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Cox, S. F., McKinley, J. D., Ferguson, A. S., O'Sullivan, G., & Kalin, R. M. (2013). Degradation of carbon disulphide (CS₂) in soils and groundwater from a CS₂ contaminated site. *Environmental Earth Sciences*, 68(7), 1935-1944. <https://doi.org/10.1007/s12665-012-1881-y>

Published in:
Environmental Earth Sciences

Document Version:
Peer reviewed version

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Degradation of carbon disulphide (CS₂) in soils and groundwater from a CS₂ contaminated site

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ABSTRACT

This study is the first investigation of biodegradation of carbon disulphide (CS₂) 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 CS₂ contaminated sites. Proposed degradation mechanisms were validated using equilibrium speciation modelling of concentrations and carbon isotope ratios. A first order degradation rate constant of $1.25 \times 10^{-2} \text{ h}^{-1}$ was obtained for biological degradation with soil. Carbonyl sulphide (COS) and hydrogen sulphide (H₂S) were found to be intermediates of degradation, but did not accumulate in vials. A ¹³C/¹²C enrichment factor of $-7.5 \pm 0.8\text{‰}$ was obtained for degradation within microcosms with both soil and groundwater whereas a ¹³C/¹²C enrichment factor of $-23.0 \pm 2.1\text{‰}$ was obtained for degradation with site

34 groundwater alone. It can be concluded that biological degradation of both CS₂ contaminated soil and
35 groundwater is likely to occur in the field suggesting that natural attenuation may be an appropriate remedial
36 tool at some sites. The presence of biodegradation by-products including COS and H₂S indicates that
37 biodegradation of CS₂ is occurring and stable carbon isotopes are a promising tool to quantify CS₂ degradation.

38

39 **KEY WORDS**

40 *Carbon disulphide, carbon disulfide, biodegradation, microcosms, natural attenuation, stable*
41 *carbon isotopes*

42

43 INTRODUCTION

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

55

56 Carbon disulphide has been produced commercially since 1880, and was used historically in
57 a variety of industries including the viscose process (Beauchamp Jr. et al., 1983). In 1973
58 approximately 65 million kilograms of CS_2 were released to the air in the US, whilst 35
59 million kilograms reached water and land (SRI, 1975 cited in Peyton et al., 1976). Although
60 demand for CS_2 has declined in recent years, it is predicted that the expanding viscose
61 industries in Asia will increase CS_2 demand by approximately 4.7% in the period 2007 to
62 2012 (Rojo et al., 2010). Carbon disulphide is also an intermediate formed during the
63 degradation of carbon tetrachloride (CCl_4) in granular sludge (van Eekert et al., 1998), and in
64 a sandy aquifer under sulphate-reducing conditions (Devlin and Müller, 1999). Davis et al.
65 (2003) reported CS_2 concentrations of up to 160 mg L^{-1} on a CCl_4 contaminated site under
66 highly reducing conditions due to abiotic degradation of CCl_4 . Given the above, it is

67 unsurprising that sites contaminated with CS₂ 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 CS₂ 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 CCl₄ (USEPA,
71 2006).

72

73 A number of abiotic techniques for the in-situ remediation of CS₂ 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 CS₂, 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 CS₂ 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;

Kelly and Smith, 1990; Plas et al., 1993; Odintsova et al., 1993; Jordan et al., 1995; Jordan, 1996; Jordan et al., 1997; Alcantara et al., 1999; Hartikainen et al., 2000; Sorokin et al., 2001; Pol et al., 2007). During the aerobic and anaerobic degradation of CS₂, carbonyl sulphate (COS) and hydrogen sulphide (H₂S) are both formed as intermediates (Smith and Kelly, 1988; Jordan et al., 1997; Alcantara et al., 1999; Hartikainen et al., 2000; Sorokin et al., 2001; Pol et al., 2007). However, under anaerobic conditions COS is reported to accumulate to a greater extent, prior to being degraded (Smith and Kelly, 1988 and Pol et al., 1997). Smith and Kelly (1988) proposed that all the carbon in CS₂ is converted first to COS and then to CO₂ during both aerobic and anaerobic degradation (Equations 1 and 2).

Under aerobic conditions subsequent oxidation of H₂S to elemental sulphur and eventually sulphate may also occur (Smith and Kelly, 1988 and Alcantara et al., 1999).

Compound specific stable isotope analysis measures the relative abundance of heavy and light isotopes in a compound (in this case ¹²C and ¹³C). Biological and abiotic reactions which break individual bonds tend to cause greater stable isotope fractionation than physical processes such as dilution, volatilisation and sorption which act on the whole molecule (Elsner et al., 2005). Therefore, stable isotope fractionation provides a powerful tool in determining whether the natural attenuation of xenobiotic compounds is occurring in the field (Sturchio et al., 1998; Hunkeler et al., 1999; Sherwood Lollar, 2001 and McKelvie et al., 2007). However, to the authors' knowledge, no studies have reported stable carbon isotope enrichment factors for the degradation of CS₂. This study investigates the biologically mediated processes of natural attenuation of CS₂, to provide information about CS₂

degradation rates, identify whether COS and H₂S are degradation intermediates and measure the carbon isotope signatures of degradation in CS₂ contaminated soils and groundwater. This information will assist determination of whether natural attenuation is occurring at CS₂ contaminated sites.

MATERIALS AND METHODS

Chemicals and materials

Experiments were carried out using general purpose reagent grade CS₂ (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 CS₂ DNAPL were exposed to a nitrogen atmosphere within a sealed glove bag to allow volatilisation of background CS₂ 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 CS₂ 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 CS₂ 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 CS₂ stock solution prepared in methanol. Concentrations in the Tedlar bag for the spiked experiments were 250 mg L⁻¹ CS₂ and 157 mg L⁻¹ methanol. Previous investigations had shown increased CS₂ degradation when sulphate was present (Cox et al., 2005), therefore Na₂SO₄ (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, CS₂ concentrations in the water in the vials (C₀) 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 H₂, 5% v/v CO₂ and 85% N₂).

Carbon disulphide free controls containing soil and groundwater (that had been exposed to a nitrogen atmosphere in a glove bag to remove CS₂ but spiked with methanol) were used to account for the microbial growth on methanol or any background carbon sources present. Groundwater microcosms containing CS₂ were set up to determine CS₂ 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 ($\delta^{13}\text{C}$) 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 $\delta^{13}\text{C}$ 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 CS₂ concentrations (normalised with respect to initial concentration, C₀) 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 CS₂ 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 CS₂ was observed in both the unspiked and spiked tests. Therefore the majority of CS₂ 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 ($k_{\text{micro soil}}$) were corrected for CS₂ losses due to volatilisation, abiotic reactions and biodegradation from groundwater using the degradation rate constant for the spiked sterilised soil with groundwater controls ($k_{\text{sterilised}}$). This gives a rate constant for the biodegradation due to soil microbes (k_{degrad}) of $>2.39 \pm 0.16 \times 10^{-2} \text{ h}^{-1}$ for the unspiked test and $1.25 \pm 0.15 \times 10^{-2} \text{ h}^{-1}$ for the spiked test (Table 1).

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

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 ($0.008\% \text{ v/v}$) 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 $0.043 \text{ \%v/v}$ and $0.287 \text{ \%v/v}$, 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 ($0.008\% \text{ v/v}$) in control vials or microcosms containing groundwater exclusively. Hydrogen
256 sulphide was also below the limit of quantification ($0.008\% \text{ v/v}$) 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 H₂S is formed as an intermediate during the anaerobic degradation of CS₂ by soil microorganisms. Indeed, the second smaller peak observed in Figure 2(b) may indicate that H₂S is also produced as a result of the subsequent degradation of COS, as proposed by Equation 2.

The generation of both COS and H₂S as intermediates during the biodegradation of CS₂ is in accordance with the mechanism for CS₂ degradation proposed by Smith and Kelly (1988) (Equations 1 and 2). In tests under anaerobic conditions, they found that both COS and H₂S accumulated (Smith and Kelly, 1988). Similarly, Pol et al., (2007) found that under anaerobic conditions, degradation of CS₂ by *Thiomonas* sp. WZW resulted in the accumulation of COS and H₂S, which finally resulted in the inhibition of CS₂ degradation. It is unclear from our results whether the microorganisms responsible for the anaerobic degradation of CS₂ were responsible for the subsequent anaerobic degradation of COS and H₂S (another microorganism in the mixed consortium may have caused this degradation). However it is encouraging that even under strictly anaerobic conditions COS and H₂S did not accumulate to sufficient concentrations to significantly inhibit CS₂ degradation.

Carbon isotope signatures

Rayleigh plots for CS₂ carbon isotopes were constructed for all experiments spiked with CS₂ (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, CS₂ 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 CS₂ concentration and a slight increase in COS
285 concentrations, indicating that CS₂ 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 CS₂ 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 $-7.5 \pm 0.8\text{‰}$ was obtained for the soil microcosms (which contained both soil and
294 groundwater), while an enrichment factor of $-23.0 \pm 2.1\text{‰}$ was obtained for the less rapid, but
295 highly fractionating degradation observed in microcosms with groundwater only.

296
297 Both enrichment factors obtained for CS₂ 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‰

(Dayan et al., 1999; Hunkeler et al., 1999; Barth et al., 2002; Hunkeler et al., 2002), which encompasses the enrichment factors found for degradation of CS₂.

The Streitweiser Limit for breaking a C-S bond is 1.050 (Huskey, 1991). This is a semi-quantitative estimate of the maximum kinetic isotope effect (KIE) ($1/\alpha$) that would be observed for a reaction that breaks a C-S bond (Elsner et al., 2005). This assumes bond cleavage at an infinitely late transition state, and therefore a more realistic estimate of the KIE may be obtained by assuming a transition state at 50% bond cleavage (Elsner et al., 2005), which corresponds to an estimated KIE of half the Streitweiser Limit (KIE = 1.025) (Elsner et al., 2005). KIEs and equivalent fractionation and enrichment factors estimated for C-S bond breakage and observed from CS₂ degradation are shown in Table 2.

Fractionation observed due to degradation with site groundwater only ($\epsilon = -23.0 \pm 2.1\%$) correlates well with the estimated values. The p-value for the gradient of the straight line regression on the Rayleigh plot for these vials is $p = 1.4 \times 10^{-7}$, indicating the null hypothesis that the straight line's true gradient is zero can be rejected comfortably (threshold p-value = 0.05). Therefore the assumption that a linear relationship exists is acceptable for these results. The coefficient of determination, R^2 , was greater than 0.90 (n=14), which is considered to be a good fit, given that vials were sacrificially sampled. A linear fit indicates that fractionation is controlled by a single reaction step (Ahad et al., 2000). The coefficient of determination, R^2 , was also greater than 0.85 (n=17), for microcosms with soil, while the p-value for the gradient was $p = 6.6 \times 10^{-8}$ ($\epsilon = -7.5 \pm 0.8\%$), however the lower enrichment factor and higher reaction rate for these vials suggests the majority of degradation is occurring via a different pathway/mechanism than degradation due to site groundwater only.

This difference in fractionation factors is seemingly anomalous, and requires further investigation to fully elucidate.

Carbonyl sulphide was initially highly depleted in ^{13}C in soil microcosms (as shown in Figure 4), as it was being formed predominantly from CS_2 molecules that contained ^{12}C rather than ^{13}C . However as COS was subsequently degraded, its carbon isotope ratio became enriched, as the CS_2 became enriched in ^{13}C and concurrently COS molecules containing ^{12}C were preferentially degraded. A similar trend in carbon isotope ratio was previously reported for intermediates produced during the degradation of chlorinated solvents (Hunkeler et al., 1999; Hunkeler et al., 2002).

Modelling of degradation product concentrations and isotope ratios

To investigate the end point of degradation in soil microcosms, concentrations and isotope ratios of CS_2 and potential degradation products (COS, CO_2 and CH_4) from soil microcosms were modelled mathematically, using a method described by Hunkeler et al. (2002). Microbial and abiotic activity with groundwater only vials were not modelled, as COS concentrations in these vials were less than the limit of quantitation of the concentration analysis.

Two illustrative models were constructed, the first assuming that CO_2 is the end point of CS_2 degradation, (Model 1, shown in Equation 7), and the second assuming that CO_2 is removed from the system. In this case, it has been assumed that CO_2 is converted to CH_4 by methanogenesis (Model 2, shown in Equation 8). Both models treat the microcosms as closed systems, with no other carbon sources contributing to the production of COS, CO_2 or CH_4 .

calculated by Models 1 and 2 shows that both models fit the CO₂ data within the error observed (Figure 8.2 in the online resources). CH₄ concentrations were also modelled adequately by Model 2 (Figure 8.3 of the online resources).

As Model 1 and Model 2 both fit the concentration data (see Figures 8.1 to 8.3 of the online resources), it is not possible from concentration data alone to determine whether the CH₄ produced in these vials was generated by methanogenic degradation of CO₂ or if another carbon source was degraded to form CH₄. To investigate this further both models were extended to consider carbon isotope effects (see Section 7 of the online resources and Cox (2008) for full details).

Again the expressions for carbon isotope ratios for CS₂ and COS are the same for both Model 1 and 2, and isotope ratios predicted by both models are shown on Figure 4. The small dip in CS₂ carbon isotope ratio observed at 100 hours may be due to an inhibitory affect caused by transitory accumulation of COS and H₂S as Pol et al. (2007) found these intermediates can inhibit CS₂ degradation. The fit for COS does not appear to be as good as for CS₂, however if any lag period was experienced before COS degradation commenced, this may explain the initial rise and fall, as the model would show more rapid enrichment over the first 100 hours, if the COS degradation rate was reduced.

Isotope data for CO₂ showed an enrichment in CO₂ isotope ratios of approximately 4‰ over the course of the experiment. This enrichment was not modelled by Model 1, but Model 2 was able to replicate this (Figure 5(a)) if the CO₂ was degraded by a highly fractionating process, such as methanogenesis, which has a reported enrichment factor (ϵ) of $-75 \pm 15\%$ (Clarke and Fritz, 1997)). But modelling of CH₄ isotope data (Figure 5(b)) demonstrated that

this process was not methanogenic conversion of CO₂ to CH₄. The actual CH₄ produced was initially depleted in ¹³C, and rapidly became more enriched in ¹³C. However, due to the low CO₂ degradation rate and high initial CO₂ concentration, the model predicts very slow enrichment if CO₂ was being converted to CH₄. Therefore, as a rapid degradation rate would not fit the CH₄ concentration data, the CH₄ present in the vials must be produced as a result of degradation of another carbon source in the microcosm, such as methanol. This is supported by the fact that CH₄ was produced in CS₂ free controls in the spiked test (which contained methanol) but not CS₂ free controls in the unspiked tests (which did not contain methanol).

Therefore it is likely that CO₂ in the vials is being consumed, possibly by assimilation into the biomass of cells. Miltner et al. (2005) have suggested CO₂ fixation is a significant factor of microbial activity in soils. This could mean that the bacteria responsible for CS₂ degradation are obtaining energy from CS₂ and carbon from CO₂, as described by Odintsova et al. (1993). However it is also possible that another microorganism is consuming CO₂.

CONCLUSIONS AND RECOMMENDATIONS

Degradation experiments demonstrated that the soil tested contained indigenous bacteria that were capable of degrading CS₂. This implies that natural attenuation could potentially be used to remediate CS₂ contaminated sites; however, further work is needed to characterise the conditions under which degradation is likely to occur in the field. Comparison of the rate constants calculated in both tests revealed that the first-order degradation rate constant decreases with increasing initial CS₂ concentration. This may be as a result of CS₂ being inhibitory to the CS₂ degrading organisms. Therefore site investigations should determine

whether natural attenuation would be restricted to down gradient portions of a CS₂ plume and whether source zone remediation would significantly enhance the performance of natural attenuation.

COS and H₂S were both shown to be intermediates of anaerobic biodegradation of CS₂ by the bacteria present in the soil; however, no COS or H₂S greater than the limit of quantitation was observed in control vials or during degradation with site groundwater alone. Therefore the presence of COS or H₂S in groundwater may be good indicators that biodegradation of CS₂ is occurring in the field; however, their absence is not indicative that biodegradation is not occurring. Even under strictly anaerobic conditions COS and H₂S did not accumulate to sufficient concentrations long enough to inhibit CS₂ degradation, which suggests that accumulation of by-products will not prevent natural attenuation from occurring in the field.

A ¹³C/¹²C enrichment factor of $-7.5 \pm 0.8\%$ was obtained for CS₂ degradation with both soil and site groundwater, whereas a ¹³C/¹²C enrichment factor of $-23.0 \pm 2.1\%$ was obtained for the less rapid degradation due to site groundwater alone, suggesting that if isotopic fractionation is observed in the field, it could indicate that degradation is occurring. However, as it appears that different mechanisms may cause differing amounts of fractionation, until a database of CS₂ enrichment factors has been established it will only be possible to quantify degradation once a site specific enrichment factor has been determined experimentally.

ACKNOWLEDGEMENTS

This work was supported by an industrial grant from AkzoNobel to Prof. Kalin. White Young Green Environmental is also acknowledged for obtaining permission to collect groundwater samples. The authors acknowledge the valuable technical assistance provided by Juliet McShane and Luc Belaid. We also thank Dr Debra Phillips and Dr Trevor Elliot for valuable advice and comments during preparation of this manuscript.

REFERENCES

- Ahad JME, Sherwood Lollar B, Edwards EA, Slater GF, Sleep BE (2000) Carbon isotope fractionation during anaerobic biodegradation of toluene: Implications for intrinsic bioremediation. *Environ Sci Technol* 34:892-896
- Alcantara S, Estrada I, Vasquez MS, Revah S (1999) Carbon disulfide oxidation by a microbial consortium from a trickling filter. *Biotechnol Lett* 21:815-819
- Ashworth J, Briggs GG, Evans AA, Matula J (1977) Inhibition of nitrification by nitrapyrin, carbon disulphide and trithiocarbonate. *J Sci Food and Agric* 28:673-683
- Barth JAC, Slater GF, Schuth C, Bill M, Downey A, Larkin MJ, Kalin RM (2002) Carbon isotope fractionation during aerobic biodegradation of trichloroethene by *Burkholderia cepacia* G4: a tool to map degradation mechanisms. *Appl Environ Microbiol* 68(4):1728-1734
- Beauchamp Jr. RO, Bus JS, Popp JA, Boreiko CJ, Goldberg L (1983) A critical review of the literature on carbon disulfide toxicity. *CRC Crit Rev Toxic* 11(3):169-278
- Bond EJ (1984) Manual of fumigation for insect control. Research Centre Agriculture Canada, Ontario
- Bremner JM, Banwart WL (1976) Sorption of sulfur gases by soils. *Soil Biol Biochem* 8(79):83
- Carey MA, Finnamore JR, Morrey MJ, Marsland PA (2000) Guidance on the assessment and monitoring of natural attenuation of contaminants in groundwater. R&D Publication 95. Bristol, UK, Environment Agency
- Cox SF, Kalin RM, McKinley JD, Morgan P, Thurgood R (2005) Preliminary investigations into biological processes of natural attenuation of carbon disulphide. Alleman BC and Kelley ME (eds). In:

477 Proceedings of the Eighth International In Situ and On-site Bioremediation Symposium. Battelle Press,
 478 Columbus, OH. Paper H3
 479 Cox SF (2008) Investigation of the biological processes of natural attenuation of carbon disulphide in the
 480 ground. Dissertation, Queen's University Belfast
 481 Clark I, Fritz P (1997) Environmental isotopes in hydrogeology. CRC Press Ltd, United States of America
 482 Crookes MJ, Diment J, Dobson S (1993) Environmental hazard assessment: carbon disulphide. TSD14. Great
 483 Britain. Department of the Environment. Directorate of Air, Climate and Toxic Substances. Toxic
 484 Substances Division, BRE bookshop
 485 Davis A, Fennemore GG, Peck C, Walker CR, McIlwraith J, Thomas S (2003) Degradation of carbon
 486 tetrachloride in a reducing groundwater environment: Implications for natural attenuation. Appl
 487 Geochem 18(4):503-525
 488 Dayan H, Abrajano T, Sturchio NC, Winsor L (1999) Carbon isotope fractionation during reductive
 489 dehalogenation of chlorinated ethenes by metallic iron. Org Geochem 30:755-763
 490 Devlin JF, Muller D (1999) Field and laboratory studies of carbon tetrachloride transformation in a sandy
 491 aquifer under sulfate reducing conditions. Environ Sci Technol 33:1021-1027
 492 Devai I, DeLaune RD, Henry CB, Lindau CW, Roberts PO (1998) Environmental significance of atmospheric
 493 emission resulting in *in situ* burning of oiled salt marsh. Mar Environ Res 45(2):157-167
 494 Dulsey R, Mausner M, Bybee G, Mercurio B, Fahrenthold P, Hazard J, McGhee C (2001) Method for oxidative
 495 destruction of carbon disulfide in soil (US 6283675 B1)
 496 Elsner M, Zwank L, Hunkeler D, Schwarzenbach RP (2005) A new concept linking observable stable isotope
 497 fractionation to transformation pathways of organic pollutants. Environ Sci Technol 39:6896-6916
 498 Gelbke H, Goen T, Maurer M, Sulsky SI (2009) A review of health effects of carbon disulphide in viscose
 499 industry and a proposal for an occupational exposure limit. Crit Rev Toxicol. 39(S2):1-126
 500 Gustafsson JP (2006) Help for Visual Minteq 2.5, KTH, Department of Land and Water Resources Engineering.
 501 Available with Visual Minteq 2.5.
 502 Gustafsson JP (2012) Visual Minteq, KTH, Department of Land and Water Resources Engineering.
 503 <http://www2.lwr.kth.se/English/OurSoftware/vminteq/>. (Accessed 22 June 2012)
 504 Hartikainen T, Ruuskanen J, Raty K, von Wright A, Martikainen PJ (2000) Physiology and taxonomy of
 505 Thiobacillus strain TJ330, which oxidizes carbon disulphide (CS₂). Journal of Appl Microbiol
 506 89(4):580-586

507 Hunkeler D, Andersen N, Aravena R, Bernasconi SM, Butler BJ (2001a) Hydrogen and carbon isotope
508 fractionation during aerobic biodegradation of benzene. *Environ Sci Technol* 35:3462-3467

509 Hunkeler D, Aravena R, Butler BJ (1999) Monitoring microbial dechlorination of tetrachloroethene (PCE) in
510 groundwater using compound-specific stable carbon isotope ratios: Microcosm and field studies.
511 *Environ Sci Technol* 33:2733-2738

512 Hunkeler D, Aravena R, Cox E (2002) Carbon isotopes as a tool to evaluate the origin and fate of vinyl chloride:
513 Laboratory experiments and modelling of isotope evolution. *Environ Sci Technol* 36:3378-3384

514 Hunkeler D, Butler BJ, Aravena R, Barker JF (2001b) Monitoring biodegradation of methyl tert-butyl ether
515 (MTBE) using compound-specific carbon isotope analysis. *Environ Sci Technol* 35:676-681

516 Huskey WP (1991) Origins and interpretations of heavy-atom isotope effects. In: Cook PF (ed) *Enzyme*
517 *mechanism from isotope effects*, 1st edn. CRC Press, Boca Raton, FL, pp 47-72

518 Jordan SL (1996) Physiology and biochemistry of bacteria which oxidise carbon disulphide. Dissertation,
519 University of London

520 Jordan SL, Kraczkiewicz-Dowjat AJ, Kelly DP, Wood AP (1995) Novel eubacteria able to grow on carbon
521 disulfide. *Arch Microbiol* 163:131-137

522 Jordan SL, McDonald IR, Kraczkiewicz-Dowjat AJ, Kelly DP, Rainey FA, Murrell JC, Wood AP (1997)
523 Autotrophic growth on carbon disulfide is a property of novel strains of *Paracoccus denitrificans*. *Arch*
524 *Microbiol* 168:225-236

525 Kalin RM, Myles P, McGeough KL (2005) Carbon disulphide sources, risks and treatment: An emerging
526 contaminant of environmental concern? Boshoff GA and Bone BD (eds). In: *Permeable Reactive*
527 *Barriers*. IAHS Press. 82-92

528 Kelly DP, Baker SC (1990) The organosulphur cycle: aerobic and anaerobic processes leading to turnover of C₁-
529 sulphur compounds. *FEMS Microbiol Rev* 87:241-246

530 Kelly DP, Smith NA (1990) Organic sulfur compounds in the environment. *Biogeochemistry, microbiology and*
531 *ecological aspects*. *Adv Microb Ecol*. 11:345-385

532 Lovelock JE, (1974) CS₂ and the natural sulphur cycle. *Nat* 248:625-626

533 Ma J, Ji J, Ding Q, Liu W, Wang S, Wang N, Chen G (2010) The effects of carbon disulfide on male sexual
534 function. *Toxicol Ind Health*. 26(6):375-382

535 Mariotti A, Germon JC, Hubert P, Kaiser P, Letolle R, Tardieux A, Tardieux P (1981) Experimental
 536 determination of nitrogen kinetic isotope fractionation: some principles; illustrations for the
 537 denitrification and nitrification processes. *Plant Soil* 62:413-430
 538 McKelvie JR, Mackay DM, Sieyes NR, Lacrampe-Couloume G, Sherwood Lollar B (2007) Quantifying MTBE
 539 biodegradation in Vandenberg Air Force Base ethanol release study using stable carbon isotopes. *J*
 540 *Contam Hydrol* 94:157-165
 541 Meckenstock RU, Morasch B, Griebler C, Richnow HH (2004) Stable isotope fractionation analysis as a tool to
 542 monitor biodegradation in contaminated aquifers. *J Contam Hydrol* 75:215-255
 543 Miltner A, Kopinke F, Kindler R, Selesi D, Hartmann A, Kastner M (2005) Non-phototrophic CO₂ fixation by
 544 soil microorganisms. *Plant Soil* 269:193-20
 545 Moret I, Gambaro A, Piazza R, Barbante C, Andreoli C, Corami F, Scarponi G (2000) The seasonal variations
 546 of dimethyl sulphide and carbon disulphide in surface waters of the Venice lagoon. *Mar Chem* 71(3-
 547 4):283-295
 548 Morgan P, Sinke A (2005) Monitored natural attenuation. *Land Contam Reclam* 13(3):247-265
 549 Odinstova EV, Wood AP, Kelly DP (1993) Chemolithoautotrophic growth of *Thiothrix ramosa*. *Arch Microbiol*
 550 160:152-157
 551 Ottengraf SPP, Meesters JJP, van den Oever, A. H. C., Rozema HR (1986) Biological elimination of volatile
 552 xenobiotic compounds in biofilters. *Bioprocess Eng* 1:61-69
 553 Peyton TO, Steele RV, Mabey WR (1976) Carbon Disulfide, Carbonyl Sulfide. Literature Review and
 554 Environmental Assessment. EPA-600/9-78-009. Washington DC, United States Environmental
 555 Protection Agency. Office of Health and Ecological Effects
 556 Plas C, Wimmer K, Holubar P, Mattanovich D, Danner H, Jelinek E, Harant H, Braun R (1993) Degradation of
 557 carbon disulphide by a *Thiobacillus* isolate. *Appl Microbiol Biotechnol* 38(6):820-823
 558 Pol A, van der Drift C, Op den Camp, H. J. M. (2007) Isolation of carbon disulfide utilizing *Thiomonas* sp. And
 559 its application in a biotrickling filter. *Appl Microb Cell Physiol* 74:439-446
 560 Rajagopal BS, Daniels L (1986) Investigation of mercaptans, organic sulfides, and inorganic sulfur compounds
 561 as sulfur sources for the growth of methanogenic bacteria. *Curr Microbiol* 14:137-144
 562 Rasmussen RA, Khalil MA, Dalluge RW (1982) Carbonyl sulfide and carbon disulfide from the eruptions of
 563 Mount St. Helens. *Sci* 215:665-667

564 Rojo N, Gallastegi G, Barona A, Gurtubay L, Ibarra-Berastegi G, Elias A (2010) Biotechnology as a alternative
565 for carbon disulfide treatment in air pollution control. *Environ Rev* 18:321-332

566 Ross I, MacLeod C, Harvey B, Burdick J, Maggs D (2008) In-situ remediation of carbon disulphide. In:
567 Consoil 2008 – CDRom edition: Proceedings of the 10th International UFZ-Deltares/UNO Conference
568 on Soil-Water Systems. E474-E475

569 Rothschild B, Butler R, Keller JR (1969) Metabolism of carbon disulfide by *Thiobacillus thiooxidans*. *Bacteriol*
570 *Proc*:64

571 Sherwood Lollar B, Slater GF, Ahad J, Sleep B, Spivack J, Brennan M, MacKenzie P (1999) Contrasting carbon
572 isotope fractionation during biodegradation of trichloroethylene and toluene: implications for intrinsic
573 bioremediation. *Org Geochem* 30:813-820

574 Sherwood Lollar B, Slater GF, Sleep B, Witt M, Klecka GM, Harkness M, Spivack J (2001) Stable carbon
575 isotope evidence for intrinsic bioremediation of tetrachloroethene and trichloroethene at Area 6, Dover
576 Air Force Base. *Environ Sci Technol* 35:261-269

577 Smith NA (1988) Metabolism of dimethyl sulphide, carbon disulphide and other volatile sulphur. Dissertation,
578 University of Warwick

579 Smith NA, Kelly DP (1988) Oxidation of carbon disulphide as the sole source of energy for the autotrophic
580 growth of *Thiobacillus thioparus* strain TK-m. *J Gen Microbiol* 134(11):3041-3048

581 Sorokin DY, Tourova TP, Lysenko AM, Kuenen JG (2001) Microbial Thiocyanate Utilisation under Highly
582 Alkaline Conditions. *Appl Environ Microbiol* 67(2):528-538

583 Sturchio NC, Clausen JL, Heraty LJ, Huang L, Holt BD, Abrajano JR (1998) Chlorine isotope investigation of
584 natural attenuation of trichloroethene in an aerobic aquifer. *Environ Sci Technol* 32(20):3037-3042

585 U.S. Environmental Protection Agency (USEPA) (2006) National Priorities List (NPL): Advanced query form.
586 U.S. Environmental Protection Agency (USEPA).
587 <http://www.epa.gov/superfund/sites/query/advquery.htm>. Accessed August, 4 2006

588 Van Eekert, M. H. A., Schroder TJ, Stams AJM, Schraa G, Field JA (1998) Degradation and fate of carbon
589 tetrachloride in unadapted methanogenic granular sludge. *Appl Environ Microbiol* 64(7):2350-2356

590 van Ras, N. J. P., Winters RO, Lieten SH, Dijkhuis JE, Henssen MJC, van Hattem WA, Lethbridge G (2007)
591 Sustainability of natural attenuation of aromatics (BTEX). 2003.1969. Rotterdam, Network for
592 industrially contaminated land in Europe (NICOLE)

593 Watts SF, (2000) The mass budgets of carbonyl sulfide, dimethyl sulfide, carbon disulfide and hydrogen sulfide.
594 Atmos Environ 34:761-779.

595 Wilson JT, Kaiser PM, Adair C (2005) Monitored Natural Attenuation of MTBE as a Risk Management Option
596 at Leaking Underground Storage Tank Sites. EPA/600/R-04/1790. Cincinnati, OH, National Risk
597 Management Research Laboratory, Office of Research and Development, USEPA
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599 Figures

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602 **Fig. 1** Plot of ln normalised CS₂ concentration versus time for an initial CS₂ 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

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(a)

(b)

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

Fig. 3 Rayleigh plot of $\Delta\delta^{13}\text{C}$ versus $-\ln(f)$ for (a) sterilised soil control, (b) groundwater microcosms (p-value (gradient) = 1.4×10^{-7}) and (c) groundwater and soil microcosms (p-value (gradient) = 6.6×10^{-8}). Circles in Fig.3(a) identify two anomalous data points

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620 **Fig. 4** Actual and modelled carbon isotope ratio of CS₂ 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 CS₂) and t = 148 hrs where n = 2 (for COS))

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Fig 5 Actual and modelled carbon isotope data versus time for $k_1 = 0.0163 \text{ h}^{-1}$, $\alpha_1 = 0.9919$, $k_2 = 0.15 \text{ h}^{-1}$, $\alpha_2 = 0.985$, $k_3 = 0.00055 \text{ h}^{-1}$ 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 ($C_0 = 100\text{mg L}^{-1}$)	$k_{\text{sterilised}} (\text{h}^{-1})$	$0.15 \pm 0.04 \times 10^{-2}$
Groundwater only ($C_0 = 40\text{mg L}^{-1}$)	$k_{\text{groundwater}} (\text{h}^{-1})$	$0.26 \pm 0.12 \times 10^{-2}$
Groundwater only ($C_0 = 100\text{mg L}^{-1}$)	$k_{\text{groundwater}} (\text{h}^{-1})$	$0.38 \pm 0.11 \times 10^{-2}$
Soil and groundwater ($C_0 = 40\text{mg L}^{-1}$)	$k_{\text{micro soil}} (\text{h}^{-1})$	$>2.54 \pm 0.15 \times 10^{-2}$
Soil and groundwater ($C_0 = 100\text{mg L}^{-1}$)	$k_{\text{micro soil}} (\text{h}^{-1})$	$1.40 \pm 0.14 \times 10^{-2}$

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