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Degradation of carbon disulphide (CS_2) in soils and groundwater from a CS_2 contaminated site

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ABSTRACT

This study is the first investigation of biodegradation of carbon disulphide (CS_2) in soil that provides estimates of degradation rates and identifies intermediate degradation products and carbon isotope signatures of degradation. Microcosm studies were undertaken under anaerobic conditions using soil and groundwater recovered from CS_2 contaminated sites. Proposed degradation mechanisms were validated using equilibrium speciation modelling of concentrations and carbon isotope ratios. A first order degradation rate constant of 1.25×10^{-2} h^{-1} was obtained for biological degradation with soil. Carbonyl sulphide (COS) and hydrogen sulphide (H_2S) were found to be intermediates of degradation, but did not accumulate in vials. A $^{13}C/^{12}C$ enrichment factor of -7.5 ± 0.8‰ was obtained for degradation within microcosms with both soil and groundwater whereas a $^{13}C/^{12}C$ enrichment factor of -23.0 ± 2.1‰ was obtained for degradation with site

groundwater alone. It can be concluded that biological degradation of both CS_2 contaminated soil and groundwater is likely to occur in the field suggesting that natural attenuation may be an appropriate remedial tool at some sites. The presence of biodegradation by-products including COS and H_2S indicates that biodegradation of CS_2 is occurring and stable carbon isotopes are a promising tool to quantify CS_2 degradation.

KEY WORDS

Carbon disulphide, carbon disulfide, biodegradation, microcosms, natural attenuation, stable carbon isotopes

INTRODUCTION

Carbon disulphide (CS_2) is a toxic, dense non-aqueous phase liquid (DNAPL) that is both highly volatile and highly flammable (Kalin et al., 2005). It is present in the environment due to anaerobic activity in sediments (Moret et al., 2000 and Lovelock, 1974), metabolism of naturally occurring sulphur compounds by soil bacteria and vegetation (Crookes et al., 1993), volcanic eruptions (Rasmussen et al., 1982), and the in-situ burning of hydrocarbon contaminated salt marsh (Devai et al., 1998). However, anthropogenic sources provide the primary source of CS_2 in the environment (Watts, 2000). Due to its high volatility and that it can ignite or explode when exposed to air (Kalin et al., 2005) remediation of CS_2 contaminated sites is difficult and hazardous. Therefore, the development of a remediation approach that removes CS_2 contamination from soil and groundwater without exposure to air is desirable.

Carbon disulphide has been produced commercially since 1880, and was used historically in a variety of industries including the viscose process (Beauchamp Jr. et al., 1983). In 1973 approximately 65 million kilograms of CS_2 were released to the air in the US, whilst 35 million kilograms reached water and land (SRI, 1975 cited in Peyton et al., 1976). Although demand for CS_2 has declined in recent years, it is predicted that the expanding viscose industries in Asia will increase CS_2 demand by approximately 4.7% in the period 2007 to 2012 (Rojo et al., 2010). Carbon disulphide is also an intermediate formed during the degradation of carbon tetrachloride (CCl_4) in granular sludge (van Eekert et al., 1998), and in a sandy aquifer under sulphate-reducing conditions (Devlin and Müller, 1999). Davis et al. (2003) reported CS_2 concentrations of up to 160 mg L^{-1} on a CCl_4 contaminated site under highly reducing conditions due to abiotic degradation of CCl_4. Given the above, it is

67 unsurprising that sites contaminated with CS_2 have been identified worldwide. In 2006, of
68 the 1244 sites listed on the USEPA's National Priorities List (NPL), 139 sites had recorded
69 CS_2 as a contaminant of concern (USEPA, 2006). This is a similar figure to the number of
70 sites that have recorded the presence of other chlorinated solvents, such as CCl_4 (USEPA,
71 2006).
72
73 A number of abiotic techniques for the in-situ remediation of CS_2 using zero-valent iron for
74 groundwater (Kalin et al. 2005) and chemical oxidation for soil (Dulsey et al. 2001 and Ross
75 et al. 2008) are available. However, to the authors' knowledge no investigations into natural
76 attenuation of CS_2, for contaminated land cleanup have been carried out. In order to
77 demonstrate natural attenuation at a contaminated site, Monitored Natural Attenuation
78 (MNA) protocols recommend a detailed site characterisation and assessment employing a
79 'lines of evidence approach' (Morgan and Sinke, 2005). Primary evidence includes the
80 demonstration that the contaminant plume is stable, shrinking or exhausted using historical
81 contaminant concentrations. However, these data alone will not indicate whether a
82 destructive attenuation mechanism is responsible for the decrease in concentrations (Carey et
83 al., 2000). Geochemical and chemical data are often used as a secondary line of evidence to
84 demonstrate whether a destructive process is causing attenuation. Secondary data includes
85 the characterisation of known intermediates and products of biodegradation and compound
86 specific isotope analysis (van Ras et al., 2007).
87
88 The degradation of CS_2 by microorganisms has been studied by a number of authors to
89 investigate the potential for their use in waste gas treatment plants for manufacturing
90 processes such as the viscose rayon process (Rothschild et al., 1969; Rajagopal and Daniels,
91 1986; Ottengraf et al., 1986; Smith, 1988; Smith and Kelly, 1988; Kelly and Baker, 1990;

92 Kelly and Smith, 1990; Plas et al., 1993; Odintsova et al., 1993; Jordan et al., 1995; Jordan,
93 1996; Jordan et al., 1997; Alcantara et al., 1999; Hartikainen et al., 2000; Sorokin et al., 2001;
94 Pol et al., 2007). During the aerobic and anaerobic degradation of CS_2, carbonyl sulphate
95 (COS) and hydrogen sulphide (H_2S) are both formed as intermediates (Smith and Kelly,
96 1988; Jordan et al., 1997; Alcantara et al., 1999; Hartikainen et al., 2000; Sorokin et al., 2001;
97 Pol et al., 2007). However, under anaerobic conditions COS is reported to accumulate to a
98 greater extent, prior to being degraded (Smith and Kelly, 1988 and Pol et al., 1997). Smith
99 and Kelly (1988) proposed that all the carbon in CS_2 is converted first to COS and then to
100 CO_2 during both aerobic and anaerobic degradation (Equations 1 and 2).

101 $$CS_2 + H_2O \longrightarrow COS + H_2S \qquad (1)$$

102 $$COS + H_2O \longrightarrow CO_2 + H_2S \qquad (2)$$

103 Under aerobic conditions subsequent oxidation of H_2S to elemental sulphur and eventually
104 sulphate may also occur (Smith and Kelly, 1988 and Alcantara et al., 1999).
105
106 Compound specific stable isotope analysis measures the relative abundance of heavy and
107 light isotopes in a compound (in this case ^{12}C and ^{13}C). Biological and abiotic reactions
108 which break individual bonds tend to cause greater stable isotope fractionation than physical
109 processes such as dilution, volatilisation and sorption which act on the whole molecule
110 (Elsner et al., 2005). Therefore, stable isotope fractionation provides a powerful tool in
111 determining whether the natural attenuation of xenobiotic compounds is occurring in the field
112 (Sturchio et al., 1998; Hunkeler et al., 1999; Sherwood Lollar, 2001 and McKelvie et al.,
113 2007). However, to the authors' knowledge, no studies have reported stable carbon isotope
114 enrichment factors for the degradation of CS_2. This study investigates the biologically
115 mediated processes of natural attenuation of CS_2, to provide information about CS_2

degradation rates, identify whether COS and H_2S are degradation intermediates and measure the carbon isotope signatures of degradation in CS_2 contaminated soils and groundwater. This information will assist determination of whether natural attenuation is occurring at CS_2 contaminated sites.

MATERIALS AND METHODS

Chemicals and materials

Experiments were carried out using general purpose reagent grade CS_2 (99.99% w/v, Hopkin and Williams).

Soils and groundwater

Soils were collected during remediation works at a former chemical manufacturing works in Stretford, Manchester, UK. Groundwater was collected from a former viscose rayon plant in Carrickfergus, Northern Ireland. All samples were transported and stored in the dark at 5°C until use. Further details of both sites are provided in Section 1 of the online resources. Soil samples contaminated with CS_2 DNAPL were exposed to a nitrogen atmosphere within a sealed glove bag to allow volatilisation of background CS_2 contamination. Soils were sieved to remove stones greater than 2.36 mm, prior to placing in thin walled plastic bags and storing in the anaerobic chamber at room temperature until ready for use.

Site groundwater was collected anaerobically in 1.92 L nitrogen purged glass jars from an area of known CS_2 contamination. Prior to sampling the borehole was purged until water quality parameters reached stable values. Groundwater was transferred under nitrogen to

collapsible Tedlar bags to ensure no headspace during preparation. The CS_2 concentration in the site groundwater, used in the unspiked tests, was approximately 87 mg L^{-1}. Because higher initial concentrations were required to facilitate carbon isotope analysis of degradation products, site groundwater was spiked with a CS_2 stock solution prepared in methanol. Concentrations in the Tedlar bag for the spiked experiments were 250 mg L^{-1} CS_2 and 157 mg L^{-1} methanol. Previous investigations had shown increased CS_2 degradation when sulphate was present (Cox et al., 2005), therefore Na_2SO_4 (20 mM) was added in both tests. Full details of soil and groundwater preparation are included in Section 2 of the online resources.

Spiked and unspiked microcosm studies

Microcosm studies were carried out in pre-sterilised 22 mL Chromacol glass vials. Soil (5 g ± 0.01 g) were added to each vial, along with 10 mL of site groundwater taken directly from the Tedlar bag using a Teflon and glass, gas tight syringe. Approximately 10.1 mL of headspace was present in each vial. Vials were sealed immediately with a Teflon faced aluminium crimp seal. Due to equilibration with the headspace in the vial, CS_2 concentrations in the water in the vials (C_0) were 40 mg L^{-1} (unspiked test) and 100 mg L^{-1} (spiked test). All setup was undertaken in an anaerobic chamber (10% v/v H_2, 5% v/v CO_2 and 85% N_2).

Carbon disulphide free controls containing soil and groundwater (that had been exposed to a nitrogen atmosphere in a glove bag to remove CS_2 but spiked with methanol) were used to account for the microbial growth on methanol or any background carbon sources present. Groundwater microcosms containing CS_2 were set up to determine CS_2 losses due to abiotic and biological degradation within groundwater exclusively. Microcosms containing soil and

groundwater, referred to as soil microcosms from this point forward, were set up to investigate what additional losses could be attributed to the presence of microorganisms in the soils. Sterilisation of soil and groundwater using mercuric chloride ($HgCl_2$) (final soil concentration of 92 mg of Hg L^{-1}) was unsuccessful (Cox, 2008). Autoclaving for 90 minutes on two occasions on consecutive days was required to successfully sterilise soil containing CS_2 degrading bacteria. For details of sterilisation trials see Section 4 of the online resources. Summary details of the composition of controls and microcosms for both the unspiked and spiked tests are shown in Tables 5.1 and 5.2 in the online resources.

Soil microcosms were prepared in triplicate, while controls and microcosms containing groundwater only were prepared in duplicate. All vials were sealed with teflon faced aluminium crimp seals, wrapped in parafilm and stored in the dark in the anaerobic chamber at room temperature. Vials were removed from the anaerobic chamber at regular intervals and sampled sacrificially to minimise potential for losses due to volatilisation over 10 days.

Analytical methods

Analysis for CS_2, COS, H_2S, CO_2 and CH_4 concentration was undertaken by GC-MS (Trace DSQ, Thermo Finnigan). Compound specific carbon isotope ratios ($\delta^{13}C$) of CS_2, COS, CO_2 and CH_4 in the vial headspace were measured using GC-C-IRMS (Isoprime, GV Instruments) for the spiked test only. All isotopes were reported using the delta notation referenced to Vienna Peedee Belemnite, VPDB. Detailed methods for all analyses are described in Section 6 of the online resources.

Quantification of isotope fractionation

Fractionation is often quantified for comparison purposes using the Rayleigh relationship (Equation 3) (Mariotti et al., 1981).

$$R = R_0 f^{(\alpha-1)} \quad (3)$$

where R is the isotopic ratio of the substrate, R_0 is the initial isotopic ratio of the substrate, f is the remaining fraction of the substrate and α is the fractionation factor. Equation 3 can be rearranged and expressed in δ‰ notation as shown in Equation 4 (Mariotti et al., 1981):

$$\ln\left(\frac{\delta/1000+1}{\delta_0/1000+1}\right) = (\alpha-1)\ln f = \frac{\varepsilon}{1000}\ln f \quad (4)$$

where δ_0 is the initial δ value, and ε is the per mil enrichment factor, which represents the isotopic difference between the contaminant and its initial degradation product (Clark and Fritz, 1997). Equation 4 can be simplified to Equation 5 for small values of δ (Mariotti et al., 1981).

$$\delta - \delta_0 = \Delta\delta \cong 10^3(\alpha-1)\ln f = \varepsilon \ln f \quad (5)$$

and therefore a plot of change in $\delta^{13}C$ against ln(f) will be a straight line of gradient ε that goes through the origin.

RESULTS AND DISCUSSION

Degradation rates

The natural log of CS_2 concentrations (normalised with respect to initial concentration, C_o) against time, are plotted in Figure 1 for soil microcosms (unspiked and spiked tests), sterilised soil with groundwater controls (spiked test) and microcosms containing groundwater-only (unspiked and spiked tests). Initial losses were considered by excluding the initial (time zero) data point and calculating the best fit line without specifying a y-intercept. Where consecutive sampling occasions showed that CS_2 concentrations were less than the limit of quantification (<0.008% v/v), the dataset has been modified to exclude the later sampling occasion, as including this data point skewed the linear regression.

In both the unspiked and spiked groundwater-only tests (Figure 1) carbon disulphide concentrations decreased by approximately 40%. Significantly more degradation was observed in soil microcosms where almost 100% degradation of CS_2 was observed in both the unspiked and spiked tests. Therefore the majority of CS_2 degradation was attributed to the biological activity within the soil. First-order degradation rate constants for soil microcosms in the unspiked test were calculated based on the modified datasets, as shown in Figure 1 and summarised in Table 1. In accordance with Equation 6, the rate constants for the unspiked and spiked soil microcosms ($k_{micro\ soil}$) were corrected for CS_2 losses due to volatilisation, abiotic reactions and biodegradation from groundwater using the degradation rate constant for the spiked sterilised soil with groundwater controls ($k_{sterilised}$). This gives a rate constant for the biodegradation due to soil microbes (k_{degrad}) of $>2.39 \pm 0.16 \times 10^{-2}\ h^{-1}$ for the unspiked test and $1.25 \pm 0.15 \times 10^{-2}\ h^{-1}$ for the spiked test (Table 1).

$$k_{\text{degrad}} = k_{microsoil} - k_{sterilised} \quad (6)$$

233

234 k_{degrad} for the spiked test is less than k_{degrad} from the unspiked test suggesting that the rate of
235 degradation decreases with increasing initial CS_2 concentration (C_0 was 40 mg L^{-1} in the
236 unspiked test and 100 mg L^{-1} in the spiked test) due to microbial inhibition. Similar
237 inhibitory effects were recorded by Plas et al. (1993) at CS_2 concentrations above 150 mg L^{-1},
238 for degradation of CS_2 by *Thiobacillus* K4, while Pol et al. (2007) found that CS_2
239 concentrations greater than 22.8 mg L^{-1} inhibited growth of *Thiomonas* sp. WZW.
240

241 **Degradation products**

242 Carbonyl sulphide was not observed above the limit of quantitation (0.008% v/v) in any
243 control vials or microcosms containing groundwater exclusively. However, following 30
244 hours incubation COS was detected in both the CS_2 unspiked and spiked soil microcosms at
245 0.043 %v/v and 0.287 %v/v, respectively (Figure 2(a)). After 150 hours incubation the
246 concentrations of COS in the spiked and unspiked soil microcosms were below the limit of
247 quantification. If the biological degradation of 1 mole of CS_2 generates 1 mole of COS
248 (Equation 1), it would be expected that a 60% reduction in COS production would be
249 observed correlating to the 60% reduction in the initial CS_2 concentration. However, the
250 observed reduction was 85%, and such discrepancies were attributed to the fact that the
251 maximum COS concentrations may not have been recorded due to the 24-hour sampling
252 interval.
253

254 As with the intermediate COS, H_2S was not detected above the limit of quantification
255 (0.008% v/v) in control vials or microcosms containing groundwater exclusively. Hydrogen
256 sulphide was also below the limit of quantification (0.008% v/v) in the unspiked soil
257 microcosms (Figure 2(b)). However, in CS_2 spiked microcosms containing soil, H_2S

concentrations increased during the first 30 hours of the experiment, before decreasing to less than the limit of quantification at approximately 50 hours, and increasing again slightly at 200 hours (Figure 2(b)). Therefore H_2S is formed as an intermediate during the anaerobic degradation of CS_2 by soil microorganisms. Indeed, the second smaller peak observed in Figure 2(b) may indicate that H_2S is also produced as a result of the subsequent degradation of COS, as proposed by Equation 2.

The generation of both COS and H_2S as intermediates during the biodegradation of CS_2 is in accordance with the mechanism for CS_2 degradation proposed by Smith and Kelly (1988) (Equations 1 and 2). In tests under anaerobic conditions, they found that both COS and H_2S accumulated (Smith and Kelly, 1988). Similarly, Pol et al., (2007) found that under anaerobic conditions, degradation of CS_2 by *Thiomonas* sp. WZW resulted in the accumulation of COS and H_2S, which finally resulted in the inhibition of CS_2 degradation. It is unclear from our results whether the microorganisms responsible for the anaerobic degradation of CS_2 were responsible for the subsequent anaerobic degradation of COS and H_2S (another microorganism in the mixed consortium may have caused this degradation). However it is encouraging that even under strictly anaerobic conditions COS and H_2S did not accumulate to sufficient concentrations to significantly inhibit CS_2 degradation.

Carbon isotope signatures

Rayleigh plots for CS_2 carbon isotopes were constructed for all experiments spiked with CS_2 (Figure 3). Apart from two anomalous data points (circled in Figure 3(a)), most data points from the sterilised soil with groundwater controls are clustered around the x-axis (zero), revealing that significant fractionation is not occurring in these vials. Therefore, CS_2 losses in the sterilised soil control vials are mostly due to non-fractionating processes such as

283 volatilisation and sorption to soil and vials. The fractionation observed in the two anomalous
284 data points coincides with a drop in CS_2 concentration and a slight increase in COS
285 concentrations, indicating that CS_2 degradation may be occurring in these vials, possibly due
286 to the presence of site groundwater or incomplete sterilisation of the soil.
287
288 The fractionation of carbon isotopes observed in microcosms containing groundwater
289 exclusively (Figure 3(b)) and with soil (Figure 3(c)) follows a Rayleigh-type relationship.
290 However, the carbon isotope enrichment factor for CS_2 degradation was different when soil
291 was present. Initial losses were again considered by excluding the initial (time zero) data
292 point and calculating the best fit line without specifying a y-intercept. An enrichment factor
293 of -7.5 ± 0.8‰ was obtained for the soil microcosms (which contained both soil and
294 groundwater), while an enrichment factor of -23.0 ± 2.1‰ was obtained for the less rapid, but
295 highly fractionating degradation observed in microcosms with groundwater only.
296
297 Both enrichment factors obtained for CS_2 degradation are within the range of enrichment
298 factors reported in literature for other organic compounds (-0.5‰ to -32.1‰) (Hunkeler et
299 al., 2001a; Meckenstock et al., 2004; Sherwood Lollar et al., 1999; Ahad et al., 2000; Dayan
300 et al., 1999; Hunkeler et al., 1999; Barth et al.; 2002; Hunkeler et al., 2002; Hunkeler et al.,
301 2001b). Of the many compounds studied previously, it would be expected that fractionation
302 would be similar to that observed for short chain chlorinated hydrocarbons, as (1) compounds
303 with greater numbers of carbon atoms would have "diluted" enrichment factors
304 (Meckenstock et al., 2004), and (2) degradation in these compounds occurs by breaking the
305 C-Cl bond, and chlorine is similar in atomic weight to sulphur (Elsner et al., 2005). Reported
306 enrichment factors for biodegradation of chlorinated ethenes range from -7.1‰ to -31.1‰

307 (Dayan et al., 1999; Hunkeler et al., 1999; Barth et al.; 2002; Hunkeler et al., 2002), which
308 encompasses the enrichment factors found for degradation of CS_2.
309
310 The Streitweiser Limit for breaking a C-S bond is 1.050 (Huskey, 1991). This is a
311 semi-quantitative estimate of the maximum kinetic isotope effect (KIE) ($1/\alpha$) that would be
312 observed for a reaction that breaks a C-S bond (Elsner et al., 2005). This assumes bond
313 cleavage at an infinitely late transition state, and therefore a more realistic estimate of the
314 KIE may be obtained by assuming a transition state at 50% bond cleavage (Elsner et al.,
315 2005), which corresponds to an estimated KIE of half the Streitweiser Limit (KIE = 1.025)
316 (Elsner et al., 2005). KIEs and equivalent fractionation and enrichment factors estimated for
317 C-S bond breakage and observed from CS_2 degradation are shown in Table 2.
318
319 Fractionation observed due to degradation with site groundwater only (ε = -23.0 ± 2.1‰)
320 correlates well with the estimated values. The p-value for the gradient of the straight line
321 regression on the Rayleigh plot for these vials is $p = 1.4 \times 10^{-7}$, indicating the null hypothesis
322 that the straight line's true gradient is zero can be rejected comfortably (threshold p-value =
323 0.05). Therefore the assumption that a linear relationship exists is acceptable for these
324 results. The coefficient of determination, R^2, was greater than 0.90 (n=14), which is
325 considered to be a good fit, given that vials were sacrificially sampled. A linear fit indicates
326 that fractionation is controlled by a single reaction step (Ahad et al., 2000). The coefficient
327 of determination, R^2, was also greater than 0.85 (n=17), for microcosms with soil, while the
328 p-value for the gradient was $p = 6.6 \times 10^{-8}$ (ε = -7.5 ± 0.8‰), however the lower enrichment
329 factor and higher reaction rate for these vials suggests the majority of degradation is
330 occurring via a different pathway/mechanism than degradation due to site groundwater only.

331 This difference in fractionation factors is seemingly anomalous, and requires further
332 investigation to fully elucidate.
333
334 Carbonyl sulphide was initially highly depleted in ^{13}C in soil microcosms (as shown in Figure
335 4), as it was being formed predominantly from CS_2 molecules that contained ^{12}C rather than
336 ^{13}C. However as COS was subsequently degraded, its carbon isotope ratio became enriched,
337 as the CS_2 became enriched in ^{13}C and concurrently COS molecules containing ^{12}C were
338 preferentially degraded. A similar trend in carbon isotope ratio was previously reported for
339 intermediates produced during the degradation of chlorinated solvents (Hunkeler et al., 1999;
340 Hunkeler et al., 2002).
341

342 **Modelling of degradation product concentrations and isotope ratios**

343 To investigate the end point of degradation in soil microcosms, concentrations and isotope
344 ratios of CS_2 and potential degradation products (COS, CO_2 and CH_4) from soil microcosms
345 were modelled mathematically, using a method described by Hunkeler et al. (2002).
346 Microbial and abiotic activity with groundwater only vials were not modelled, as COS
347 concentrations in these vials were less than the limit of quantitation of the concentration
348 analysis.
349
350 Two illustrative models were constructed, the first assuming that CO_2 is the end point of CS_2
351 degradation, (Model 1, shown in Equation 7), and the second assuming that CO_2 is removed
352 from the system. In this case, it has been assumed that CO_2 is converted to CH_4 by
353 methanogenesis (Model 2, shown in Equation 8). Both models treat the microcosms as
354 closed systems, with no other carbon sources contributing to the production of COS, CO_2 or
355 CH_4.

356

357 Model 1 $CS_2 \xrightarrow{k_1} COS \xrightarrow{k_2} CO_2$ (7)

358 Model 2 $CS_2 \xrightarrow{k_1} COS \xrightarrow{k_2} CO_2 \xrightarrow{k_3} CH_4$ (8)

359

360 The models also assume that all degradation processes are first-order (as has been
361 demonstrated for degradation of CS_2), with first-order rate constants, k_1, k_2 and k_3. Full
362 details of the assumptions used in this modelling are provided in Section 7 of the online
363 resources and Cox (2008). The fits of modelled data to actual concentration and isotope data
364 were obtained visually, as modelling was undertaken as a proof of concept, rather than to
365 obtain accurate values for rate constants.

366

367 Concentrations of CS_2 and COS are the same in both Model 1 and Model 2, and were
368 obtained by curve fitting as shown in Figure 8.1 in the online resources. A preliminary
369 estimate of the first-order degradation rate for COS degradation was found to be 0.15 h^{-1},
370 which is considerably faster than the first-order degradation rate for CS_2 under the same
371 conditions. Concentrations of CO_2 were elevated in all vials as the anaerobic growth gas
372 used in the anaerobic chamber contained 5% v/v CO_2. Headspace CO_2 concentrations
373 fluctuated greatly during initial stages of the experiment due to dissociation of gaseous CO_2
374 to H_2CO_3, HCO_3^- and CO_3^{2-}. In order to evaluate total inorganic carbon (TIC), and further
375 investigate CO_2 production, data were inputted into Visual Minteq (version 2.51)
376 (Gustafsson, 2006; Gustafsson, 2012) incorporating ion and pH data (for further details see
377 Cox (2008)). This revealed that the initial peak observed in headspace CO_2 data is consistent
378 with the change in pH and ion content observed in these vials, however significant variation
379 was still apparent between microcosms. Comparison of CO_2 concentrations (as TIC)

380 calculated by Models 1 and 2 shows that both models fit the CO_2 data within the error
381 observed (Figure 8.2 in the online resources). CH_4 concentrations were also modelled
382 adequately by Model 2 (Figure 8.3 of the online resources).
383
384 As Model 1 and Model 2 both fit the concentration data (see Figures 8.1 to 8.3 of the online
385 resources), it is not possible from concentration data alone to determine whether the CH_4
386 produced in these vials was generated by methanogenic degradation of CO_2 or if another
387 carbon source was degraded to form CH_4. To investigate this further both models were
388 extended to consider carbon isotope effects (see Section 7 of the online resources and Cox
389 (2008) for full details).
390
391 Again the expressions for carbon isotope ratios for CS_2 and COS are the same for both Model
392 1 and 2, and isotope ratios predicted by both models are shown on Figure 4. The small dip in
393 CS_2 carbon isotope ratio observed at 100 hours may be due to an inhibitory affect caused by
394 transitory accumulation of COS and H_2S as Pol et al. (2007) found these intermediates can
395 inhibit CS_2 degradation. The fit for COS does not appear to be as good as for CS_2, however
396 if any lag period was experienced before COS degradation commenced, this may explain the
397 initial rise and fall, as the model would show more rapid enrichment over the first 100 hours,
398 if the COS degradation rate was reduced.
399
400 Isotope data for CO_2 showed an enrichment in CO_2 isotope ratios of approximately 4‰ over
401 the course of the experiment. This enrichment was not modelled by Model 1, but Model 2
402 was able to replicate this (Figure 5(a)) if the CO_2 was degraded by a highly fractionating
403 process, such as methanogenesis, which has a reported enrichment factor (ε) of -75 ± 15‰
404 (Clarke and Fritz, 1997)). But modelling of CH_4 isotope data (Figure 5(b)) demonstrated that

405 this process was not methanogenic conversion of CO_2 to CH_4. The actual CH_4 produced was
406 initially depleted in ^{13}C, and rapidly became more enriched in ^{13}C. However, due to the low
407 CO_2 degradation rate and high initial CO_2 concentration, the model predicts very slow
408 enrichment if CO_2 was being converted to CH_4. Therefore, as a rapid degradation rate would
409 not fit the CH_4 concentration data, the CH_4 present in the vials must be produced as a result
410 of degradation of another carbon source in the microcosm, such as methanol. This is
411 supported by the fact that CH_4 was produced in CS_2 free controls in the spiked test (which
412 contained methanol) but not CS_2 free controls in the unspiked tests (which did not contain
413 methanol).
414
415 Therefore it is likely that CO_2 in the vials is being consumed, possibly by assimilation into
416 the biomass of cells. Miltner et al. (2005) have suggested CO_2 fixation is a significant factor
417 of microbial activity in soils. This could mean that the bacteria responsible for CS_2
418 degradation are obtaining energy from CS_2 and carbon from CO_2, as described by Odintsova
419 et al. (1993). However it is also possible that another microorganism is consuming CO_2.
420

421 CONCLUSIONS AND RECOMMENDATIONS

422 Degradation experiments demonstrated that the soil tested contained indigenous bacteria that
423 were capable of degrading CS_2. This implies that natural attenuation could potentially be
424 used to remediate CS_2 contaminated sites; however, further work is needed to characterise the
425 conditions under which degradation is likely to occur in the field. Comparison of the rate
426 constants calculated in both tests revealed that the first-order degradation rate constant
427 decreases with increasing initial CS_2 concentration. This may be as a result of CS_2 being
428 inhibitory to the CS_2 degrading organisms. Therefore site investigations should determine

429 whether natural attenuation would be restricted to down gradient portions of a CS_2 plume and
430 whether source zone remediation would significantly enhance the performance of natural
431 attenuation.
432
433 COS and H_2S were both shown to be intermediates of anaerobic biodegradation of CS_2 by the
434 bacteria present in the soil; however, no COS or H_2S greater than the limit of quantitation
435 was observed in control vials or during degradation with site groundwater alone. Therefore
436 the presence of COS or H_2S in groundwater may be good indicators that biodegradation of
437 CS_2 is occurring in the field; however, their absence is not indicative that biodegradation is
438 not occurring. Even under strictly anaerobic conditions COS and H_2S did not accumulate to
439 sufficient concentrations long enough to inhibit CS_2 degradation, which suggests that
440 accumulation of by-products will not prevent natural attenuation from occurring in the field.
441
442 A $^{13}C/^{12}C$ enrichment factor of -7.5 ± 0.8‰ was obtained for CS_2 degradation with both soil
443 and site groundwater, whereas a $^{13}C/^{12}C$ enrichment factor of -23.0 ± 2.1‰ was obtained for
444 the less rapid degradation due to site groundwater alone, suggesting that if isotopic
445 fractionation is observed in the field, it could indicate that degradation is occurring.
446 However, as it appears that different mechanisms may cause differing amounts of
447 fractionation, until a database of CS_2 enrichment factors has been established it will only be
448 possible to quantify degradation once a site specific enrichment factor has been determined
449 experimentally.
450

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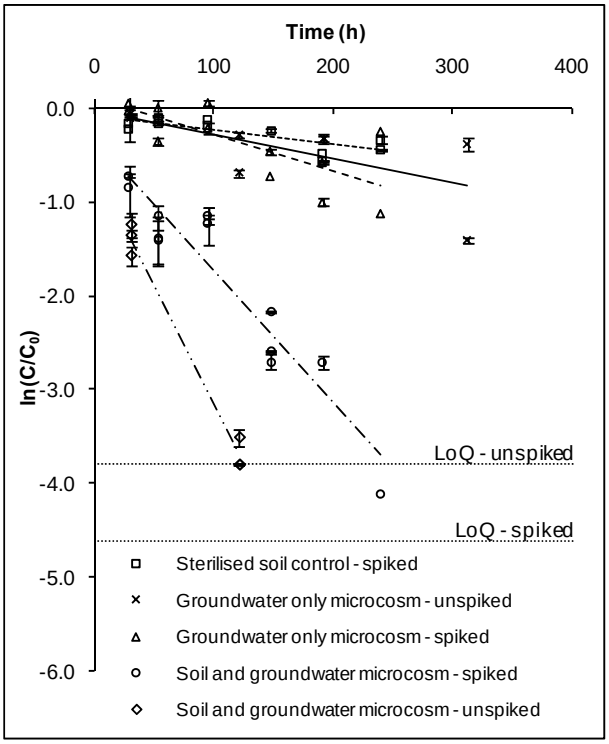
598

599 **Figures**

600

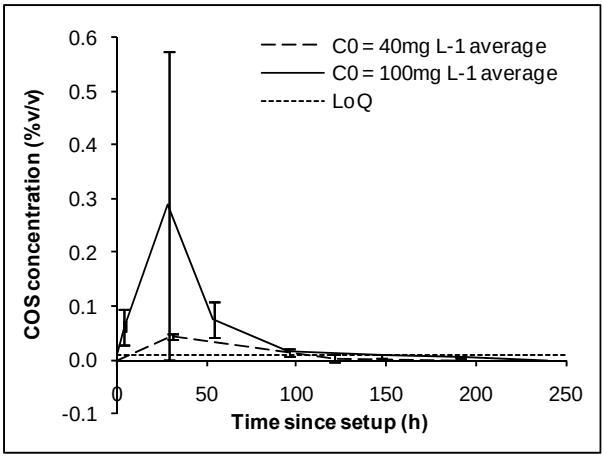


601

602 **Fig. 1** Plot of ln normalised CS_2 concentration versus time for an initial CS_2 concentration of 40 mg L^{-1}
603 (unspiked test) and 100 mg L^{-1} (spiked test). Error bars are two standard errors of three replicate measurements,
604 and therefore depict error associated with method of analysis. LoQ is limit of quantification

605

606 (a)



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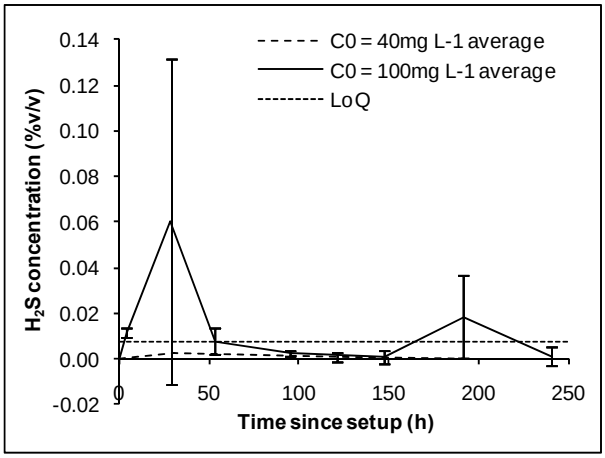
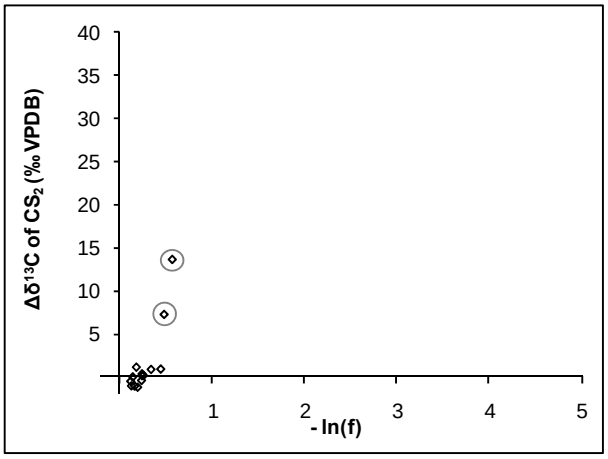


608 **Fig. 2** Headspace concentration versus time for an initial CS_2 concentration of 40 mg L^{-1} (unspiked test) and
609 100 mg L^{-1} (spiked test) for (a) COS and (b) H_2S. Limit of Quantification (LoQ) is 0.008% v/v for both COS
610 and H_2S. Error bars are two standard errors of three independent samples

611 (a)



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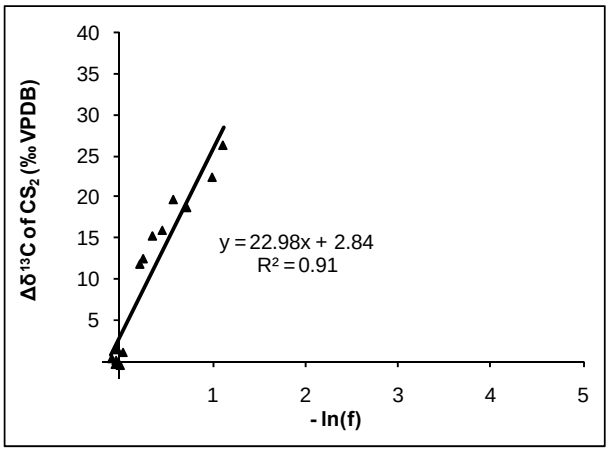

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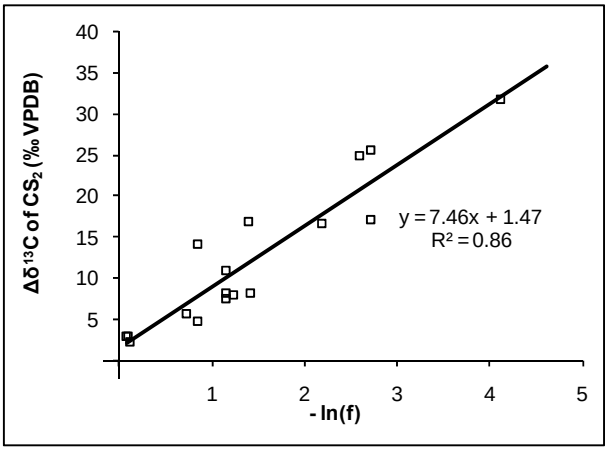


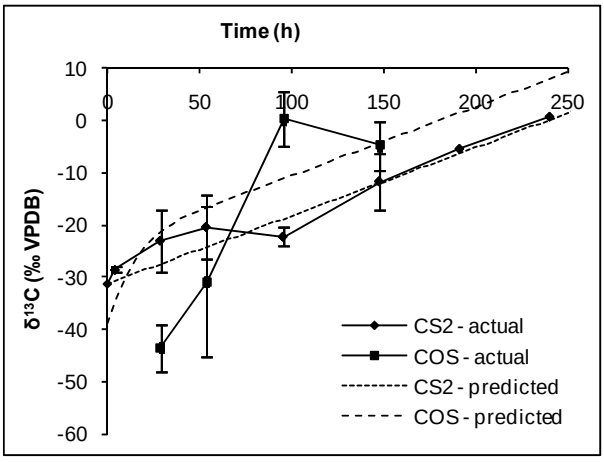
614 **Fig. 3** Rayleigh plot of $\Delta\delta^{13}C$ versus –ln(f) for (a) sterilised soil control, (b) groundwater microcosms (p-value

615 (gradient) = 1.4×10^{-7}) and (c) groundwater and soil microcosms (p-value (gradient) = 6.6×10^{-8}). Circles in

616 Fig.3(a) identify two anomalous data points

617

618

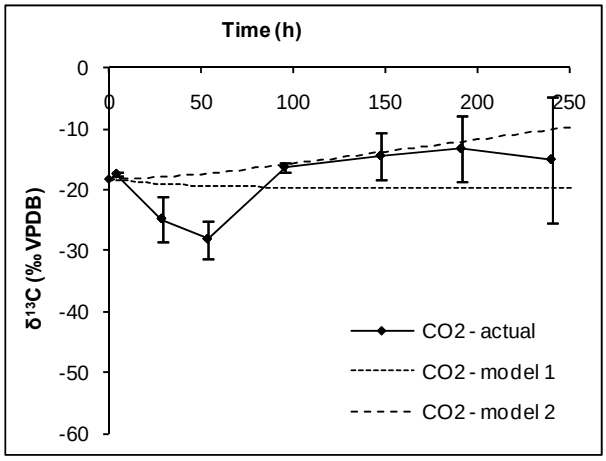


619

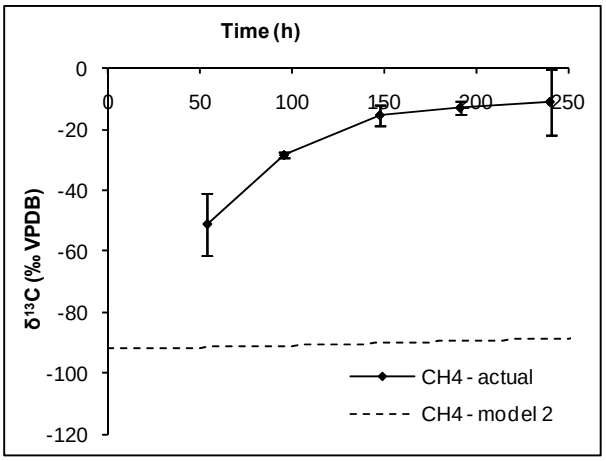
620 **Fig. 4** Actual and modelled carbon isotope ratio of CS_2 and COS versus time for microcosms with soil and
621 site groundwater. Error bars are two standard errors of three independent samples (except t = 191 hrs and 240
622 hrs, where n = 1 (for CS_2) and t = 148 hrs where n = 2 (for COS))

623

624



(a)



(b)

Fig 5 Actual and modelled carbon isotope data versus time for $k_1 = 0.0163\ h^{-1}$, $\alpha_1 = 0.9919$, $k_2 = 0.15\ h^{-1}$, $\alpha_2 = 0.985$, $k_3 = 0.00055\ h^{-1}$ and $\alpha_3 = 0.925$ for (a) CO_2 and (b) CH_4. Error bars are two standard errors of three independent samples

Tables

Sterilised soil control (C_0 = 100mg L^{-1})	$k_{sterilised}$ (h^{-1})	0.15 ± 0.04 x 10^{-2}
Groundwater only (C_0 = 40mg L^{-1})	$k_{groundwater}$ (h^{-1})	0.26 ± 0.12 x 10^{-2}
Groundwater only (C_0 = 100mg L^{-1})	$k_{groundwater}$ (h^{-1})	0.38 ± 0.11 x 10^{-2}
Soil and groundwater (C_0 = 40mg L^{-1})	$k_{micro\ soil}$ (h^{-1})	>2.54 ± 0.15 x 10^{-2}
Soil and groundwater (C_0 = 100mg L^{-1})	$k_{micro\ soil}$ (h^{-1})	1.40 ± 0.14 x 10^{-2}

Table 1 First-order degradation rate constants (h^{-1}) for unspiked and spiked tests. Uncertainties are one standard error

	KIE	α	ε (‰)
Steitweiser limit for C-S bond[a]	1.050	0.952	-48
More realistic estimate of KIE[a]	1.025	0.976	-24
Degradation due to site groundwater	1.0235	0.9770	-23.0
Degradation due to soil and site groundwater	1.0076	0.9925	-7.5

[a] taken from Huskey (1991)

Table 2 KIE, fractionation factor (α) and enrichment factor (ε) calculated for C-S bond breakage and observed during degradation of CS_2