha$ X-rays at 2.31 keV, but Tc $K\alpha$ X-rays at 18.38 keV do not overlap in energy with any other elemental X-rays measured here and so these confirm the presence of Tc.

XAS analysis, Tc microcosms were prepared so that the final concentration of Tc in the solid phases was several hundred ppm. Both X-ray absorption near edge structure (XANES) and extended X-ray

absorption fine structure (EXAFS) analysis showed that Tc(VII) was reduced to Tc(IV) in all samples (Burke et al., 2005). Modeling of the local coordination environment in all Humber Estuary samples

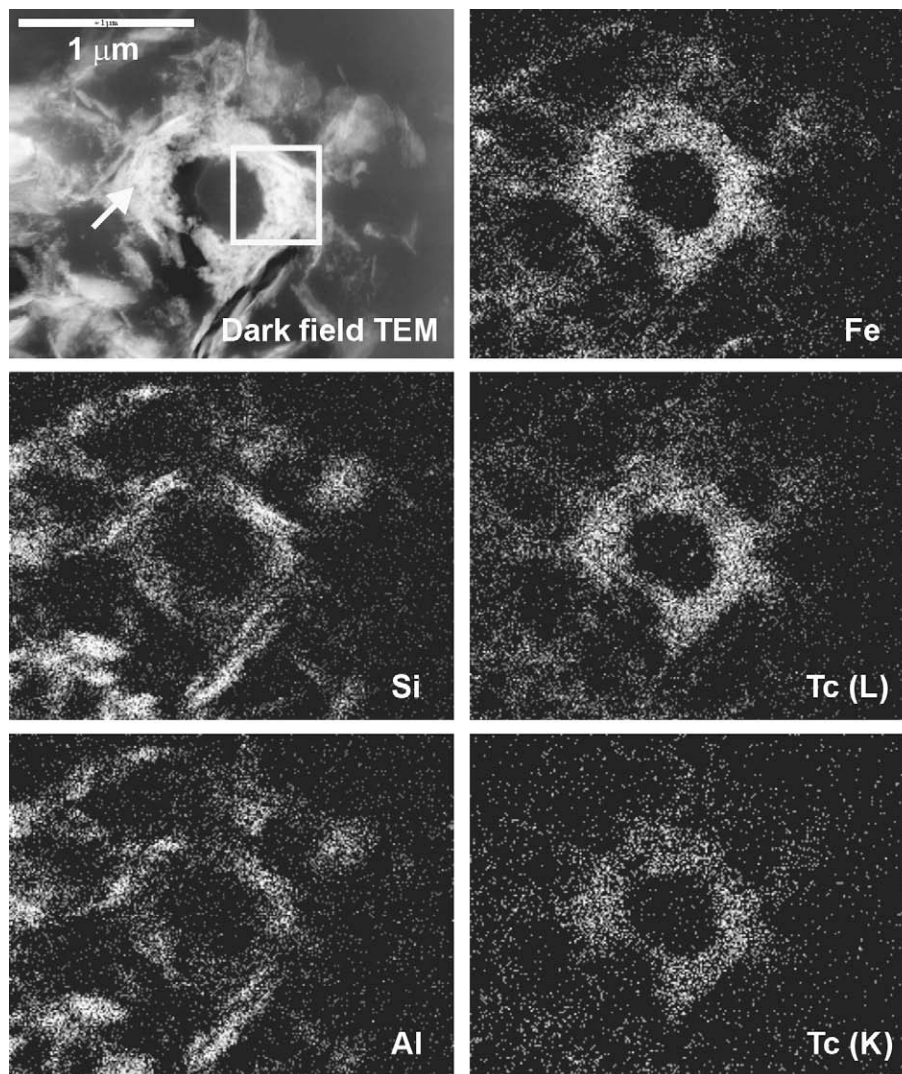


Fig. 6. Annular dark field STEM micrograph and elemental X-ray maps showing the distribution of Fe, Si, Al and Tc ($L\alpha$ X-rays and $K\alpha$ X-rays) in SO_4^{2-} -reducing Humber Estuary sediments incubated with 0.5% (w/w) Tc. Both the Tc ($L\alpha$) and Tc ($K\alpha$) X-ray maps indicate similar Tc distributions suggesting that there is very little or no contribution from S ($K\alpha$ X-rays) in the Tc $L\alpha$ map. Clearly Tc is co-localised with Fe, and Si is co-localised with Al.

was remarkably consistent, and suggested that hydrous Tc(IV)O₂-like phases formed on bioreduction of Tc under Fe(III)-reducing, SO₄²⁻-reducing and progressive anoxia conditions. Indeed, the presence of similar hydrous TcO₂-like phases with both abiotic and biotic reduction has been reported in a wide range of environmental materials (Fredrickson et al., 2004; Maes et al., 2004; Wildung et al., 2004; McBeth et al., 2007; Morris et al., 2008; Begg et al., 2008; Peretyazhko et al., 2008). There are, of course, certain limitations of the XAS technique. For example, it does require elevated Tc concentrations that are unlikely in all but extremely contaminated environments; therefore, Tc speciation determined by XAS may be different to that found at lower concentrations. Nonetheless, the solubility of TcO₂ at pH 7 (~10⁻⁸ mol L⁻¹; Meyer et al., 1991) means that in this study experiments will be oversaturated with respect to TcO₂ in reduced sediment systems (except for the ^{99m}Tc experiments, Section 3.5). Finally, XAS is an averaging technique so that the environment of an 'average' Tc atom within the beam (for these samples the beam was collimated to an area of several mm²) is probed so no information on micro-scale heterogeneity was obtained. Recently, μ-scale XAS mapping has been performed on Tc labelled bioreduced sediments and showed Tc(IV) as co-located with Fe within millimeter sized bioreduced sediment particles (Fredrickson et al., 2009) although XAS approaches currently are unable to resolve nano-scale heterogeneities.

3.4. Fate of Tc: direct visualisation

In order to investigate further the associations of Tc in sediments at the nano-scale, (S)TEM analysis was carried out using SO₄²⁻-reducing Humber Estuary sediments reacted with 10 mmol L⁻¹ TcO₄⁻ for 48 h and producing a maximum Tc concentration in sediments of approximately 0.5% (w/w). This maximum Tc concentration is clearly elevated compared to all but the most contaminated environments, yet was selected as it is at the bottom end of the detectable Tc concentration in TEM-energy dispersive X-ray (EDX) spectroscopy unless elements are concentrated within samples in hotspots. In low resolution EDX maps, Tc was indeed found to occur

in localised hotspots within sediments, consistent with previous reports (Fredrickson et al., 2004; Lear et al., 2009). The EDX spectrum of a representative hotspot is shown in Fig. 5. In STEM/EDX elemental maps of this area (Fig. 6), Tc Kα X-rays were found to coincide with Fe Kα X-rays but not with Al or Si Kα X-rays, which were associated with separate, larger particles that can be viewed in Fig. 8. A background-stripped, energy loss spectrum containing the Fe L_{2,3} core edge (Fig. 7) taken from within the Fe/Tc area identified in STEM/EDX, indicates the presence of Fe(II) in these Tc-labelled sediments because the L₃ edge has a peak maximum on its low energy side (at 707 eV energy loss) with a small shoulder on the high energy side (~709 eV) (Calvert et al., 2005; Garvie and Buseck, 1998; van Aken and Liebscher, 2002; van Aken et al., 1998). A bright field close-up image (Fig. 8) shows that the Fe and Tc are found in a network of fine particles of ca. 10 nm size. Selected area electron diffraction patterns collected from these particles identified them as predominately amorphous in nature. These results provide direct evidence that estuarine sediments amended with high concentrations of Tc, contain Tc associated with nanometre sized Fe(II)-rich nano-particulates. This finding is consistent with evidence from sediment microcosm and XAS experiments (Sections 3.1 and 3.3) where Tc-reduction is strongly linked to the accumulation of microbially-generated, sediment-associated Fe(II). The Tc-rich nanoparticles are likely to have been formed by the reduction of Tc(VII) to Tc(IV) coupled to the oxidation of microbially produced Fe(II) to Fe(III). Although the sediments used in this experiment did contain aqueous Fe (~560 μmol L⁻¹ at pH ~7.6, Table 1) that is likely to be present as Fe(II), this is only sufficient to reduce approximately 2% of the total Tc added to Tc(IV). The majority of Tc-reduction in this TEM sample is therefore mediated via reaction with solid phase Fe(II). The microbially-generated 0.5 M HCl extractable Fe(II) present in these experiments was equivalent to 30.6 ± 2.1 mmol L⁻¹ Fe(II) (Burke et al., 2006), sufficient to reduce all of the 10 mmol L⁻¹ spike to Tc(IV). In addition, previous studies have found that although the reduction of Tc(VII) by aqueous Fe(II) can initially occur in solution (at pH ≥ 7) the reduction processes is dominated by the redox reaction of Tc(VII) with Fe(II) that becomes sorbed to any solid Fe(III) oxides that form (Zachara et al., 2007). Indeed,

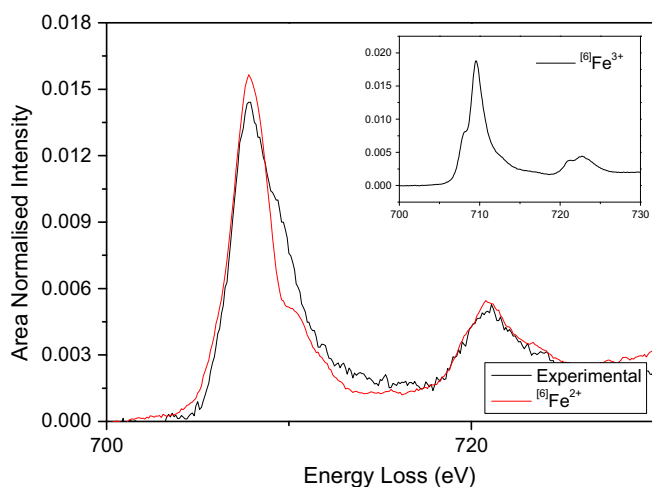


Fig. 7. Background stripped Fe L_{2,3}-edge electron energy loss spectrum (black line) acquired from the Fe–Tc containing sediment (marked by the white arrow in Fig. 6). The general shape of the edge and the position of the L₃ peak maxima (at 707 eV energy loss) is consistent with a spectrum from an Fe(II) rich region. For reference a spectrum from a well characterised Fe(II) bearing mineral (hedenbergite; CaFe-Si₂O₆, where the Fe is octahedrally co-ordinated to O [6]) is shown in red and inset is a reference spectrum from a well characterised Fe(III) bearing mineral (hematite; α-Fe₂O₃). Reference data provided by Pan et al. (2009) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.).

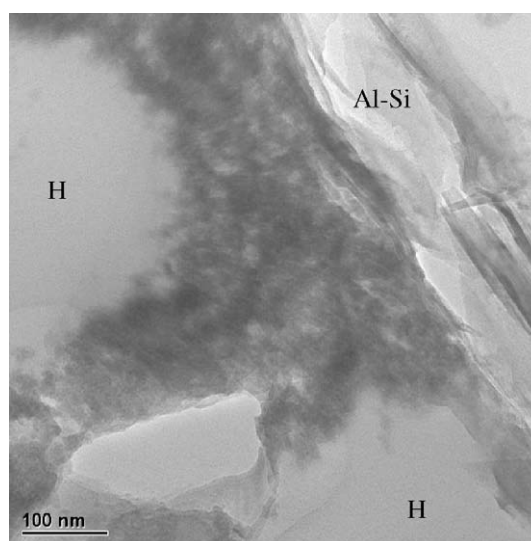


Fig. 8. Bright field TEM micrograph of the sediment shown to be Fe- and Tc-rich (inside white box in Fig. 6; central dark area above) showing a network of ~10 nm particles that are amorphous (by electron diffraction) and Fe(II) rich (Fig. 7). There are holes evident in the resin section where larger more crystalline particles have been pulled out under the action of the knife during sectioning (H). Larger crystalline Al- and Si-rich particles (Al-Si, as identified by EDX mapping, Fig. 6) can be seen in the upper right light area.

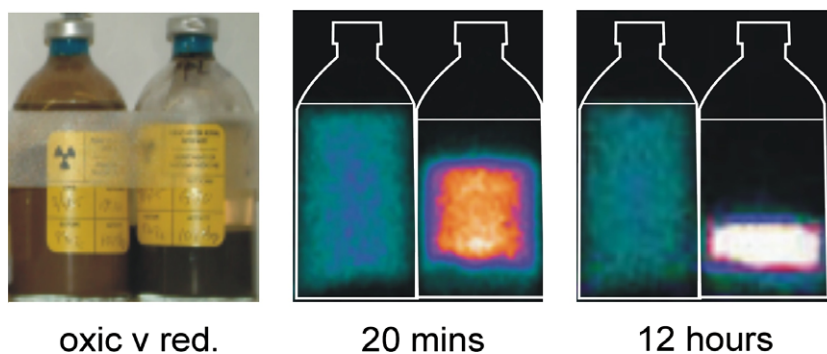


Fig. 9. Gamma camera images of microcosms containing oxidised and SO_4^{2-} -reducing sediments incubated with 10 MBq $^{99\text{m}}\text{Tc}$. As the sediment settles during the experiment, the gamma camera clearly shows that Tc is associated with sediments in the reducing experiment (right bottles) and that Tc remains evenly dispersed in solution in the oxidised experiment (left bottles).

the reaction of Tc(VII) with Fe(II) sorbed to mineral surfaces is reportedly almost instantaneous at pH greater than 7 (Peretyazhko et al., 2008). Therefore, it is likely that the reaction of Tc(VII) with sorbed Fe(II) produces the nano-particles visualised in these experiments. It is, however, possible that these Tc-containing, Fe(II)-rich, nano-particles will only form if elevated Tc concentrations are present and that different reduction mechanisms may be more important in environments where lower concentrations of Tc exist.

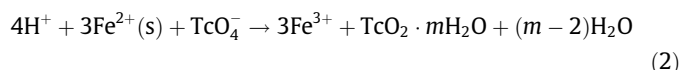
3.5. Fate of Tc: gamma camera experiments

Direct spectroscopic and imaging techniques such as XAS and TEM require very high concentrations of Tc (0.05–0.5 wt.%). This is some 3–4 orders of magnitude above the lower concentrations used in the batch and chemical extraction experiments (Sections 3.1 and 3.2) and several-more orders of magnitude higher than Tc-levels found in contaminated environments (10^{-7} – 10^{-10} mol L^{-1}) (Istok et al., 2004; McBeth et al., 2007; McCubbin et al., 2006; Morris et al., 2000). Thus, it is important to verify if the results found in experiments using higher Tc concentrations provide insights relevant to Tc behaviour in the far field environment. One attraction of gamma camera imaging is that concentrations in the order of *ca.* 10^{-11} – 10^{-12} mol L^{-1} are used, typical of many Tc contamination scenarios. In $^{99\text{m}}\text{Tc}$ imaging experiments using bioreduced and oxic Humber Estuary sediments, the concentration of Tc added in these experiments was *ca.* 5×10^{-12} mol L^{-1} . The gamma camera images (Fig. 9) show that, as the sediments in the bottles spiked with $^{99\text{m}}\text{TcO}_4^-$ settle, Tc is associated with the bioreduced sediments, presumably due to reduction and retention of Tc(IV), whereas the $^{99\text{m}}\text{Tc}$ remains evenly distributed in solution in experiments with oxic sediments. The concentrations of Tc used in these experiments was below the solubility limits for hydrous Tc(IV) O_2 ($\sim 10^{-9}$ mol L^{-1} at pH 7–9 (Meyer et al., 1991)), suggesting that sorption of Tc(IV) to mineral surfaces is an important process causing Tc retention. Indeed, Lear et al. (2009) have recently reported a dynamic $^{99\text{m}}\text{Tc}$ imaging experiment in which a $\sim 10^{-12}$ mol L^{-1} Tc solution flowing into Fe(III)-reducing FRC sediment becomes rapidly reduced and removed from solution.

4. Summary

Microcosm experiments show that more than 99% of TcO_4^- is removed from solution both in experiments where active microbially-mediated ingrowth of Fe(II) occurs, and by contact with pre-reduced Fe(III)- and SO_4^{2-} -reducing sediments. Indeed, the Tc K_d values of between 10^3 and 10^4 L kg^{-1} are in close agreement with other studies. EXAFS analysis of reduced sediments has shown that, at high concentrations (*ca.* 10^{-3} – 10^{-4} mol L^{-1} added to

microcosms; 50–500 ppm on sediments), hydrous Tc(VI) O_2 -like phases are the product of reduction in Humber Estuary sediments (Burke et al., 2005) and in a wide range of other environmentally relevant materials (Fredrickson et al., 2004; Maes et al., 2004; McBeth et al., 2007; Wharton et al., 2000; Wildung et al., 2004; Morris et al., 2008; Begg et al., 2008; Peretyazhko et al., 2008). Sequential extractions using the Tessier scheme suggest that most of the Tc ($\sim 70\%$) leached into the organic matter associated fraction, whilst alkaline extraction of an organic fraction shows that only 28–30% of Tc was found in the humic substances fraction. These results are contradictory, but given that hydrous Tc(IV) O_2 forms in both organic-rich and organic-poor sediments, this result may simply reflect artefacts of the indirect leaching methodologies. In the Tessier procedure Tc(IV) may be oxidised to form soluble TcO_4^- on exposure to H_2O_2 , the first strong oxidant used, and in alkaline extractions soluble Tc(IV) species may form at high pH. In (S)TEM micrographs and EDX elemental mapping performed on pre-reduced Humber Estuary sediments reacted with pertechnetate it was found that Tc was located in nano-scale amorphous, Fe(II)-rich particles. The mechanism of reduction in Humber Estuary sediments is therefore most likely to be through reaction with Fe(II) produced by microbial reduction in sediments:



It is useful to understand the mechanism of Tc-reduction since the reaction of TcO_4^- with Fe(II), originating from stimulation of indigenous Fe(III)-reducing bacteria in sediments, is now being proposed as a possible *in situ* treatment for contaminated sites (Istok et al., 2004; Lloyd et al., 2003; Lovley, 2001; Lovley and Lloyd, 2000; Michalsen et al., 2006). In this context, it is also important to understand potential reoxidation processes which could lead to remobilisation of Tc from sediments. Recent work suggests that hydrous Tc(IV) O_2 is resistant to reoxidation and remobilisation (Tagami and Uchida, 1999; Wharton et al., 2000; Abdelouas et al., 2005; Burke et al., 2006; McBeth et al., 2007; Begg et al., 2008; Fredrickson et al., 2009; Jaisi et al., 2009), so these bioremediation techniques are promising as a means to treat ^{99}Tc contamination.

Acknowledgements

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