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DELIVERABLE D1.2

Effects of radiation and microbial degradation of ILW organic polymers

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Publishable Summary

Work Package 1 (WP1) of the Microbiology in Nuclear waste Disposal (MIND) project addresses remaining key issues for the geological disposal of organic-containing intermediate level wastes. Specifically, its focus is on the long-term behaviour, fate and consequences of these organic materials. This report forms Deliverable 1.2 of the MIND project, which summarises WP1 research carried out by UNIMAN on the microbial degradation of two polymers and related compounds previously identified as priority targets for MIND research; cellulose and polyvinylchloride (PVC). In particular, the effects of gamma irradiation in combination with high pH on the microbial degradation of these materials were addressed. The results are presented here, along with discussion on their implications for the safe disposal of nuclear waste and recommendations for future study.

The impact of irradiation on the abiotic alkaline hydrolysis of cellulose was studied in batch experiments containing laboratory grade tissue paper immersed in a saturated $Ca(OH)_2$ solution (pH 12.7), and irradiated with 1 MGy of γ-radiation. Irradiation caused physical degradation of the tissue paper, and a change in the colour of the solid and solution. It also enhanced the rate of the abiotic alkaline hydrolysis of cellulose, through decreasing its degree of polymerisation and the number of crystalline domains. Batch sacrificial microcosms, containing a sediment sample from a high pH contaminated site, showed enhanced microbial activity in the samples that were supplemented with irradiated tissue paper compared to those containing non-irradiated tissue paper. The samples containing irradiated tissue paper showed a drop in pH, H_2 and acetate production compared to the samples containing non-irradiated tissue paper.

A series of microcosm experiments were conducted with the goal of assessing whether PVC could support microbial metabolism via nitrate reduction at high pH. In order to address the potential role of common PVC additives in these processes, a plasticised form of PVC sheet previously used in tenting operations within the nuclear industry was used, in addition to a pure PVC powder lacking additives. Samples of both forms were submerged in saturated calcium hydroxide at pH 12.4, and half of these were subject to a total cumulative dose of 1 MGy gamma irradiation. These PVC samples, all of which were subject to high pH conditions for several weeks, and some were additionally irradiated, were supplied to pH 10 microcosms as the sole source of organic carbon and electron donors for nitrate reduction. Microcosms

were inoculated with sediment from an anthropogenic high pH environment known to contain denitrifying bacteria. The results show that plasticised PVC is used to fuel nitrate reduction to nitrite, whether irradiated or not, though irradiated PVC sheet supported less nitrate reduction. In contrast, pure non-irradiated PVC powder did not support nitrate reduction, while irradiated powder supported minor amounts. Additional experiments with two of the PVC sheet additives identified with pyrolysis GC-MS (triphenyl phosphate and phthalate) did not support the extent of nitrate reduction observed with PVC sheet, indicating that other additives were fuelling this process. Measurements of dissolved organic carbon indicate that alkaline hydrolysis was occurring on all PVC materials, and no significant difference was observed between irradiated and non-irradiated, or sterile and live, microcosms.

Results from the cellulose degradation experiments provide further evidence of the role of microorganisms in cellulose degradation under cementitious ILW conditions that may overall reduce the complexation effect of the organic alkaline hydrolysis products generated. The study of PVC materials demonstrates the potential for alkaline degradation and biodegradation mainly of additives present. Further research may be required to examine the complexation properties and stability of specific PVC additives. Both studies provide data constraining the extent and rate of degradation that is of relevance to predicting gas generating processes from organic ILW.

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1 Introduction

Work Package 1 (WP1) of the MIND project addresses remaining key issues for the geological disposal of ILW concerning the long-term behaviour, fate and consequences of organic materials in nuclear waste, along with H_2 generated by corrosion and radiolysis. The objectives of WP1 are thus to reduce the uncertainty of safety-relevant microbial processes controlling radionuclide, chemical and gas release from organic-containing long-lived intermediate level wastes (ILW). This report constitutes Deliverable 1.2 of the MIND project, which serves to summarise the work carried out by researchers at the University of Manchester (UNIMAN) during the first two years of the project on effects of radiation and microbial degradation of organic polymers. The work is focused on two organic polymers, cellulose and polyvinylchloride (PVC), and their degradation products and additives, respectively. These materials were identified as priority targets in the MIND project proposal and are further discussed in Deliverable 1.1, the review of organic wastes and their biodegradation under ILW repository conditions (Abrahamsen *et al.*, 2015). Understanding the fate of these materials is important since they represent significant volumes of ILW inventories in Europe, and the cellulose alkaline hydrolysis products and PVC additives are known to form complexes with radionuclides that can increase their mobility in groundwater. To date a conservative approach has been adopted regarding the stability of such organic complexants and the effects of microbiological processes on fully degrading soluble organic hydrolysis products and organic additives to thermodynamically stable CO_2 and CH_4 has been largely ignored. Gas generation fuelled by such organic wastes and mediated by microbial processes is an additional concern that is being examined by the MIND project. Understanding the rates and extent of organic polymer degradation will provide information relevant to estimating the rates of CH_4 gas generation.

Although other partners within the MIND consortium are also working on organic-containing wastes as part of WP1, the experimental work remains in its early stages, hence this report is dedicated to the completed research concerning cellulose and PVC.

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1.1 Background on cellulose degradation

A major constituent of organic matter in low level waste (LLW) and ILW that will be consigned to near surface and geological disposal facilities will be cellulose based material, for example wood derivatives (paper and cardboard), and clothing and other cotton derivatives (Leschine, 1995). Cellulose is a polymer of glucose subunits linked by β-1,4 glycosidic bonds (Beguin and Aubert, 1994), and forms highly ordered crystalline domains that are interspersed by more disordered amorphous regions (Leschine, 1995; Lynd *et al.*, 2002). Under high pH conditions, similar to those expected in a geological disposal facility (GDF), cellulose undergoes chemical hydrolysis (van Loon *et al.*, 1999; Knill and Kennedy, 2003), to produce isosaccharinic, lactic, formic, acetic, glycolic, glyceric, butyric, threonic, adipic, succinic, pyruvic, and propionic acid (Glaus *et al.*, 1999), the major one being isosaccharinic acid (ISA). Extensive studies have been undertaken on the formation of water soluble, alkali stable complexes between ISA and various metalloids, metals and radionuclides (for example Ca, Ni, U, Np, Th, Am, and Eu) relevant to an ILW-GDF, and stability and solubility constants have been calculated for various complexes (Warwick *et al.*, 2003, 2004, 2006; Rai *et al.*, 1998, 2003; Vercammen *et al.*, 2001; Wieland *et al.*, 2002; Tits *et al.*, 2005).

The rate of the alkaline hydrolysis of cellulose depends on the chemistry of the environment where the cellulosic material will be present, which is, in turn, dependent on the evolution of the porewater chemistry in the GDF. Assuming that the GDF will be anaerobic and dominated by hyperalkaline pH (pH value of 12.5) and a Ca^{2+} concentration of about 20 mM (Berner, 1992), the most recent studies had proposed that the complete hydrolysis of cellulose would require between 1,000 and 5,000 years after the groundwater gets into contact with the cellulose inside the ILW canisters (Glaus and van Loon, 2008). Furthermore, similar models estimated that the concentration of ISA in the ILW-GDF will be about 44 mM (van Loon, Glaus and Vercammen, 1999), although, this will greatly depend on the groundwater flow rate, the amount of cellulose loaded, the sorption of ISA to solid phases and the formation of sparingly soluble salts with cations. These models did not include the effect of radiation and biological degradation, and considered these to be negligible in an ILW-GDF (van Loon and Glaus, 1997; van Loon *et al.*, 1999).

Many studies have shown that a variety of microorganisms belonging to the fungal and bacterial kingdoms are able to degrade cellulose under aerobic and anaerobic conditions at circumneutral pH (Lynd *et al.*, 2002). The enzymes and mechanisms involved in the

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biodegradation of cellulose have been identified in these neutrophilic microorganisms (Mosier *et al.*, 1999; Leschine, 1995; Beguin and Aubert, 1994; Zvereva *et al.*, 2006). However, these are not directly relevant to the GDF, where hyperalkaline conditions will dominate. A recent study showed that bacteria (belonging to the genus *Clostridium*) were able to degrade tissue paper, and ferment the degradation products to acetate under anaerobic, hyperalkaline conditions (starting pH value of 12.2) (Bassil *et al.*, 2015).

Previous studies showed that irradiation of dry cotton-filters, hardwood and softwood with an electron beam, led to a decrease in the degree of polymerisation of cellulose fibres, along with the formation of carbonyl and carboxyl groups (Bouchard *et al.*, 2006; Lawton *et al.*, 1951). Similar observations were made when cotton-wool and softwood were immersed in water and exposed to various doses of gamma-radiation, which led to an increase in the amount of depolymerisation and mid chain scissions with increasing dosage, especially in the presence of oxygen (Glegg and Kertesz, 1957; Blouin and Arthur, 1958). Irradiation of dry microcrystalline cellulose in air with increasing dose from an electron beam (100-1000 KGy) led to an increase in the relative crystallinity index and the molecular weight, and to an increase in the surface area of the cellulose (Driscoll *et al.*, 2009). These make it more susceptible to chemical hydrolysis by alkali. To our knowledge, there are no studies on the effect of irradiation on the abiotic alkaline hydrolysis of cellulose, and indeed, on the bioavailability of irradiated cellulose under hyperalkaline conditions. In this respect, we conducted such experiments as part of the MIND project, and the results acquired are presented in this report.

The microbial degradation of ISA under different biogeochemical conditions has attracted much attention in recent years due to its impact to the safety case for a ILW-GDF. Three bacterial strains had been previously isolated that were able to degrade ISA under aerobic conditions at circumneutral pH (Strand *et al.*, 1984; Bailey, 1986). Furthermore, studies have shown that microorganisms can utilise pure ISA (Kuippers *et al.*, 2015; Maset *et al.*, 2006; Kyeremeh *et al.*, 2016), or a mixture of alkaline cellulose degradation products (Rout *et al.*, 2015; Rout *et al.*, 2014) to fuel the biogeochemical redox progression from aerobic to methanogenesis at circumneutral pH, which are relevant to the far-field conditions. Furthermore, alkaliphilic and alkalitolerant bacteria utilised pure ISA to fuel biogeochemical processes from aerobic to Fe(III) reduction at pH 10 (Bassil *et al.*, 2015). Also, ISA fermentation and to some extent methanogenesis were observed in the presence of a mixture of alkaline cellulose degradation products at pH 11 (Charles *et al.*, 2015; Rout *et al.*, 2015). A novel alkaliphilic bacterial strain that utilises ISA for the reduction of nitrate was recently

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isolated as part of the MIND project (Bassil and Lloyd, 2017a), and its genome sequenced (Bassil and Lloyd, 2017b).

1.2 Background on PVC

In addition to cellulose, plastics represent a significant volumetric contribution of organic material in the inventory of ILW and LLW in a number of countries throughout Europe (Abrahamsen *et al.*, 2015). In the UK, halogenated plastics constitute the largest component of the organic-containing waste inventory (NDA/DECC, 2014). Much of this waste arises from the miscellaneous use of these plastics in maintenance and decommissioning operations at nuclear power plants, as well as from reprocessing plants and laboratories. PVC is widely used to manufacture glove box posting bags, protective suits and in tenting operations. The bulk of the PVC in the UK National Inventory is expected to be flexible films and sheets of PVC from these activities (Smith *et al*., 2013).

Owing to strong intermolecular forces between polymer chains, PVC in its pure form is a rigid, mainly amorphous material with little flexibility. As such, PVC must be rendered flexible in order to be of use in the nuclear industry, which necessitates the addition of a variety of additives, including plasticisers, heat stabilisers, fillers, pigments, flame retardants, UV absorbers, colorants and anti-oxidants (Coaker, 2003). Of these, plasticisers are typically present in the largest quantities, accounting for between 30% and 50% by volume. Plasticisers are not covalently bonded to the PVC polymer, rather they sit between and serve to lubricate otherwise rigid polymer layers. There is concern that these additives may diffuse out of the PVC material under the conditions of a geological disposal facility (GDF), which may have implications for microbial activity and for radionuclide mobility (Dawson, 2013). PVC additives, and in particular the plasticisers, have therefore been a major focus of the work reported here.

Historically, the most widely used plasticisers have been phthalate esters, which represented 93% of plasticisers declared in 1993 (European Commission, 2000). Common esters include di-ethylhexyl phthalate (DEHP; sometimes referred to as di-octyl phthalate, DOP); di-isononyl phthalate (DINP) and di-isodecyl phthalate (DIDP) (NDA, 2012**).** These esters share similar chemical structures (a phenol group linked to two aliphatic side-chains via ester linkages), the difference between these three compounds being the size and nature of their side-chains. Phthalate esters are widely known to be bioavailable to common soil

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microorganisms, with more rapid degradation on lower molecular weight phthalates (Engehardt *et al*., 1975, Engelhardt and Wallnöfer, 1978). Whilst many bacteria can degrade these esters, some can fully oxidise them to carbon dioxide and water, the mechanism for which involves sequential ester hydrolysis to remove alkyl groups, after which the phthalic acid is further metabolised and ultimately broken down via the tricarboxylic acid cycle (Kurane *et al*., 1980; Jianlong *et al*., 1995). Despite their known biodegradability, concern over the hazards of phthalate esters to human health has led to restrictions of their use as plasticisers in recent decades (European Parliament, 2005). Consequently, their use in the nuclear industry, and therefore their contribution to organic-containing wastes, is expected to decline in future, though their contribution to current arisings is expected to be significant. Phthalates are known to form stable complexants with uranium (Vazquez *et al*., 2008, 2009), europium (Zhou *et al*., 2004) and curium (Panak *et al*., 1995), therefore their presence and fate in a GDF is of importance to the safety case for nuclear waste disposal.

Whilst the high chlorine content (~57%) of pure PVC renders the polymer inherently flame retardant, addition of phthalate esters and other organic additives contribute to increased flammability (Coaker, 2003). A common approach to mediate this is to supplement the volume of phthalate plasticisers with flame retardant compounds, most typically phosphate esters (Moy, 1998). Triaryl phosphate esters, such as triphenyl phosphate (TPP), are desirable since they serve as plasticisers as well as flame retardants (Coaker, 2003). They have been shown to serve as the sole carbon source for some microbial communities, and some are known to readily degrade aerobically and anaerobically in soil (Anderson *et al*., 1993; Pickard *et al*., 1975). Often added to the type of PVC sheeting that is widely used in the nuclear industry, it is anticipated that TPP and other phosphate esters will be present in the waste inventory.

A small number of studies have addressed the degradability of PVC and common additives under GDF-relevant conditions. The PVC base polymer is widely known to degrade upon irradiation, with direct formation of double radicals leading to chain scissions and the formation of hydrochloric acid. Irradiation of PVC in contact with water led to a greater decrease in pH than that in contact with calcium hydroxide, the latter presumably buffering the pH change (Dawson, 2013). The presence of air during irradiation leads to more chain scissions to the polymer (Wypych, 2015). Irradiation of plasticised PVC liberates 100 times more organic compounds than that of pure PVC, the majority of which were found to be additives leached out from the polymer and their degradation products (Colombani *et al*.,

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2009). These additives appear to leach out from irradiated PVC in the absence of an aqueous phase, as evidenced by a sticky residue on the polymer surface after dry irradiation (Shashoua, 2008; Dawson, 2013).

PVC and its additives are known to degrade under high pH conditions alone. One experiment compared the effects of ageing plasticised PVC in deionized water (pH 7) compared with calcium hydroxide (pH 12), and the latter gave rise to a 30% mass loss compared with 2% in water (Baston and Dawson, 2012). These results indicate that alkaline hydrolysis of the material occurs at the high pH conditions of a GDF. The same mechanism appears to break ester linkages of common phthalate plasticisers, liberating aliphatic alcohols and phthalic acid (Baston and Dawson, 2012).

The likelihood of the degradation of the PVC base polymer and its stabiliser and filler additives under GDF conditions is thought to be low. In contrast, it is likely that phthalate esters will diffuse from the polymer and be broken down to their constituent phthalate anions and aliphatic alcohols. Preferential leaching of these phthalates may lead to the formation of non-aqueous phase liquids (NAPLs), which in a GDF could potentially accelerate radionuclide release. It is therefore essential to consider these phthalate additives in any assessment of the biodegradability and bioavailability of PVC materials in nuclear waste. In contrast, little is known about the fate of phosphate esters at high pH and in response to ionising radiation. Triphenyl phosphate, a commonly used flame retardant plasticiser, is considered a low risk substance with respect to the human health (Brooke et al., 2009), therefore its use in PVC is unlikely to be restricted in future.

Here we report results from microcosm experiments in which the bioavailability of plasticised PVC for microbial nitrate reduction at high pH was assessed. The effect of gamma irradiation on PVC bioavailability was also investigated, in addition to the bioavailability of two relevant additives (TPP and phthalate). The implications for nuclear waste disposal are discussed, and future research priorities suggested.

2 Methods

2.1 Materials studied

For cellulose experiments, laboratory-grade tissues (Kimwipes) were purchased from Sigma-Aldrich.

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For PVC experiments, two forms of PVC were used. The first was pure PVC powder (average M_w ~43,000, average M_n ~22,000, CAS 9002-86-2, Sigma-Aldrich, UK). The second form was PVC sheeting (Romar, Whitehaven, UK), previously used in a tenting operation at the UK National Nuclear Laboratory, and thus represents a highly relevant form of plasticized PVC to study here.

2.2 Irradiation

Materials were irradiated using a ^{60}Co source at the University of Manchester Dalton Nuclear Facility, Cumbria, UK. All materials were irradiated in saturated calcium hydroxide solution (1.5 g $Ca(OH)_2$ per litre deionised water) to achieve a pH of 12.4. For cellulose irradiations, pieces of Kimwipe high grade laboratory tissue measuring 5.50 x 5.25 cm were transferred to 5 ml pre-scored glass ampules (Wheaton, Millville NJ, USA), and 2 ml saturated $Ca(OH)_2$ added. For PVC powder irradiations, 0.5 g of powder was added, and for PVC sheet the approximate equivalent weight was added in the form of small (0.75 cm^2) squares. All vials were flame sealed with an air headspace using a blowtorch. Vials were irradiated at a rate of about 1 Gy/min over a 16 hours period until the materials had received a total cumulative dose of 1 ± 0.3 MGy. This high dose was chosen not in an attempt to precisely replicate conditions anticipated in a GDF, but rather to compare the overall effects of irradiation on biodegradation and bioavailability. The high dose was therefore chosen with such a comparison in mind.

2.3 Microcosm setup

Sediment samples were collected from a depth of ~20 cm from the surface of a site, at Harpur Hill, Buxton, UK, that had been contaminated for decades by high pH legacy lime works. The sediments at the site have a circumneutral to alkaline pH and contain high calcium and silicate concentrations, analogous to a cementitious radioactive waste repository (Rizoulis *et al.*, 2012; Rout *et al.*, 2015; Smith *et al.*, 2016).

2.3.1 Cellulose experiments

Sacrificial microcosms were prepared in 20 ml serum bottles (Agilent), containing 10 ml of a 1 g/L $Ca(OH)_2$ solution that was previously flushed with N_2, 2.5% (w/v) sediment from the high pH analogue site, and loaded with 2.5% (w/v) irradiated or non-irradiated Kimwipes. These samples were closed with rubber butyl stoppers and incubated at 20°C for the length of

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the experiment. Control samples that did not contain the sediment were also prepared. Three replicates for each condition were selected at each time point for analysis of the gas phase. After analysis of the gas phase, the serum bottles were opened in an anaerobic chamber and the solid and liquid phases were separated by centrifugation at 4000 *g* for 10 min. Aliquots were collected from the supernatants for measurements of pH, E_h, the concentrations of ISA and acetate, and dissolved organic and inorganic carbon. Aliquots were also taken from the solid phase for light and scanning electron microscopy. The remainder of the liquid and the solid phases were stored at -20°C.

2.3.2 PVC experiments

To assess the bioavailability of PVC, microcosms were established in which pure and plasticised PVC (irradiated and not irradiated) were supplied as the sole source of carbon and electron donors for nitrate reduction by the same high pH-adapted microbial community that was used for the preparation of the cellulose microcosms. The contents of each of the four batches of PVC ampules (powder and sheet, irradiated and non-irradiated) were homogenised so that PVC added to microcosms was equivalent. For experiments with PVC powder, 1.5 ml of powder-leachate slurry was added to 35 ml sterile serum vials inside an anaerobic chamber, and 15 ml anoxic sterile medium was added as appropriate. For experiments with PVC sheet, 12 PVC squares (each measuring 0.75 cm^2) and 1 ml of leachate were transferred to serum vials containing 15 ml medium. The basal medium used across the experiments comprised (in g per L): $NaHCO_3$ (2.0), NH_4Cl (0.25), $NaH_2PO_4.H_2O$ (0.06) and KCl (0.1), and was supplemented with filter sterilised, anoxic vitamin and mineral mixes (Lovley *et al.*, 1984). Nitrate reduction tests were amended with 20 mM $NaNO_3$ as the terminal electron acceptor, and 5 mM each of lactate and acetate was added to positive controls as electron donors. All experiments were inoculated with 1% v/v water-sediment slurry collected from a legacy lime works in Harpur Hill, Buxton, Derbyshire, UK. This high pH (11 to 12) environment is analogous to a cementitious radioactive waste repository (Rizoulis *et al*., 2012) and is known to harbour high pH-adapted microorganisms including nitrate-reducing bacteria (Bassil *et al*., 2015). Prior to inoculation, approximately 30 ml turbid sediment-laden water was transferred from a master sample to two sealed anoxic sterile serum vials inside an anaerobic chamber. One was autoclaved and served as the inoculum for sterile controls, whilst the other was used for live tests. All microcosms were pH-corrected to pH 10 using 10 N sodium hydroxide, and incubated at 20°C in the dark for a total of 97 days.

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Based on results from organic geochemistry measurements, additional microcosm experiments were conducted to test the potential for additives triphenyl phosphate (TPP; Sigma-Aldrich, UK) and phthalate (in the form of phthalic acid; Sigma-Aldrich, UK) to fuel nitrate reduction at pH 10. Phthalate was used instead of a specific phthalate ester plasticiser since these esters are known to break down into their constituent aliphatic alcohols and phthalate at high pH (Baston and Dawson, 2012). The same conditions were used in these experiments as outlined above, with the exception that these compounds were supplied as the sole source of carbon and electron donors instead of PVC powder/sheet. TPP and phthalate were tested separately by adding 2 mM to the basal medium described above. Positive, negative and sterile controls were established in the same way as in PVC microcosm experiments. After pH-correcting as above, TPP and phthalate microcosms were inoculated with the same batch of sediment (1% v/v) as the PVC microcosms.

2.4 Analytical

The concentrations of ISA, acetate and nitrate and nitrite in all microcosm experiments were determined by ion exchange chromatography as described previously (Bassil *et al.*, 2015). The percentage of CH_4 and H_2 in the gas phase were measured by gas chromatography as described previously (Kuippers *et al.*, 2015).

Pyrolysis gas chromatography coupled to mass spectrometry (Pyrolysis GC-MS) was used to assess the bulk organic geochemistry of samples of PVC powder and sheet before and after addition of $Ca(OH)_2$ and flame sealing in glass ampules (with or without irradiation). Specifically, small samples of each material were placed into a clean fire-polished quartz tube and pyrolised step-wise at 300°C followed by 750°C. This approach was chosen in an attempt to analyse the additives (not covalently bonded to the PVC; targeted by lower temperature) independently to the PVC polymer itself (higher temperature). At each temperature, samples were heated in a CDS-5200 pyroprobe for 5 seconds in a flow of helium. The pyrolised material was transferred to an Agilent 7980A gas chromatograph (GC) via a heated transfer line. The GC was fitted with an Zebron ZB-5MS column coupled to an Agilent 5975 MSD singe quadrapole mass spectrometer (MS) in scan mode. The pyrolysis probe was set to 300°C or 750°C and the heated GC interface to 280°C. The carrier gas for sample transfer was helium, and the sample itself was introduced in split mode (ratio 75:1, constant flow of 1 ml min^{-1}, gas saver mode active). The oven was programmed from 40°C to 300°C increasing

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at 4°C min^{-1}. The final temperature was held for 10 minutes and run for a total of 78 minutes per sample. Major compounds in each sample were identified by comparison of relative retention times and spectra to those in the NIST library.

The dissolved organic carbon (DOC) concentration in the liquid phase of the microcosm experiments was measured using the high-temperature catalytic oxidation method (680°C) using a total organic carbon (TOC) analyser (Shimadzu TOC-V_{CPN}, Japan). Specifically, 600 µl samples of supernatant after centrifugation at room temperature (14,000 *g* for 7 min) were diluted 25-fold and introduced to the TOC analyser using an autosampler (ASI-V, Shimadzu, Milton Keynes, UK). Concentrations of total dissolved carbon and dissolved inorganic carbon were obtained from five-point potassium hydrogen phthalate and sodium carbonate standard calibration curves, respectively.

Environmental Scanning Electron Microscopy (ESEM) was used to obtain images of the non-irradiated and irradiated PVC sheet and associated microbial cells. Single PVC squares were taken from the end-points of microcosms using ethanol-sterilised tweezers inside an anaerobic chamber (Coy, Grass Lake, MI, USA). PVC squares were submerged in deionised water and gently agitated for a few seconds in order to remove salts, and subsequently air-dried overnight prior to gold-coated for observation in high vacuum mode with an ESEM-Field Emission Gun (ESEM-FEG) (FEI XL30).

3 Cellulose degradation

Irradiation of Kimwipes with 1 MGy of γ-radiation induced physical degradation and a change in the colour of the tissue paper and the solution (Figure 1 A and B), and induced breakage of the cellulose fibers into short chains, as observed by light and scanning electron microscopy (Figure 1 C-F). Irradiation also enhanced the production of ISA and acetate by the alkali hydrolysis of cellulose, which was more prominent after 6 months of incubation in a saturated solution of $Ca(OH)_2$ (Figure 1 G-I). A slight drop in pH (from pH 12.7 to 12.5) was also noted in the irradiated samples, compared to the unirradiate samples.

Irradiated cellulose samples, inoculated with sediments from the alkaline contaminated site, showed a significant drop in pH compared to the unirradiated control (Figure 2 A and D). Some samples showed significant production of H_2 (up to 12%) after 3 and 6 months of

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incubation at 20°C (Figure 2 B and E). The dissolved organic carbon and ISA concentrations were significantly higher in the irradiated cellulose samples (Figure 3 A, B, D, E). The concentration of acetate increased at the 3 and 6 months time points, and dropped after 12 months of incubation, in the irradiated and unirradiated samples; however, it was more prominent in the irradiated samples (Figure 3 C and F). It is important to note here that the same sample that showed the highest percentage of hydrogen in the headspace at the 6 months time point (Figure 2 E), also showed the lowest concentration of ISA (Figure 3 E), and the highest concentration of acetate in solution (Figure 3 F).

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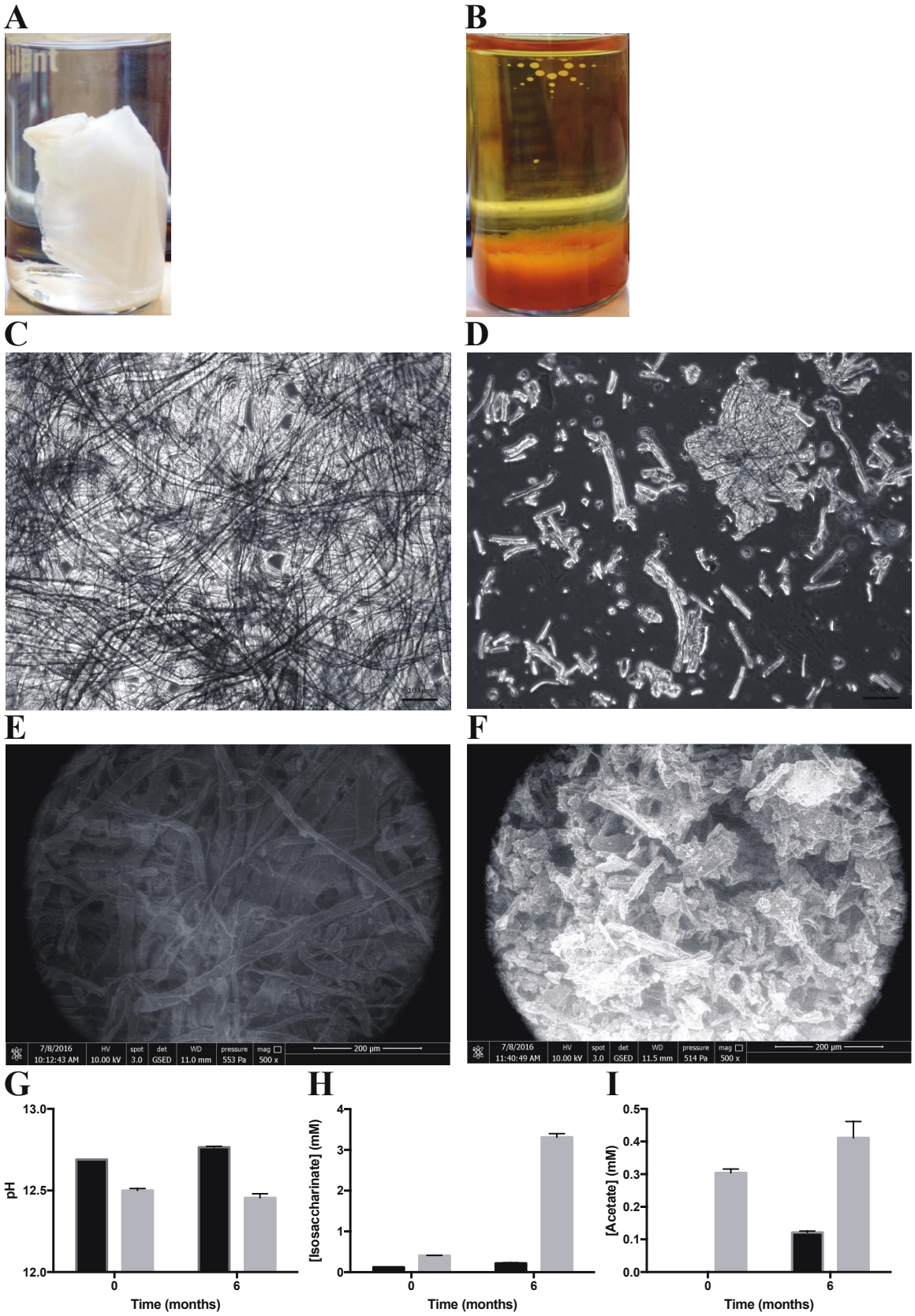

Figure 1: Kimwipes irradiated with 1 MGy of γ-radiation in a saturated solution of $Ca(OH)_2$ showed physical and chemical degradation compared to the unirradiated control. A and B, pictures of unirradiated and irradiated Kimwipes, respectively. C and D, light microscope images of unirradiated and irradiate Kimwipes, respectively; bar is 0.1 mm. E and F, Scanning

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electron micrographs of unirradiated and irradiate Kimwipes, respectively; bar is 0.2 mm. G, pH of the solution directly after irradiation and 6 months after irradiation; black bars are unirradiated samples and grey bars are irradiated samples. H, the concentration of ISA in solution directly after irradiation and 6 months after irradiation; black bars are unirradiated samples and grey bars are irradiated samples. I, the concentration of acetate in solution directly after irradiation and 6 months after irradiation; black bars are unirradiated samples and grey bars are irradiated samples.

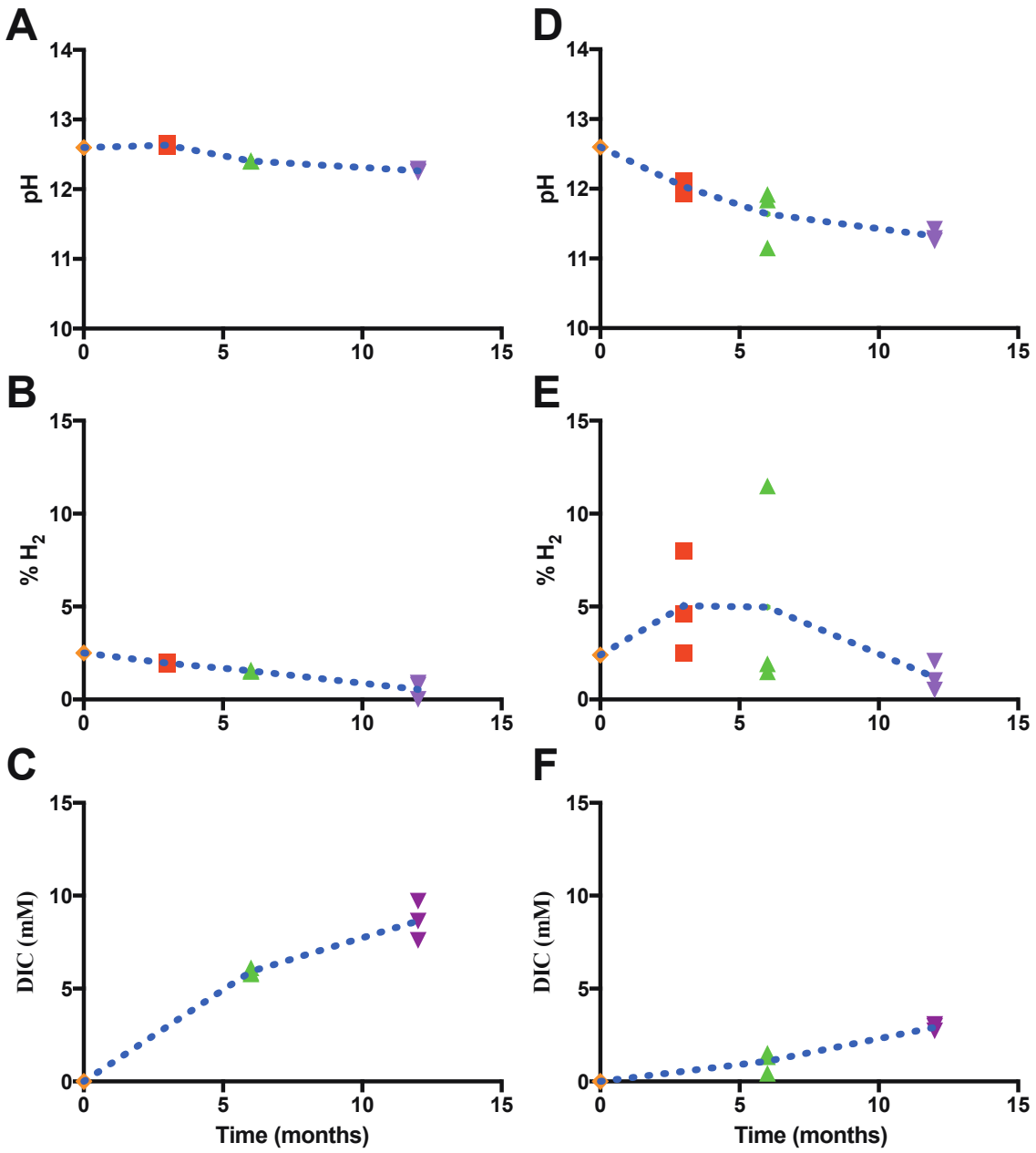

Figure 2: Inoculated microcosms containing Kimwipes, irradiated with 1 MGy of γ-radiation (D-F), and in a saturated solution of $Ca(OH)_2$ showed enhanced microbial activity compared to the unirradiated controls (A-C). A and D, pH in samples sacrificed at 0, 3, 6 and 12 months after incubation at 20°C. B and E, H_2 percentage in the headspace of the same samples. C and F, concentration of dissolved inorganic carbon in mM, in the same samples.

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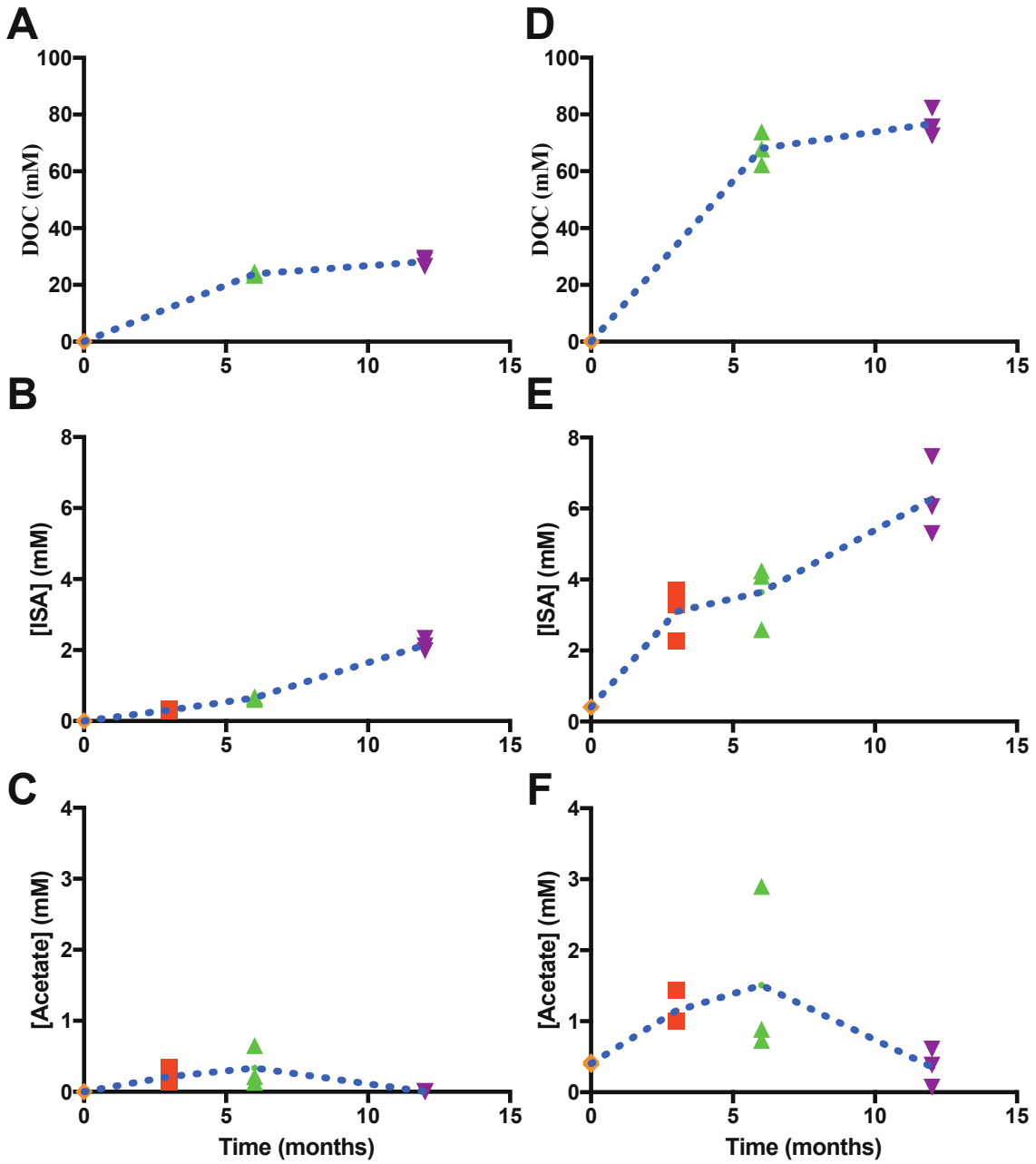

Figure 3 Inoculated microcosms containing Kimwipes, irradiated with 1 MGy of γ-radiation (D-F), and in a saturated solution of $Ca(OH)_2$ showed enhanced microbial activity compared to the unirradiated controls (A-C). A and D, concentration of dissolved organic carbon in mM, in the samples sacrificed at 0, 3, 6 and 12 months after incubation at 20°C. B and E, concentration of ISA in mM in the same samples. C and F, concentration of acetate in mM, in the same samples.

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4 PVC degradation

Irradiation of pure PVC powder and plasticised PVC sheet with 1 MGy γ-radiation led to noticeable changes in the appearance of these materials, as shown in Figure 4. In particular, the PVC powder turned from white to black, whilst the PVC sheet turned from bluish translucent to a brown opaque colour. The pH of the homogenised leachate from irradiated PVC powder and sheet vials was measured at 0.3 and 6.6, respectively. In contrast, the pH of the leachate from non-irradiated PVC powder was 10.2 and that of PVC sheet was 8.4. This difference is presumably owing to higher surface area and lack of additives in the powder compared with the sheet material, and hence higher susceptibility of the former to radiolysis. That the pH in the non-irradiated microcosms had dropped below pH 12.4 indicates that alkaline hydrolysis was also taking place. The effects of irradiation on the PVC sheet are also evident at the microscopic scale, characterised by a change from a smooth to a rough surface with large cracks shown in Figure 5.

Figure 4: The effect of radiation on the appearance of PVC materials. Imaged are flame-sealed vials containing saturated $Ca(OH)_2$ and (from left to right) non-irradiated PVC powder; irradiated PVC powder; non-irradiated PVC sheet; irradiated PVC sheet. Note the change in colour of the PVC powder from white to black, and of the PVC sheet from clear translucent to light brown opaque.

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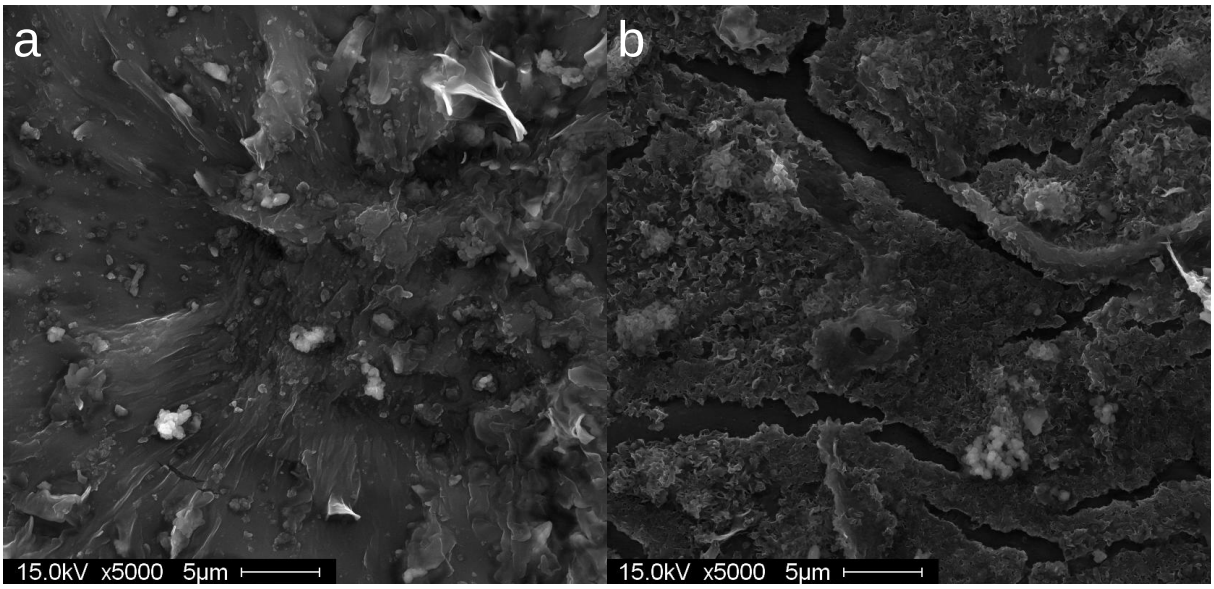

Figure 5: The effects of radiation on PVC sheet at the microscopic scale. Pictured are representative SEM micrographs of non-irradiated (a) and irradiated (b) PVC sheet, exhibiting relatively smooth surface features in the former compared with the coarse surface and appearance of cracks in the latter.

The chemical changes incurred by the materials under high pH and irradiating conditions are less pronounced than the physical changes. The pyrolysis products of the PVC polymer itself are consistent with those reported elsewhere (O'Mara, 1970; Ma *et al*., 2004). Figure 6 summarises pyrolysis-GC-MS results of the PVC sheet from the 300°C step. This data represent the additives that were desorbed at this lower pyrolysis temperature in the starting material (Fig 6a) and samples exposed to both high pH (Fig 6b) and high pH as well as ionising radiation (Fig 6c). The two prominent compound classes evident in this data are aryl phosphate esters (predominantly TPP), and phthalate esters (predominantly di-isononyl phthalate). Although some of the peaks are less pronounced in those exposed to high pH and radiation compared with the starting material, overall the compound groups detected are consistent, indicating that no preferential loss of one over the other of these groups of additives occurred.

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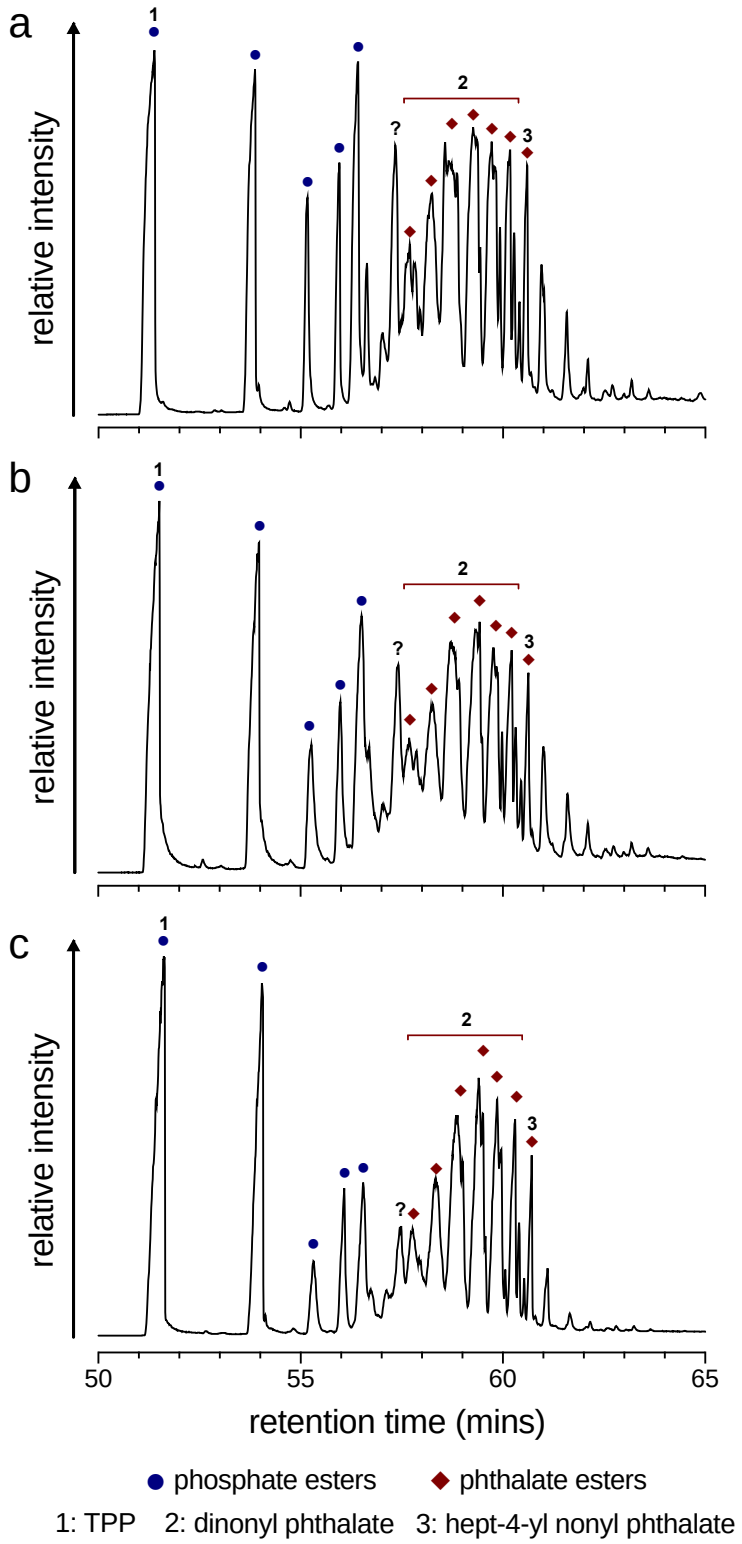

Figure 6: Detection of additives in PVC sheet starting material (a); after prolonged contact with pH 12.4 aqueous solution (b); and after irradiation in addition to prolonged contact with pH 12.4 aqueous solution (c). Data were obtained using pyrolysis GC-MS at 300℃, and chromatograms show a subset of data taken from between 50 and 65 minutes into each pyrolysis-GC-MS run.

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Results from PVC experiments show that nitrate reduction is coupled to PVC sheet, whether irradiated or not, though more nitrate reduction occurred in the presence of non-irradiated material than irradiated. In contrast, non-irradiated PVC powder does not support nitrate reduction, though irradiated powder supports minor amounts (see Figure 7). Given that the major difference between the two types of PVC materials tested is the presence of additives in the plasticised sheet, the results indicate that PVC additives are fuelling this microbial process. However, results from separate experiments with two identified additives (TPP and phthalate) do not account for the extent of nitrate reduction measured with PVC (see Figure 8).

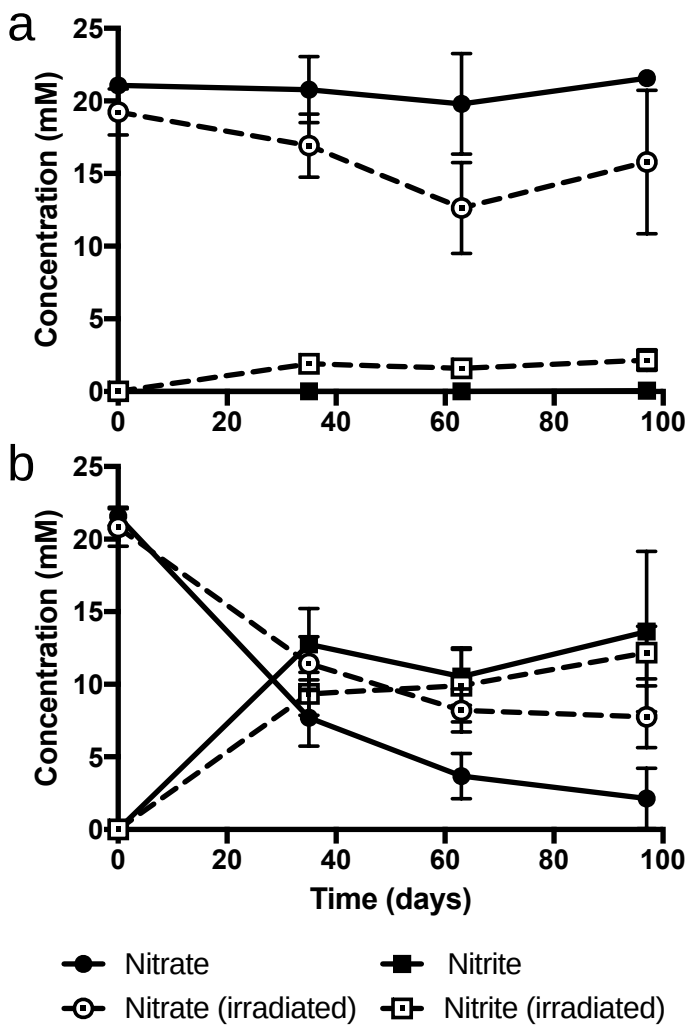

Figure 7: Results from pH 10 nitrate reduction microcosm experiments with non-irradiated and irradiated PVC powder (a) and sheet (b). Results are expressed as average mM concentrations of nitrate (circles) and nitrite (squares) over the 97 days of incubation, as measured by ion chromatography. Dashed lines and open shapes denote irradiated microcosms. Error bars represent standard deviation of triplicate measurements.

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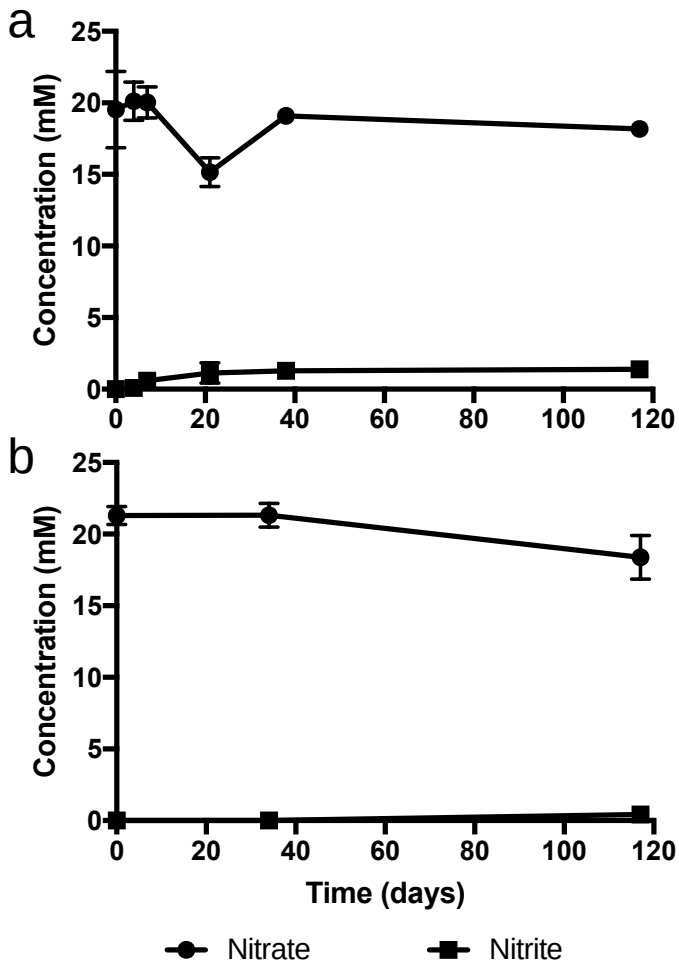

Figure 8: Results from pH 10 nitrate reduction microcosm experiments with TPP (a) and phthalate (b). Results are expressed as average mM concentrations of nitrate (circles) and nitrite (squares) over the 117 days of incubation, as measured by ion chromatography.

Measurements of dissolved organic carbon show an increase in concentration throughout the PVC microcosm experiments, with no significant different between sterile and live tests or between irradiated or non-irradiated tests with the same material (see Figure 9). DOC in the PVC sheet experiments is consistently higher than those containing PVC powder, owing to the presence of additional organic carbon compounds, yet the rate of increase across all experiments appears the same. Given that all microcosms were conducted at pH 10, yet not all were irradiated or inoculated with live sediment, the increased organic carbon can be attributed to alkaline hydrolysis acting on all PVC materials.

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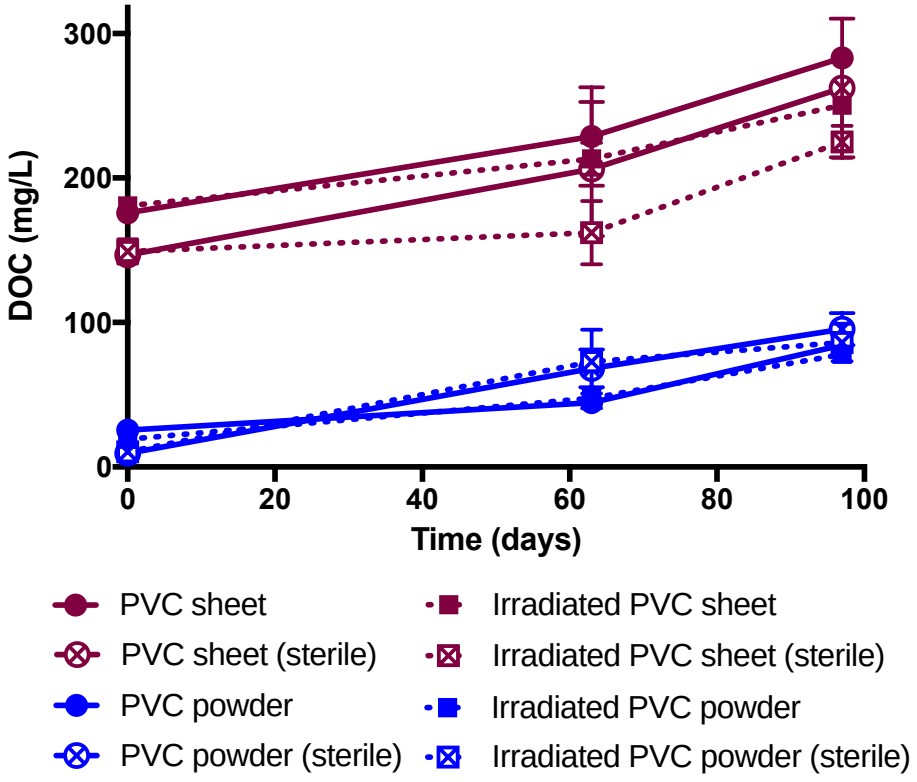

Figure 9: Concentration of DOC measured in PVC microcosm experiments, expressed as mg per liter with time. The aqueous phase of PVC powder microcosms (blue) contain less organic carbon than that of the PVC sheet microcosms owing to the lack of additives. There is no significant difference between both irradiated vs non-irradiated and sterile vs live tests (2 tailed student's t-test, p values > 0.05).

Collectively, these results show that PVC additives are available to microorganisms as a source of carbon and electron donors, whereas pure PVC is recalcitrant unless subject to radiation. In spite of this, irradiated PVC sheet is rendered less available for microbial metabolism. Additives other than TPP and phthalate tested here must be responsible for the extent of nitrate reduction observed. Alkaline hydrolysis acts on the PVC base polymer at high pH and liberates organic carbon to the aqueous phase over time.

5 Discussion and Conclusions

The rate of radionuclide transport from the GDF is dependent on a number of factors, which include (i) the geology and hydrogeology of the host site for the GDF, (ii) the rate of organic polymer alkaline hydrolysis (including cellulose), and the production and release of water soluble organic ligands (including ISA, gluconate, nitrilotriacetic acid, and

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ethylenediaminetetraacetic acid) (Glaus and van Loon, 2008; Haas *et al.*, 1967; Keith-Roach, 2008), (iii) the stability and solubility of the ligand-radionuclide complexes, and the affinity of the ligand to radionuclides in the presence of competing cations like Ca^{2+}, which will be present in high concentrations due to the extensive use of cement (Gaona *et al.*, 2008; Berner, 1992), (iv) the sorption of the ligand and the ligand-radionuclide complexes to the solid phases already present or produced over time in the GDF (Tits *et al.*, 2005; Wieland *et al.*, 2002; Vercammen *et al.*, 2001; Moyce *et al.*, 2014). Another factor that is overlooked in studies on radionuclide transport from the GDF is the role of microorganisms.

Microorganisms may be present in the GDF, or in the chemically disturbed zone surrounding the GDF, where lower pH values are expected to dominate. The heterogeneity of the ILW may create lower pH niches where microorganisms may survive inside the wasteforms (Askarieh *et al.*, 2000). In addition, a recent study has shown that microorganisms can form flocs, made from extracellular polymeric substances (EPS), where the pH inside the flocs was significantly lower than the outside (Charles *et al.*, 2017). In this respect, extremophilic microorganisms that could grow under GDF-similar conditions, may play a significant role in reducing radionuclide transport to the biosphere through (i) the direct or indirect bioreduction of radionuclides, (ii) biomineralisation into insoluble solid phases, or (iii) bioaccumulation into or biosorption onto the surface of microorganisms (Newsome *et al.*, 2014). It is also safe to assume that the biodegradation of these organic ligands (or their precursor polymers) would favour the immobilisation of radionuclides in the GDF or surrounding chemically disturbed zone.

Indeed, the microbial degradation of cellulosic material under hyperalkaline conditions caused a drop in the pH of the solution, which in turn caused a stop in the abiotic production of ISA from the alkaline hydrolysis of cellulose (Bassil *et al.*, 2015). Realistically, cementitious material in the GDF will probably buffer the pH for a very long time, however, the microbial degradation of cellulose under these hyperalkaline conditions, may have an effect on the amount of cellulose available and the rate of the abiotic hydrolysis, and therefore should be considered in the safety case for a cementitious ILW-GDF. Here we showed that cellulose irradiation enhances the rate of the abiotic cellulose hydrolysis by alkaline, and produces predominantly higher amounts of ISA. Irradiation also made the cellulose more bioavailable for bacterial degradation, presumably through exposing the crystalline areas. Although the irradiation rate will be much lower in the GDF than that tested here, this study shows that irradiation will have an effect on the abiotic and biological degradation of cellulose, and the

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fermentation of the degradation products. It also highlights that methane was not produced under these hyperalkaline conditions. This further work on microbial degradation of cellulose under GDF conditions builds on recent research (Bassil *et al.*, 2015; Charles *et al.*, 2015; Rout *et al.*, 2015; Bassil and Lloyd, 2017a) concerning the beneficial effect of biodegradation processes in lowering the significance of ISA and other cellulose degradation products on radionuclide mobility.

Results from the PVC and additive experiments demonstrate that plasticised PVC is bioavailable at pH conditions relevant to the GDF, whether irradiated or not, though irradiation appears to render this material slightly recalcitrant to microbial metabolism. TPP and phthalate were not found to fuel the nitrate reduction observed with PVC sheet, yet results from PVC powder experiments clearly highlight that it is the presence of additives in the plasticised polymer that fuel microbial metabolism.

Given that phthalate esters are known to form complexes with radionuclides (Vacquez *et al*., 2008, 2009; Zhou *et al*., 2004; Panak *et al*., 1995), and that they are anticipated to comprise a significant component of PVC wastes accumulated to date, the results from these experiments suggest that microbial activity in the GDF will not reduce any phthalate-driven radionuclide mobilisation. Uncertainties remain over the identification of additives that were responsible for the microbial activity observed here, which warrants further study. This should be approached through the study of solvent-extracted PVC additives and their bioavailability for nitrate-reduction and other relevant microbial metabolisms. This would offer the opportunity to conduct more targeted analysis of the aqueous organic inventory and any changes observed throughout microbial incubations, with the view to identify bioavailable additives and their degradation products. Abiotic batch experiments with bulk-extracted additives and radionuclides, such as uranium and technetium, would allow for an assessment of radionuclide mobility in the presence of representative PVC additives in the ILW inventory.

In conclusion, the study of cellulose degradation provides further evidence of the role of microorganisms in cellulose degradation under cementitious ILW conditions that may overall reduce the complexation effect of the abiotically generated alkaline cellulose hydrolysis products. The study of PVC materials demonstrates the potential for alkaline degradation and biodegradation mainly of the additives present. Further research may be required to examine the complexation properties and stability of specific PVC additives. Both studies provide data constraining the extent and rate of degradation that is of relevance to predicting gas generating processes from organic ILW.

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References

Abrahamsen L, Arnold T, Brinkmann H, Leys N, Merroun M, Mijnendonckx K, Moll H, Polvika P, Ševců A, Small J, Vikman M, Wouters K. (2015). A Review of Anthropogenic Organic Wastes and Their Degradation Behaviour. MIND Project Deliverable D1.1.

Anderson C, Wischer D, Schmieder A, Spiteller M. (1993). Fate of triphenyl phosphate in soil. *Chemosphere*, **27**:869-879.

Askarieh M, Chambers A, Daniel F, FitzGerald P, Holtom G, Pilkington N, *et al.* (2000). The chemical and microbial degradation of cellulose in the near field of a repository for radioactive wastes. *Waste Manag* **20**:93–106.

Bailey M. (1986). Utilization of glucoisosaccharinic acid by a bacterial isolate unable to metabolize glucose. *Appl Microbiol Biotechnol* **1206**:493–498.

Bassil NM, Bewsher AD, Thompson OR, Lloyd JR. (2015). Microbial degradation of cellulosic material under intermediate-level waste simulated conditions. *Mineral Mag* **79**:1–9.

Bassil NM, Bryan N, Lloyd JR. (2015). Microbial degradation of isosaccharinic acid at high pH. *ISME J* **9**:310–320.

Bassil NM, Lloyd JR. (2017). *Anaerobacillus alkaliisosaccharinicus* sp. nov., an alkaliphilic bacterium which degrades isosaccharinic acid. *Prep*.

Bassil NM, Lloyd JR. (2017). Draft genome sequences of four alkaliphilic bacteria belonging to the *Anaerobacillus* genus. *Genome Announc* **5**:e01493-16.

Baston G, Dawson J. (2012). NAPL Generation from PVC Aged in Aqueous Solutions. AMEC Report, AMEC/PPE/002834/003.

Beguin P, Aubert JP. (1994). The biological degradation of cellulose. *FEMS Microbiol Rev* **13**:25–58.

Berner UR. (1992). Evolution of pore water chemistry during degradation of cement in a radioactive waste repository environment. *Waste Manag* **12**:201–219.

Blouin FA, Arthur JC. (1958). The effects of gamma radiation on cotton Part I: Some of the properties of purified cotton irradiated in oxygen and nitrogen atmospheres. *Text Res J* **28**:198–204.

Bouchard J, Méthot M, Jordan B. (2006). The effects of ionizing radiation on the cellulose of woodfree paper. *Cellulose* **13**:601–610.

Brooke DN, Crookes MJ, Quaterman P, Burns J (2009). Environmental risk evaluation report: Triphenyl phosphate (CAS no. 115-8-6). Envrionmental Agency Report, Envirnmental

The MIND-project has received funding from the European Union's Euratom research and training program (Horizon2020) under grant agreement 661880.

Agency, SCHO0809BQUK-E-P.

Charles CJ, Rout SP, Garratt EJ, Patel K, Laws AP, Humphreys PN. (2015). The enrichment of an alkaliphilic biofilm consortia capable of the anaerobic degradation of isosaccharinic acid from cellulosic materials incubated within an anthropogenic, hyperalkaline environment. *FEMS Microbiol Ecol* **91**: fiv085.

Charles CJ, Rout SP, Patel KA, Akbar S, Laws AP, Jackson BR, *et al.* (2017). Floc formation reduces the pH stress experienced by microorganisms living in alkaline environments. *Appl Environ Microbiol* **83**: AEM.02985-16.

Coaker AW (2003) Fire and flame retardants for PVC. *Journal of Vinyl and Additive Technology*, **9**:108-115.

Colombani J, Herbette G, Rossi C, Joussot-Dubien C, Labed V, Gilardi T (2009). Leaching of plasticized PVC: Effect of irradiation. *Journal of Applied Polymer Science*, **112**:1372–1377.

Dawson, J., (2013) The potential for non-aqueous phase liquid production from irradiated PVC and vinylesterstyrene (VES). Report to NDA RWMD. AMEC/PPE-1008/001 Issue 02.

Engelhardt G, Wallnöfer PR, Hurtzinger O. (1975) The microbial metabolism of di-*n*-butyl phthalate and related dialkyl phthalates. *Bulletin of Environmental Contamination and Toxicology*, **13**:342-347

Engelhardt G, Wallnöfer PR (1978) Metabolism of di- and mono-*n*-butyl phthalate by soil bacteria. *Applied and Environmental Microbiology*, **35**:243-246.

European Commission (2000) Green Paper: Environmental Issues of PVC. COM(2000)469.

European Parliament (2005) Directive 2005/84/EC.

Driscoll M, Stipanovic A, Winter W, Cheng K, Manning M, Spiese J, *et al.* (2009). Electron beam irradiation of cellulose. *Radiat Phys Chem* **78**:539–542.

Gaona X, Montoya V, Colàs E, Grivé M, Duro L. (2008). Review of the complexation of tetravalent actinides by ISA and gluconate under alkaline to hyperalkaline conditions. *J Contam Hydrol* **102**:217–27.

Glaus MA, van Loon LR, Achatz S, Chodura A, Fischer K. (1999). Degradation of cellulosic materials under the alkaline conditions of a cementitious repository for low and intermediate level radioactive waste. Part I: Identification of degradation products. *Anal Chim Acta* **398**:111–122.

Glaus MA, van Loon LR. (2008). Degradation of cellulose under alkaline conditions: New insights from a 12 years degradation study. *Environ Sci Technol* **42**:2906–2911.

The MIND-project has received funding from the European Union's Euratom research and training program (Horizon2020) under grant agreement 661880.

Glegg RE, Kertesz ZI. (1957). Effect of gamma-radiation on cellulose. *J Polym Sci* **26**:289–297.

Haas DW, Hrutfiord BF, Sarkanen KV. (1967). Kinetic study on the alkaline degradation of cotton hydrocellulose. *J Appl Polym Sci* **11**:587–600.

Jianlong W, Ping L, Yi Q. (1995). Microbial degradation of di-*n*-butyl phthalate.*Chemosphere*, **31**:4051-4065.

Keith-Roach MJ. (2008). The speciation, stability, solubility and biodegradation of organic co-contaminant radionuclide complexes: A review. *Sci Total Environ* **396**:1–11.

Knill CJ, Kennedy JF. (2003). Degradation of cellulose under alkaline conditions. *Carbohydr Polym* **51**:281–300.

Kuippers G, Bassil NM, Boothman C, Bryan N, Lloyd JR. (2015). Microbial degradation of isosaccharinic acid under conditions representative for the far field of radioactive waste disposal facilities. *Mineral Mag* **79**:1443–1454.

Kurane R, Suzuki T, Takahara Y. (1980). Metabolic pathway of phthalate esters by *Nocardia erythropolis*. *Agric. Biol. Chem*, **44**:523-527.

Kyeremeh IA, Charles CJ, Rout SP, Laws AP, Humphreys PN. (2016). Microbial community evolution is significantly impacted by the use of calcium isosaccharinic acid as an analogue for the products of alkaline cellulose degradation. *PLoS One* **11**:1–10.

Lawton EJ, Bellamy WD, Hungate RE, Bryant MP, Hall E. (1951). Some effects of high velocity electrons on wood. *Science* **113**:380–382.

Leschine SB. (1995). Cellulose degradation in anaerobic environments. *Annu Rev Microbiol* **49**:399–426.

Lovley DR, Greening RC, Ferry JG. (1984). Rapidly growing rumen methanogenic organism that synthesizes Coenzyme M and has a high affinity for formate. *Appl Environ Microbiol* **48**: 81–87.

van Loon LR, Glaus MA. (1997). Review of the kinetics of alkaline degradation of cellulose in view of its relevance for safety assessment of radioactive waste repositories. *J Environ Polym Degrad* **5**:97–109.

van Loon LR, Glaus MA, Laube A, Stallone S. (1999). Degradation of cellulosic materials under the alkaline conditions of a cementitious repository for low- and intermediate-level radioactive waste. II. Degradation kinetics. *J Polym Environ* **7**:41–51.

van Loon LR, Glaus MA, Vercammen K. (1999). Solubility products of calcium isosaccharinate and calcium gluconate. *Acta Chem Scand* **53**:235–240.

Lynd LR, Weimer PJ, van Zyl WH, Pretorius IS. (2002). Microbial cellulose utilization:

The MIND-project has received funding from the European Union's Euratom research and training program (Horizon2020) under grant agreement 661880.

Fundamentals and biotechnology. *Microbiol Mol Biol Rev* **66**:506–577.

Maset ER, Sidhu SH, Fisher A, Heydon A, Worsfold PJ, Cartwright AJ, *et al.* (2006). Effect of organic co-contaminants on Technetium and Rhenium speciation and solubility under reducing conditions. *Environ Sci Technol* **40**:5472–5477.

Mosier NS, Hall P, Ladisch CM, Ladisch MR. (1999). Reaction kinetics, molecular action, and mechanisms of cellulolytic proteins. *Adv Biochem Eng Biotechnol* **65**:23–40.

Moy PY. (1998). FR characteristics of phosphate ester plasticisers with inorganic addities in PVC. *Journal of Vunyl and Additive Technology*, **4**:22-25.

Moyce EBA, Rochelle C, Morris K, Milodowski AE, Chen X, Thornton S, *et al.* (2014). Rock alteration in alkaline cement waters over 15 years and its relevance to the geological disposal of nuclear waste. *Appl Geochemistry* **50**:91–105.

NDA (2012) NDA Technical Note 17779488, Geological Disposal Summary of NAPL studies, March 2010.

NDA/DECC (2014). Radioactive Wastes in the UK: A Summary of the 2013 Inventory. URN 14D039; NDA/ST/STY (14) 0006; February 2014.

Newsome L, Morris K, Lloyd JR. (2014). The biogeochemistry and bioremediation of uranium and other priority radionuclides. *Chem Geol* **363**:164–184.

Panak P, Klenze R, Kim JI, Wimmer H. (1995). A study of intramolecular energy transfer in Cm(III) complexes with aromatic ligands by time-resolved laser fluorescence spectroscopy. *Journal of Alloys and Compounds*, **225**:261–266.

Pickard MA, Whelihan JA, Westlake DWS. (1975). Utilization of triaryl phophates my a mixed bacterial population. *Canadian Journal of Microbiology*, **21**:140-145.

Rai D, Hess NJ, Xia Y, Rao L, Cho HM, Moore RC, *et al.* (2003). Comprehensive thermodynamic model applicable to highly acidic to basic conditions for isosaccharinate reactions with Ca(II) and Np(IV). *J Solution Chem* **32**:665–689.

Rai D, Rao L, Xia Y. (1998). Solubility of crystalline calcium isosaccharinate. *J Solution Chem* **27**:1109–1122.

Rizoulis A, Steele HM, Morris K, Lloyd JR. (2012). The potential impact of anaerobic microbial metabolism during the geological disposal of intermediate-level waste. *Mineral Mag* **76**:3261–3270.

Rout SP, Charles CJ, Doulgeris C, McCarthy AJ, Rooks DJ, Loughnane JP, *et al.* (2015). Anoxic biodegradation of isosaccharinic acids at alkaline pH by natural microbial communities. *PLoS One* **10**. doi:10.1371/journal.pone.0137682.

Rout SP, Charles CJ, Garratt EJ, Laws AP, Gunn J, Humphreys PN. (2015). Evidence of the

The MIND-project has received funding from the European Union's Euratom research and training program (Horizon2020) under grant agreement 661880.

generation of isosaccharinic acids and their subsequent degradation by local microbial consortia within hyper-alkaline contaminated soils, with relevance to intermediate level radioactive waste disposal. *PLoS One* **10**:1–13.

Rout SP, Radford J, Laws AP, Sweeney F, Elmekawy A, Gillie LJ, *et al.* (2014). Biodegradation of the Alkaline Cellulose Degradation Products Generated during Radioactive Waste Disposal. *PLoS One* **9**:e107433.

Shashoua Y (2008) Conservation of Plastics, In: Materials Science, Degradation and Preservation, Elsevier Science & Technology, p 186.

Smith V, Magalhaes S, Schneider S, (2013) The role of PVC additives in the potential formation of NAPLs. Report to NDA RWMD. AMEC/PPE-2834/001.

Smith SL, Rizoulis A, West JM, Lloyd JR. (2016). The Microbial Ecology of a Hyper-Alkaline Spring , and Impacts of an Alkali-Tolerant Community During Sandstone Batch and Column Experiments Representative of a Geological Disposal Facility for Intermediate-Level Radioactive Waste Community During Sandstone Batch and Column Experiments Representative. *Geomicrobiol J* **33**:455–467.

Strand S, Dykes J, Chiang V. (1984). Aerobic microbial degradation of glucoisosaccharinic acid. *Appl Environ Microbiol* **47**:268–271.

Tits J, Wieland E, Bradbury MH. (2005). The effect of isosaccharinic acid and gluconic acid on the retention of Eu(III), Am(III) and Th(IV) by calcite. *Appl Geochemistry* **20**:2082–2096.

Vazquez GJ, Dodge CJ, Francis AJ, (2008). Interaction of Uranium(VI) with Phthalic Acid. *Inorganic Chemistry* **47**:10739–10743.

Vazquez GJ, Dodge CJ, Francis AJ, (2009). Bioreduction of U(VI)-phthalate to a polymeric U(IV)-phthalate colloid. *Inorganic Chemistry,* **48**:9485–9490.

Vercammen K, Glaus MA, van Loon LR. (2001). Complexation of Th(IV) and Eu(III) by α-isosaccharinic acid under alkaline conditions. *Radiochim Acta* **89**:393–401.

Warwick P, Evans N, Hall T, Vines S. (2003). Complexation of Ni(II) by α-isosaccharinic acid and gluconic acid from pH 7 to pH 13. *Radiochim Acta* **91**:233–240.

Warwick P, Evans N, Hall T, Vines S. (2004). Stability constants of uranium(IV)-α-isosaccharinic acid and gluconic acid complexes. *Radiochim Acta* **92**:897–902.

Warwick P, Evans N, Vines S. (2006). Studies on some divalent metal α-isosaccharinic acid complexes. *Radiochim Acta* **94**:363–368.

Wieland E, Tits J, Dobler JP, Spieler P. (2002). The effect of α-isosaccharinic acid on the stability of and Th(IV) uptake by hardened cement paste. *Radiochim Acta* **90**:683–688.

The MIND-project has received funding from the European Union's Euratom research and training program (Horizon2020) under grant agreement 661880.

Wypych G (2015). PVC Degradation and Stabilization. 3rd Edition. Elsevier. doi:10.1016/B978-1-895198-85-0.50001-7.

Zhou Z, Shu W, Ruan J, Huang B, Liu Y, (2004). Synthesis, characteristics, and fluorescence properties of complexes of europium with benzoic acid and its derivatives. *Rare Metals* **23**:306-310.

Zvereva EA, Fedorova T V, Kevbrin VV., Zhilina TN, Rabinovich ML. (2006). Cellulase activity of a haloalkaliphilic anaerobic bacterium, strain Z-7026. *Extremophiles* **10**:53–60.

The MIND-project has received funding from the European Union's Euratom research and training program (Horizon2020) under grant agreement 661880.