Impact of two contrasting biochars on the bioaccessibility of ^{14}C-naphthalene in soil

Uchenna, O. Ogbonnaya[1,2], James Thomas[1], Sunday, A. Fasina[2], Kirk T. Semple[1]

[1] Lancaster Environment Centre, Lancaster University, LA1 4YQ, United Kingdom.

[2] Department of Soil Science and Land Resources Management, Federal University Oye-Ekiti, Nigeria

*Corresponding author: +234 8178844908; uchenna.ogbonnaya@fuoye.edu.ng

Abstract

This study investigated the impact of two different wood biochars (BioC1 and BioC2) on the extractability and biodegradation of ^{14}C-naphthalene in soil. Both biochars had contrasting properties due to difference in feedstocks and pyrolytic conditions (450 – 500 ^{o}C and 900 – 1000 ^{o}C, designated as BioC1 and BioC2, respectively). This study investigated effects of biochar on the relationship between ^{14}C-naphthalene mineralisation and calcium chloride ($CaCl_2$), hydroxypropyl-β-cyclodextrin (HPCD) or methanol extraction in soil amended with 0%, 0.1%, 0.5% and 1% BioC1 and BioC2 after 1, 18, 36 and 72 d contact times. Total extents of ^{14}C-naphthalene mineralisation and extraction were reduced with increasing concentrations of biochar; however, BioC2 showed greater sorptive capacity. Good linear correlation existed between total extents of ^{14}C-naphthalene mineralisation and HPCD extractions in BioC1 (slope = 0.86, r^2 = 0.92) and BioC2 (slope = 0.86, r^2 = 0.94) amended soils. However $CaCl_2$ and methanol extractions underestimated and overestimated extents of mineralisation, respectively. These results indicate that biochar can reduce the bioaccessibility of PAHs and the corresponding risk of exposure to biota, whilst HPCD extraction estimated the bioaccessible fraction of PAHs in soil. Bioaccessibility assessment is vital in evaluation of biodegradation potential and suitability of bioremediation as a remediation option.

Keywords: Biochar; mineralisation; HPCD; naphthalene; bioaccessibility; macroporous

1. Introduction

Black carbon (BC) encompasses naturally occurring soot and char in the environment as well as some others produced as a by-product of natural and anthropogenic activities [1,2]. Previous studies have investigated the ability of biochar to sequester atmospheric CO_2 in soil to aid climate change mitigation [3,4]. Additionally, biochar has been shown to increase soil nutrients to encourage plant growth [5], improve soil characteristics [6] and stimulate other biological functions [7]. Furthermore, biochar has an intrinsic ability to effectively sequester organic contaminants, such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzo-*p*-dioxins, and bisphenol A [8-12]. The organic contaminant sorption characteristics of biochar have been attributed to large surface area [13] and high porosity [14], which results in decreased mobility and bioaccessibility of the contaminants [15,16]. Some factors exist which affect biochar properties and consequently the capacity to influence the contaminant bioavailability in soils. These factors include (a) the source biomass (feedstock) and (b) the production method (pyrolysis) [12,17]. Therefore, the biomass feedstock for the pyrolysis process is important in determining the resulting biochar properties. Varying biochar characteristics occur as feedstock biomass materials differ; wood chip, tree bark and crop residues, others can be sourced from poultry litter, dairy manure and sewage sludge [18,19].

Contaminated land practitioners also require reliable and robust techniques to determine the applicability of biodegradation and reduce the exposure of contaminants to receptors. Hydroxypropyl-β-cyclodextrin (HPCD) extraction has been shown to predict extents of microbial mineralisation of spiked PAHs at varying concentrations, time and in different soils [20-25]. Semple et al. [26] referred the endpoint of

biodegradation as the bioaccessible fraction. HPCD extraction has further been effective in predicting biodegradation of co-contaminated soils [27,28], field contaminated soils [29,30] and sediments [31]. HPCD extraction clearly represents the fraction of PAHs loosely partitioned to soil matrix and fraction of PAH in the aqueous phase available for biodegradation [32].

Moreover, Rhodes et al. [2] investigated the potential of HPCD extractability to predict ^{14}C-phenanthrene mineralisation in activated carbon (AC) amended soils. The authors showed that HPCD extraction underestimated extent of ^{14}C-phenanthrene mineralisation in >0.1% AC amended soils. In addition, Rhodes and collaborators suggested that such concentrations of AC in soils affect bioaccessibility of PAHs and would affect regulatory procedures. Consequently, the presence of such BC substances can influence the exposure of contaminants to receptors. Therefore the aim of this study was to test investigate (i) the effect of two contrasting wood biochars on the mineralisation of ^{14}C-naphthalene by indigenous microflora; (ii) the extractability of ^{14}C-naphthalene using calcium chloride ($CaCl_2$), HPCD and methanol solutions; (iii) the correlation between amounts of ^{14}C-naphthalene mineralised to ^{14}C-naphthalene extracted; (iv) the correlation between maximum rate of ^{14}C-naphthalene mineralisation to amount of ^{14}C-naphthalene extracted.

2. Materials and Methods

2.1. Chemicals

Non-labelled (^{12}C) naphthalene was obtained from BDH laboratory supplies, UK and [9-^{14}C] naphthalene (>95% radioactive purity) was obtained from Sigma Aldrich Co., Ltd, UK. Goldstar multipurpose liquid scintillation fluid was obtained from Meridian,

97 UK. Hydroxypropyl-β-cyclodextrin (HPCD) was obtained from Fischer Scientific,
98 UK. Calcium chloride (≥99.0%) was obtained from Sigma Aldrich Co., Ltd, UK.
99 Methanol was obtained from Fisher scientific, UK. Sample oxidizer cocktails
100 (Carbotrap and Carbocount) were from Meridian, UK, and Combustaid from Perkin
101 Elmer, USA.
102
103 ***2.2. Soil preparation***
104 An uncontaminated soil (Myerscough soil) classified as surface texture of sandy loam
105 was used in this study. The physicochemical characteristics of the soil can be found in
106 Table 1. The soil was air-dried for 24 h and passed through a 2 mm sieve to remove
107 stones and plant roots. The moisture content of the soil was determined by drying 2 g
108 samples of the soil (n = 3) in porcelain crucibles at 105 ^{o}C for 24 h. After drying, the
109 samples were then cooled in a dessicator (1 h) and weighed again.
110
111 ***2.2. Biochars***
112 The first biochar (BioC1) was obtained from Yorkshire Charcoal Co., UK and the
113 second biochar (BioC2) was obtained from O-Gen UK. Plate count agar and agar-agar
114 were supplied by Oxoid, UK. BioC1 was produced by slow pyrolysis (16 - 18 hours
115 duration at 450 – 500 ^{o}C) of a feedstock containing approximately 90% *Acer*, and the
116 remaining 10% a mixture of *Quercus* and *Fraxinus* sp of wood. BioC2 was produced
117 by gasification (1 hour duration at 1,000 ^{o}C) of a feedstock containing demolition
118 wood waste. Both were sieved to ≤2 mm particle size in preparation for amendment to
119 the soil. Ash content was measured by heating biochar samples at 760 ^{o}C for 6 hours
120 [33] using Carbolite Furnace RHF 1400 and calculated using the equation:
121

$$\text{Ash content (\%)} = \frac{Bb - Ba}{Bb \times 100} \quad \text{(Eq. 1)}$$

where Ba and Bb were biochar weight after and before heating, respectively [34]. Result showed that BioC1 and BioC2 exhibited 13.7% and 34.0% ash, respectively. Biochar pH analysis was measured in triplicate at 1% (w/v) (1 g biochar to 100 ml distilled water) slurry, where BioC1 and BioC2 had pH of 9.6 and 11.2, respectively. The mixture was shaken for 24 hours at 100 rpm and then measured using a digital pH meter. The total pore volume analysis and surface area were measured by using Lab-Tools NMR Cryoporometer (Version 2) [35]. BioC2 exhibited significantly ($P < 0.05$) greater total pore volume (4.10 ml g^{-1}) compared to BioC1 (1.39). BioC1 and BioC2 were both macroporous in nature as they possessed 87.1% and 95.7% macropores ($\geq$50 nm), respectively. BioC2 had surface area of 209 m^2 g^{-1}, whilst BioC1 had significantly lower ($P < 0.01$) surface area of 79 m^2 g^{-1}.

2.4. Soil amendment and spiking

The air-dried soil was rehydrated back to the original field moisture content of 21% (regional average approximately 21 °C) using deionised water. Following rehydration, the soil was spiked with ^{12}C-naphthalene and labelled ^{14}C-naphthalene at 46.67 Bq g^{-1} soil following the method demonstrated by Doick et al. [36], using toluene as a solvent carrier. This achieved a naphthalene concentration of 50 mg kg^{-1}. The soil was then separated and amended with biochar concentrations of 0%, 0.1%, 0.5%, and 1.0% (w/w) by blending the specific quantities into each soil through the use of a stainless steel spoon. This was carried out individually for both BioC1 and BioC2. Blank soils were also prepared for blank corrections. After spiking, 100 g soils were sealed in amber glass jars and then incubated in darkness at room temperature for 1,

18, 36, and 72 days, after which, the soils were analysed as described in the following sections.

2.5. Determination of total ^{14}C-naphthalene-associated activity in soil

The ^{14}C-naphthalene associated activity was determined by combustion using a Packard 307 sample oxidiser at each sampling point of aging (1, 18, 36 and 72 d). Soil samples (1 g; n = 2) were weighed into cellulose combustion cones with an addition of 200 µl Combustaid and combusted (3 min). Carbotrap (10 ml) and Carbocount (10 ml) were used to trap $^{14}CO_2$. The trapping efficiency was >95%. ^{14}C-Activity was quantified by liquid scintillation counting (LSC) (Canberra Packard TriCarb 2300 TR, UK) using standard calibration and quench correction techniques [37].

2.6. Extraction of ^{14}C-naphthalene-associated activity by calcium chloride solution ($CaCl_2$), hydroxypropyl-β-cyclodextrin (HPCD) and methanol

Determination of ^{14}C-naphthalene extractability using $CaCl_2$ was carried out at each sampling point (1, 18, 36 and 72 d). Calcium chloride solutions (10 mM) were prepared using deionised water. Soils (2 g) were weighed into 50 ml Teflon centrifuge tubes (n = 3) and 30 ml $CaCl_2$ solution added to each. Determination of ^{14}C-naphthalene extractability using HPCD was carried out at each sampling point (1, 18, 36 and 72 d) as described by Reid et al. [20]. HPCD solutions (50 mM) were prepared using deionised water. Soils (1.25 g) were weighed into 50 ml Teflon centrifuge tubes (n = 3) and 25 ml HPCD solution added to each. The determination of ^{14}C-naphthalene extractability using methanol solvent was done at each sampling point (1, 18, 36 and 72 d). Soils (1 g) were weighed into 30 ml Teflon centrifuge tubes (n = 3) and 15 ml of methanol solvent (1:15) was added to each tube.

The tubes were placed onto an orbital shaker at 100 rpm for 22 h. The tubes were then centrifuged at 3000 rpm (Rotanta 460 Centrifuge, Hettich, Germany) for 1 h and 5 ml supernatant was pipetted into 20 ml glass scintillation vials containing Goldstar scintillation cocktail (15 ml). The ^{14}C-labeled radioactivity in the resultant solution was then quantified using the LSC. After extraction, the soil pellet remaining was air dried, weighed into combust cones and then oxidised using the method of determination of ^{14}C-associated activity in soil pellet. This was to establish a mass balance of ^{14}C-associated activity before and after desorption.

2.7. Mineralisation of ^{14}C-naphthalene in soil

This process was used to determine the rate and extent of ^{14}C-mineralisation of naphthalene by the indigenous soil microorganisms. Mineralisation assays were carried out in respirometers to assess the catabolism of ^{14}C-naphthalene by the soil indigenous microflora. Respirometers were modified Schott bottles as described in Reid et al. [38]. These were set up in triplicates and into each was added 10 ± 0.2 g soil (dry weight) containing either BioC1 or BioC2 (0%, 0.1%, 0.5%, 1.0%) as well as 30 ml minimum basal salts (MBS). The respirometers incorporated a CO_2 trap containing 1 M NaOH (1 ml) within a suspended 7 ml glass scintillation vial. The respirometers were placed on an orbital shaker set at 100 rpm and 25 ^{o}C over a period of 14 days. Evolved $^{14}CO_2$ as a result of ^{14}C-naphthalene catabolism was trapped in 1 M NaOH with ^{14}C-activity assessed daily by adding Ultima Gold (5 ml) and then utilising liquid scintillation counting (LSC).

2.8. Statistical Analysis

Statistical analysis of data was conducted using SigmaStat software (Ver 2.0; Systat, Richmond, CA, USA). One way ANOVA ($P < 0.05$) was used to demonstrate differences in extent of mineralisation and extractions amongst each biochar amendment at each time point. Student's *t*-test was used to compare differences in extent of mineralisation and extractability by $CaCl_2$, HPCD and methanol. Linear regression was used to correlate extent of mineralisation to individual chemical extraction.

3. Results

3.1. Loss of ^{14}C-naphthalene-associated activity from biochar-amended soils

At each contact time (1, 18, 36 and 72 d), the total amount of ^{14}C-naphthalene-associated activity was determined. Following an increase in soil-PAH contact time, there were statistically significant ($P < 0.05$) losses of ^{14}C-naphthalene associated activity in control and 0.1% biochar amended soils. Following 18 d soil-PAH contact time, >22% loss of total amount of spiked ^{14}C-naphthalene activity in both 0% and 0.1% biochar amendments regardless of biochar type (Figure 1). However, $\leq$20% of ^{14}C-naphthalene activity was lost in 0.5% and 1% biochar amended soils. Following subsequent increasing soil-PAH contact time (36 and 72 d), there were further significant ($P < 0.05$) loss in ^{14}C-naphthalene associated activity in 0%, 0.1% and 0.5% BioC1 and BioC2 amendments. Interestingly, despite 72 d soil-PAH contact time, there was no significant ($P > 0.05$) loss in activity in 1% BioC2 amended soil as no greater than 10% ^{14}C-naphthalene activity was lost (Figure 1).

3.2. Extraction of ^{14}C-naphthalene-associated activity by $CaCl_2$, HPCD, and methanol

The extractability of ^{14}C-naphthalene-associated activity using $CaCl_2$, HPCD, and methanol was measured over time in unamended and biochar amended soils. $CaCl_2$ extraction removed significantly ($P < 0.05$) less ^{14}C-naphthalene-associated activity compared to HPCD or methanol across all contact times. At 1 d time point, all three concentrations (0.1%, 0.5% and 1%) of BioC2 significantly reduced ($P < 0.001$) HPCD extractability; whereas, only 0.5% and 1% BioC1 amendments had similar effects. This trend was similar to $CaCl_2$ extractability. The BioC2 amendments often showed stronger reduction in amounts of ^{14}C-naphthalene removed by $CaCl_2$ extraction compared to BioC1, where 40.4%, 10.2%, 1.6% and 1.5% were removed from soil amended with 0%, 0.1%, 0.5% and 1% BioC2, respectively (Table 2). In HPCD extraction, 72.9%, 39.9%, 22.2% and 7.9% were extracted from soils amended with 0%, 0.1%, 0.5% and 1% BioC2 amended soils, respectively (Table 2). However, only the 1% of both biochars (BioC1 and BioC2) significantly reduced ($P < 0.05$) ^{14}C-naphthalene extractability by methanol.

Following increasing soil-PAH contact time (18, 36, and 72 d), the increasing concentration of biochar amendments resulted in further reduction ($P < 0.05$) in HPCD extractability of ^{14}C-naphthalene (Table 2) compared to the control soil. However, BioC2 often showed lower extent of $CaCl_2$ and HPCD extractions compared to BioC1 extractions after 18 d soil-PAH contact time (Table 2). Noticeably, 1% BioC2 amended soils exhibited the lowest ($P < 0.001$) extent of extraction compared to other concentrations and BioC1. However, methanol and $CaCl_2$ extraction methods resulted to greater and lower ($P < 0.05$) ^{14}C-naphthalene

247 extractability, respectively, compared to HPCD extraction. After 36 and 72 d contact
248 times, BioC1 and BioC2 had no significant effect on $CaCl_2$ extractability ($P > 0.05$)
249 (Table 2). Also, $CaCl_2$ and HPCD could extract no greater than 10% and 20% ^{14}C-
250 naphthalene, respectively at later contact times (36 and 72 d) (Table 2).
251
252 ***3.3. Mineralisation of ^{14}C-naphthalene in soil***
253 The mineralisation of ^{14}C-phenanthrene was monitored over a period of 14 d
254 incubation in soil amended with 0%, 0.1%, 0.5% and 1.0% biochars (BioC1 and
255 BioC2) after 1, 18, 36 and 72 d soil-PAH contact times. The lag phases, rates and
256 extents of mineralisation were calculated and analysed for significant impacts of
257 biochar on mineralisation. The lag phases were measured and defined as the time
258 taken for the extent of ^{14}C-naphthalene mineralisation to exceed 5%. Increasing
259 concentrations of biochar amendment largely served to increase the lag phase of ^{14}C-
260 naphthalene mineralisation (Figure 2 and Table 3). Lag phases for control and BioC1
261 amendments were between 2.5 and 3 days at 1 d soil-PAH time point. Noticeably, 1%
262 BioC2 amendment caused a significant increase ($P < 0.01$) in lag phase to 8 days
263 compared to control and 1% BioC1. Following 18 d aging period, lag phases were
264 below 2 days in control and BioC1 amended soils, whilst BioC2 amendments resulted
265 in further increases ($P < 0.001$) in lag phases (Figure 2, Table 3). For example, 0.1%
266 and 0.5% BioC2 extended ($P < 0.001$) the lag phases to 9 and 14 d, respectively,
267 whilst lag phase was immeasurable in 1% BioC2 amended soils (Table 3). This trend
268 was consistent following subsequent aging (36 and 72 d), where 0.5% and 1% BioC2
269 and 1% BioC1 showed immeasurable lag phases beyond 14 days (Table 3). Despite
270 this, there was no significant difference ($P > 0.05$) in lag phase between 0.1% BioC1
271 and BioC2 after 36 and 72 day time points (Table 3).

The mean maximum rates of mineralisation per day were generally shown to be lower with increasing biochar concentration and soil-PAH contact time. However, at 1 d soil-PAH contact time, the highest maximum rate of ^{14}C-naphthalene mineralisation was 35% d^{-1} and was achieved after 3 days of mineralisation in 0.1% BioC1 amended soils. Generally, BioC1 amendments had no significant effect ($P > 0.05$) on rate of mineralisation, except for 1% BioC1. In contrast, all concentrations of BioC2 amendments (0.1%, 0.5% and 1.0%) demonstrated significant reductions ($P < 0.001$) in maximum rates of ^{14}C-naphthalene mineralisation of 5.81% d^{-1}, 2.52% d^{-1} and 1.32% d^{-1}, respectively (Table 3) compared to control. Noticeably, the increase in soil-PAH time developed consistent decreases in maximum rates of ^{14}C-naphthalene mineralisation, except for 0.1% BioC1. It was also observed that BioC2 amendments significantly reduced ($P < 0.05$) the rates of mineralisation compared to BioC1 at 1, 18 and 36 d contact time (Table 3).

The total extents of ^{14}C-naphthalene mineralisation were monitored over 14 days and showed decrease with increasing biochar concentrations (Figure 2 and Table 3). This occurred for both types of biochar (BioC1 and BioC2) and after each contact time (1, 18, 36 and 72 d). For instance, the total extents of mineralisation after 1 d contact time for 0%, 0.1%, 0.5% and 1.0% BioC1 were 62.0%, 58.8%, 52.6%, 29.0%, respectively (Table 3). Similarly, fractions of ^{14}C-naphthalene mineralised in 0.1%, 0.5% and 1% BioC2 amended soils were 25%, 17.3% and 9.9%, respectively. The total extents of ^{14}C-naphthalene mineralised in soil amended with 1% BioC1 and 0.5% and 1% BioC2 were often 50% less of the control soil (0%) at all contact time points. Furthermore, the addition of BioC2 to the soil reduced the extents of mineralisation by $\geq$50%

297 compared to BioC1 (Figure 2 and Table 3). Following increases in soil-PAH contact
298 time, the mineralisation of ^{14}C-naphthalene significantly decreased ($P < 0.05$); this
299 was apparently observed irrespective of biochar amendment in the soils. It is
300 noteworthy that microbial activity was not invigorated by further spiking of ^{14}C-
301 naphthalene into the respirometry assays nor was there any addition of naphthalene
302 degrading inoculum. This was to evaluate the potential of intrinsic microbial inoculum
303 to degrade bioaccessible fraction of ^{14}C-naphthalene. In the control soil (0%), for
304 instance, the total extents of mineralisation was 62.0%, 34.1%, 17.6% and 10.1% after
305 1, 18, 36 and 72 d soil-PAH contact time (Figure 2 and Table 3). All three (0.1%,
306 0.5% and 1%) concentrations of both biochars showed significant decrease ($P <$
307 0.001) in extents of ^{14}C-naphthalene mineralisation with increase in soil-PAH contact
308 time.
309
310 ***3.4. Relationship between extraction and mineralisation of ^{14}C-naphthalene***
311 The relationship between the maximum rates of ^{14}C-naphthalene mineralisation and
312 either of $CaCl_2$, HPCD or methanol extractability was assessed to test the ability of
313 either extraction method to predict microbial degradation rate of the compound in
314 biochar amended soils. Equally, the total extents of ^{14}C-naphthalene mineralisation
315 were also correlated individually to $CaCl_2$, HPCD or methanol extractability. Figures
316 3 and 4 (A - C) shows the relationship between rates of ^{14}C-naphthalene
317 mineralisation to $CaCl_2$, HPCD, and methanol extractability, individually. There was
318 very good agreement between rate of ^{14}C-naphthalene mineralisation d^{-1} and $CaCl_2$ in
319 BioC1 and BioC2 amended soils (slope of 0.82, $r^2 = 0.89$, intercept = -1.63; slope of
320 0.59, $r^2 = 0.97$, intercept = -0.24), respectively (Figures 3 and 4). In support, there was
321 no significant difference ($P > 0.05$) between the amount extracted by $CaCl_2$ and the

rate of mineralisation at each contact time in biochar-amended soils. However, both HPCD and methanol extractions overestimated the rates of ^{14}C-naphthalene mineralisation in BioC1 and BioC2 amended soil (Figures 3 and 4). Figures 5 and 6 (A - C) illustrate relationship between total extents of ^{14}C-naphthalene mineralisation individually to $CaCl_2$, HPCD and methanol extraction.

Results showed that $CalCl_2$ extractability of ^{14}C-naphthalene underestimated the extents of mineralisation (slope of 1.58, r^2 = 0.93, intercept = 5.34), (slope of 1.53, r^2 = 0.90, intercept = 4.15) for BioC1 and BioC2, respectively. However, HPCD extraction of ^{14}C-naphthalene showed better agreement with slope of 0.86 for both biochar amendments and r^2 of 0.92 (intercept = 0.74) and r^2 of 0.94 (intercept = -1.23), respectively, for BioC1 and BioC2 amendments (Figures 5A and 6A). Also the slope was approximated to 1 (0.86). Whereas, methanol extractability overestimated the extents of mineralisation (slope of 0.74, r^2 = 0.49, intercept = -16.30) and (slope of 0.30, r^2 = 0.12, intercept = -4.40) of BioC1 and BioC2 amended soils, respectively.

4. Discussions

4.1. Loss of ^{14}C-naphthalene-associated activity

The overall losses of ^{14}C-naphthalene-associated activity in controls and 0.1% biochars amended soils were mainly attributed to degradation and volatilisation [22,39]. The inherent biodegradation of the bioaccessible fraction of ^{14}C-naphthalene would have occurred during the aging period since naphthalene catabolic potential can be found diversely in the environment [40,41]. Biochar is a form of recalcitrant organic matter produced through pyrolysis of biomass [42,43] and reduces the bioavailability of PAHs and TCDDs in soil by sorption [11,15]. This property caused

insignificant loss ($P > 0.05$) of ^{14}C-naphthalene-associated activity in 0.5% and 1% biochar amended soils compared to control. This was also attributed to the enhanced level of sequestration due to higher concentrations of biochar, which reduced any loss of naphthalene in the soil

4.2. Extractability of ^{14}C-naphthalene-associated activity using $CaCl_2$, HPCD, and methanol extraction techniques

This study tested the ability of different non-exhaustive extraction techniques ($CaCl_2$, HPCD and methanol) to remove labile fractions of naphthalene [26]. $CaCl_2$ and HPCD extractions showed significant decreasing extractability ($P < 0.05$) with increasing biochar concentrations (Table 2). This was attributed to sequestration processes, including sorption via partitioning and physical entrapment of the ^{14}C-naphthalene-associated activity to biochar particles [44-46]. Sorption may occur via physical adsorption through weak binding force, entrapment into nanopores and/or chemical or internal adsorption through strong hydrophobic and binding force [47]. There were differences in the amounts of ^{14}C-naphthalene extracted from soil with differing biochar particles (BioC1 and BioC2), mainly due to the difference in total pore volume of individual biochars which accommodated the ^{14}C-naphthalene [48]. Obviously, the biochars differed in feedstock and production process. For example, BioC2 exhibited greater pore volume which clearly sequestered more ^{14}C-naphthalene than BioC1. This was because of the higher temperature of BioC2 production, whilst BioC1 was produced at 450 ^{o}C [49]. Since the biochars contain less internal surface area and micropores, PAHs tend to accumulate within the macroporous region [46], which is dominantly in BioC2. This study supports Zhang et al. [50], in which biochar

produced at 700 ^{o}C incorporated in soil effectively sorbed phenanthrene to greater extent compared to a 350 ^{o}C biochar.

Following increasing soil-PAH contact time, there was a general reduction in amounts of ^{14}C-naphthalene removed by $CaCl_2$ or HPCD irrespective of biochar concentrations. When organic contaminants are in contact with soil, there is a rapid uptake of the organic compounds via fast and slow stages (hours to days) through partitioning and adsorption within pores of soil matrix [51]. The inability of $CaCl_2$ to extract ^{14}C-naphthalene in control and biochar amended soils was attributed to the poor extractability of the solution, inability of solution to penetrate into nanopore regions containing ^{14}C-naphthalene to desorb the contaminant [23]. Despite HPCD being an effective extracting solution [23,26,30,37], ^{14}C-naphthalene was shown to be irreversibly extractable due to significant adsorption and partitioning within nanopore sites [8,15,52]. This was better explained as methanol solvent extraction described the physical entrapment of naphthalene within soil-biochar matrix following intra-organic matter diffusion [15,53].

4.3. Mineralisation of ^{14}C-naphthalene-associated activity from soil

Although biochar affects the extent of biodegradation or organic contaminants, the degree to which different biochars impact on biodegradation differs considerably when incorporated into soils [9,16,25,54]. Extents of ^{14}C-naphthalene mineralisation were consistently lower as the concentration of biochar amendments increased (0% > 0.1% > 0.5% > 1%). Rhodes et al. [2,55], Marchal et al. [16] and Ogbonnaya et al. [25] confirmed that the addition of AC and biochar to soils reduced the extents of ^{14}C-PAH mineralisation through sorption and reduction of the PAH in aqueous phase.

396 Similarly, biochar reduced extents of ^{14}C-naphthalene mineralisation and the reduction
397 was more pronounced in the BioC2 amended soils; thus, the degree of sorption differs
398 amongst biochar materials. This is often attributed to differences in physical
399 properties, owing to difference in feedstock material and production processes
400 [50,56]. Indeed, Chen and Yuan [8], Bornemann et al. [49] and Zhang et al. [50]
401 illustrated that higher temperature biochar tend to sorb organic contaminants to a
402 greater degree. Biochar strongly sequesters naphthalene molecules within its
403 micropore network [1] and resists desorption even while experiencing shaking in
404 slurry assay, thereby reducing the bioavailable/bioaccessible fractions. High pore
405 volumes were observed for both biochars, but it was greater in BioC2 and
406 accompanied with higher surface area which resulted in the higher extent of sorption
407 that governed the bioaccessibility of naphthalene. BioC1 initially sustained rate of
408 mineralisation but increasing biochar concentrations and contact time accompanied
409 increases in lag phases and reductions in the rates and extents of biodegradation [12].
410 Reduction in extents of ^{14}C-naphthalene mineralisation with increase in soil-PAH
411 contact time is in agreement with other related studies [2,22,25,37,55].
412
413 Semple et al. [26] clearly described bioavailability as a good descriptor of the rate of
414 biodegradation of an organic contaminant; whilst bioaccessibility described the
415 biodegradation end-point. Based on these definitions, the rates and extents of ^{14}C-
416 naphthalene mineralisation were individually compared to its HPCD, $CaCl_2$ and
417 methanol extractability to utilise a suitable chemical extraction technique to determine
418 the bioavailability and bioaccessibility of ^{14}C-naphthalene in biochar-amended soils.
419 Linear regression was used to statistically test correlation between $CaCl_2$, HPCD and
420 methanol extracts to rates and extents of ^{14}C-naphthalene mineralisation by indigenous

soil microflora. $CaCl_2$ extraction estimated the maximum rates of ^{14}C-naphthalene mineralisation (bioavailable) in all soils irrespective of biochar concentrations. Previous studies demonstrated that HPCD extractability of PAHs represents its bioavailable fraction [22,57,58]. However, HPCD and methanol extractions overestimated bioavailability, thus they don't illustrate the chemically active fraction but HPCD extraction illustrated the bioaccessible fractions irrespective of the concentration and type of biochar. The interior cavity of HPCD is hydrophobic in nature and capable of forming complexes with HOCs, whilst its exterior is hydrophilic in nature [59,60]. A HPCD initiated 'host-guest' complex [61] means that HPCD can readily form inclusion complex with naphthalene [62], enabling the extraction of the bioaccessible fraction of the contaminant in soil [21,22,25,29,37] irrespective of biochar concentration. This is because, HPCD can access the macroporous exterior cavity of biochar where majority of PAHs are often entrapped [46] and form complexes with the compounds of question for extraction. Additionally, the macroporous cavity is also accessible to microorganisms for biodegradation of naphthalene. In contrast, Rhodes et al. [2] showed that hydrophobicity and microporosity of activated charcoal extensively reduces the extractability of HPCD from hydrophobic matrices. The other chemical extraction techniques ($CaCl_2$ and methanol) underestimated and overestimated the extents of ^{14}C-naphthalene mineralisation, respectively. This study validates the applicability of HPCD extraction to predict extents of PAH biodegradation soils where biochar has been incorporated to reduce bioaccessibility and the corresponding risk of exposure.

Conclusions

This current study tested extractability of ^{14}C-naphthalene spiked soils containing 2 different biochar particles (BioC1 and BioC2). Despite the influence of individual biochar on biodegradation of naphthalene, HPCD extraction was capable of predicting the extents of mineralisation and influence of biochar on biodegradation, whilst $CaCl_2$ extraction predicted the maximum rate of mineralisation. Thus extending the use of HPCD extraction to biochar amended soils. Additionally, this study has demonstrated that biochar reduces the bioaccessibility of naphthalene in soil and this depends on its production process and feedstock which affects physical properties. Thus, with different biochar concentrations and porous nature, the risk of contaminants in soil can be reduced and yet HPCD can predict the extent of biodegradation of the contaminants. Biochar being cheaper than AC can be used in PAH contaminated land sites to immobilise contaminants. However, this study is based on single spiked soil, field contaminated soils can contain mixtures of contaminants and are exposed to more hostile conditions. Further research should focus on the applicability of biochar in field contaminated soils.

Acknowledgements

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734 Table 1 Physiochemical properties of uncontaminated Myerscough (sandy loam) soil

Soil particle analysis						**pH**		**Elemental analysis**
Texture	Clay	Silt	Sand[a]			dH_2O	$CaCl_2$	OM[b]
			C	M	F			
Sandy loam	19.5	20.0	0.12	6.9	53.3	6.53	5.18	2.7

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736 [a] coarse, medium, and fine sand, [b] organic matter content (%)

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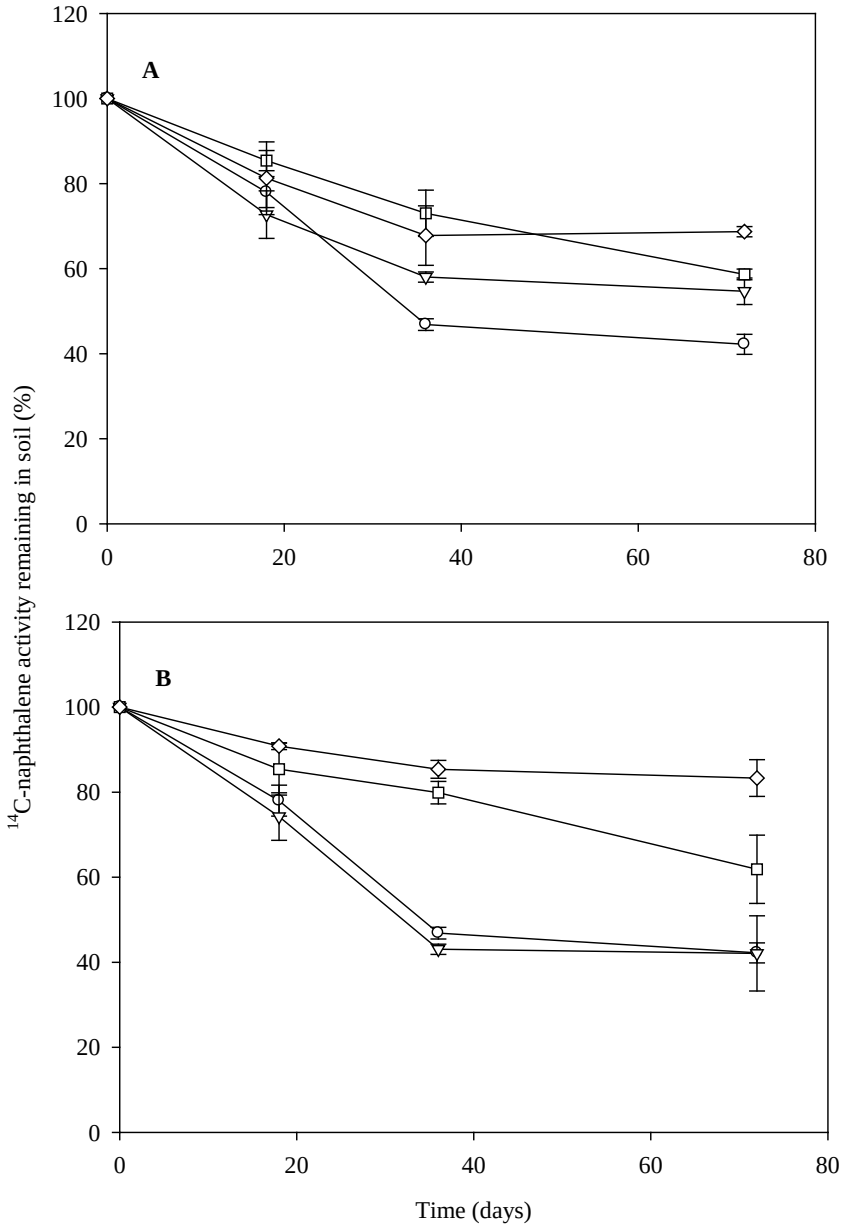

Figure 1 Total ^{14}C-naphthalene-associated activity remaining in soil amended with 0% (○), 0.1% (∇), 0.5% (□) and 1% (◊) BioC1 (A) and BioC2 (B) over 72 days incubation period. Error bars represent standard deviation (n = 3)

769 Table 2 ^{14}C-naphthalene extracted (%) by $CaCl_2$, HPCD and methanol ± standard
770 deviation of triplicate samples (n = 3)

Soil-PAH contact (d)	Biochar type	Amendment (%)	$CaCl_2$	HPCD	Methanol
1		0	40.42 ± 0.62	72.92 ± 0.34	86.55 ± 4.98
	BioC1	0.1	34.25 ± 0.12	67.53 ± 0.39	72.05 ± 2.37
		0.5	26.13 ± 2.28	50.28 ± 1.01	74.88 ± 3.17
		1	10.10 ± 3.45	46.63 ± 2.36	61.23 ± 1.81
	BioC2	0.1	10.18 ± 0.94	39.89 ± 0.14	74.00 ± 0.74
		0.5	1.56 ± 0.11	22.18 ± 0.24	67.84 ± 2.99
		1	1.54 ± 0.64	7.90 ± 0.36	58.52 ± 4.18
18		0	14.21 ± 0.42	34.57 ± 3.15	62.80 ± 1.55
	BioC1	0.1	8.59 ± 0.10	21.85 ± 1.01	55.51 ± 5.91
		0.5	7.02 ± 1.77	11.53 ± 1.97	55.60 ± 2.45
		1	4.16 ± 0.59	11.29 ± 2.19	54.00 ± 5.53
	BioC2	0.1	3.14 ± 0.55	11.74 ± 3.28	61.80 ± 2.32
		0.5	2.85 ± 0.50	10.21 ± 1.21	65.21 ± 2.40
		1	1.41 ± 0.10	4.07 ± 0.59	56.49 ± 5.34
36		0	6.49 ± 0.83	17.41 ± 1.68	32.30 ± 1.48
	BioC1	0.1	6.33 ± 1.48	12.90 ± 2.87	33.30 ± 6.23
		0.5	3.72 ± 1.03	12.10 ± 2.82	53.40 ± 5.71
		1	2.35 ± 0.05	7.66 ± 1.15	49.26 ± 2.76
	BioC2	0.1	3.40 ± 0.80	12.59 ± 2.71	57.10 ± 2.59
		0.5	1.93 ± 0.21	6.62 ± 0.71	73.70 ± 3.14
		1	1.66 ± 0.09	4.55 ± 0.21	56.90 ± 4.62
72		0	5.99 ± 1.07	17.34 ± 1.34	26.30 ± 0.72
	BioC1	0.1	4.66 ± 0.28	12.96 ± 1.03	24.45 ± 1.65
		0.5	4.44 ± 0.83	14.93 ± 2.33	43.65 ± 4.73
		1	3.33 ± 0.76	8.27 ± 1.28	69.33 ± 7.09
	BioC2	0.1	2.54 ± 0.28	10.89 ± 1.06	34.45 ± 3.03
		0.5	2.58 ± 0.57	6.49 ± 2.11	59.28 ± 0.34
		1	1.29 ± 0.08	5.04 ± 0.12	50.40 ± 3.58

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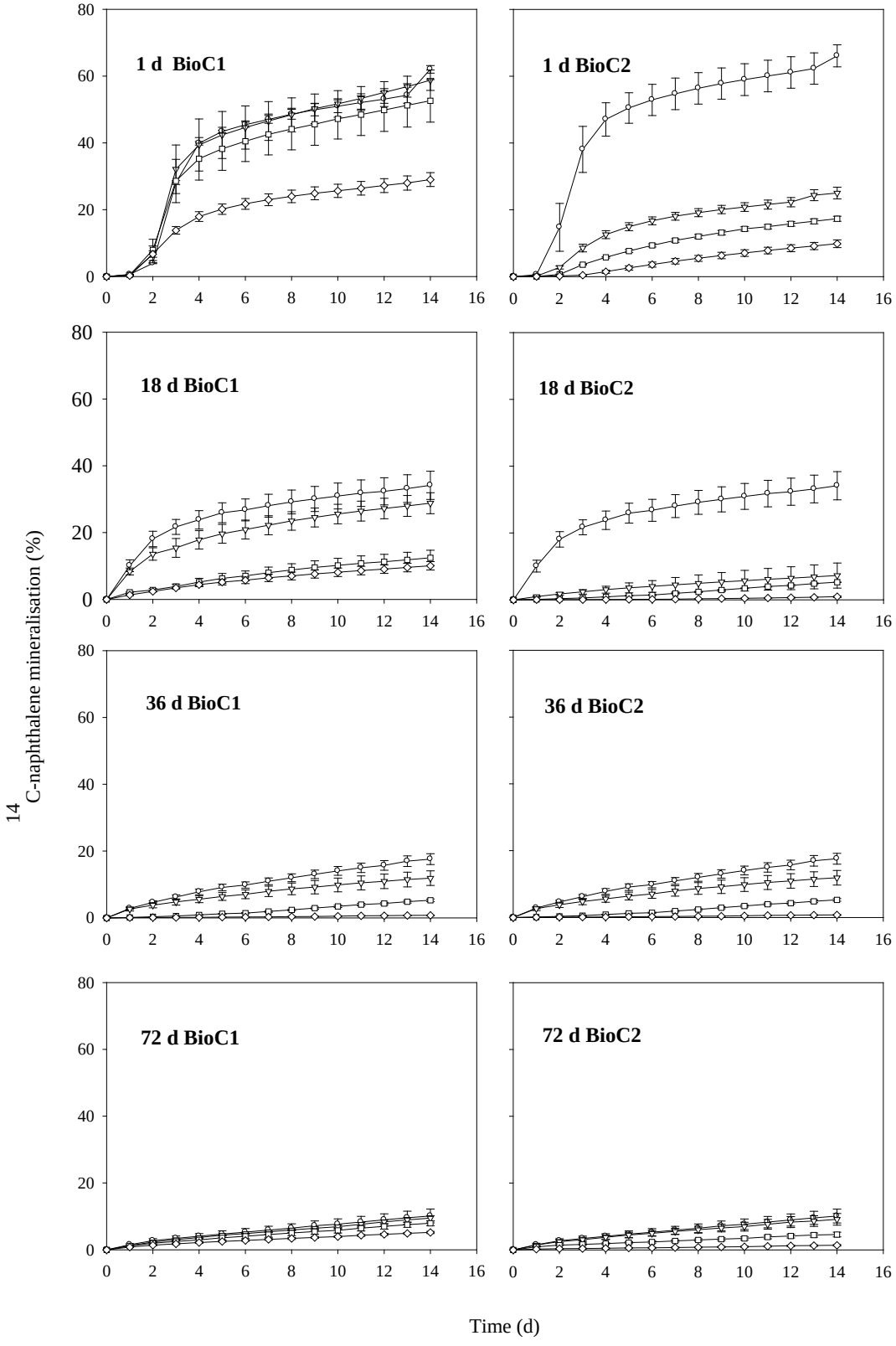
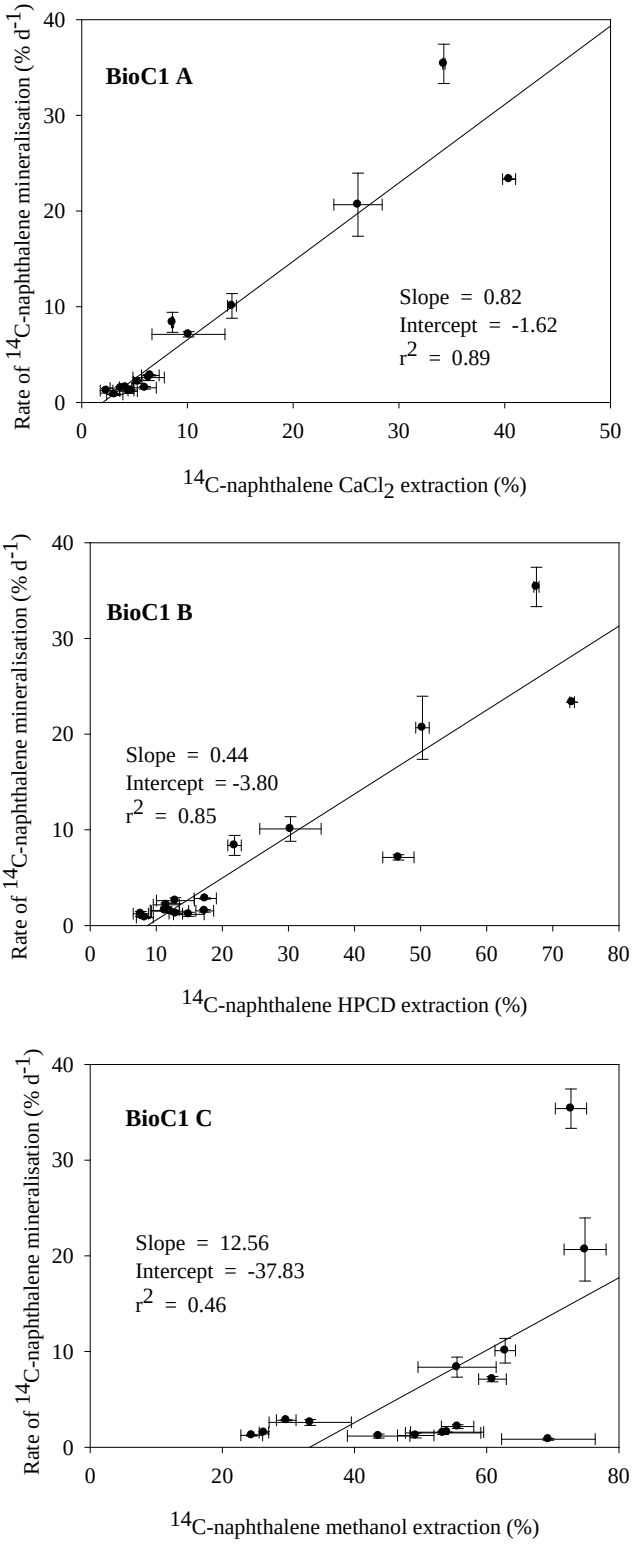


Figure 2 Mineralisation of ^{14}C-naphthalene in Myerscough soil amended with 0% (○), 0.1% (∇), 0.5% (□) and 1% (◊) of BioC1 and BioC2. Error bars represent standard error of mineralisation (SEM) of triplicate samples (n = 3).

782 Table 3 Mineralisation of ^{14}C-naphthalene in Myerscough soil amended with 0%,
783 0.1%, 0.5% and 1 % of biochar 1 and 2 ± standard error of mineralisation (SEM) of
784 triplicate samples (n = 3)

Soil-PAH contact (d)	Biochar type	Amendment (%)	Lag phase (d)	Maximum rates (d^{-1})	Total extent
1		0	3.04 ± 0.01	23.33 ± 0.01	62.03 ± 1.15
	BioC1	0.1	2.87 ± 0.19	35.38 ± 2.06	58.78 ± 3.06
		0.5	2.72 ± 0.28	20.66 ± 3.30	52.62 ± 6.38
		1	2.77 ± 0.13	7.12 ± 0.28	29.04 ± 2.08
	BioC2	0.1	3.51 ± 0.11	5.81 ± 0.81	25.04 ± 1.74
		0.5	4.65 ± 0.05	2.52 ± 0.03	17.34 ± 0.75
		1	8.70 ± 1.04	1.32 ± 0.13	9.87 ± 1.16
18		0	1.51 ± 0.08	10.09 ± 1.29	34.14 ± 2.00
	BioC1	0.1	1.62 ± 0.08	8.37 ± 1.04	28.80 ± 3.14
		0.5	5.25 ± 0.83	2.16 ± 0.20	12.45 ± 2.30
		1	6.50 ± 0.91	1.57 ± 0.01	10.09 ± 1.27
	BioC2	0.1	9.11 ± 0.58	2.06 ± 0.01	7.28 ± 0.12
		0.5	14.21 ± 0.33	0.55 ± 0.03	5.28 ± 0.03
		1	N/A	0.27 ± 0.03	0.95 ± 0.23
36		0	3.23 ± 0.01	2.82 ± 0.06	17.58 ± 1.62
	BioC1	0.1	3.68 ± 0.68	2.59 ± 0.31	15.88 ± 2.10
		0.5	5.82 ± 0.02	1.50 ± 0.17	10.84 ± 0.20
		1	N/A	1.22 ± 0.25	5.81 ± 0.63
	BioC2	0.1	4.63 ± 1.41	1.94 ± 0.05	11.89 ± 2.19
		0.5	N/A	0.91 ± 0.01	5.36 ± 0.39
		1	N/A	0.12 ± 0.04	0.78 ± 0.11
72		0	10.21 ± 2.85	1.53 ± 0.14	10.13 ± 2.08
	BioC1	0.1	7.31 ± 0.30	1.24 ± 0.08	9.47 ± 0.78
		0.5	9.71 ± 1.22	1.17 ± 0.21	7.94 ± 0.72
		1	N/A	0.83 ± 0.10	5.20 ± 0.33
	BioC2	0.1	8.97 ± 0.66	1.65 ± 0.23	7.32 ± 0.25
		0.5	N/A	0.94 ± 0.08	4.62 ± 0.70
		1	N/A	0.23 ± 0.09	1.40 ± 0.22



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786 Figure 3 Correlation between maximum rate ^{14}C-naphthalene mineralised and ^{14}C-
787 naphthalene extracted with (A) $CaCl_2$ (B) HPCD (C) methanol after 24 h with BioC1
788 amendment.

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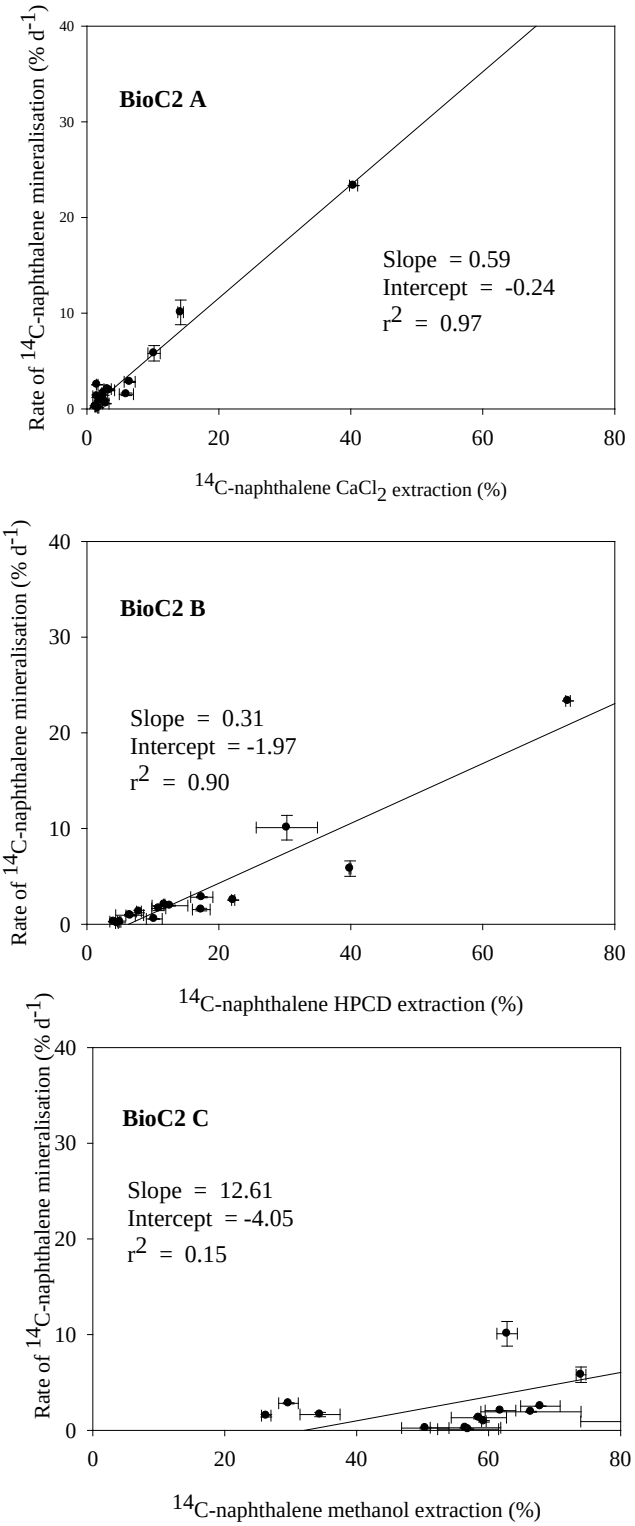

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791 Figure 4 Correlation between maximum rate ^{14}C-naphthalene mineralised and ^{14}C-
792 naphthalene extracted with (A) $CaCl_2$ (B) HPCD (C) methanol after 24 h with BioC2
793 amendment.

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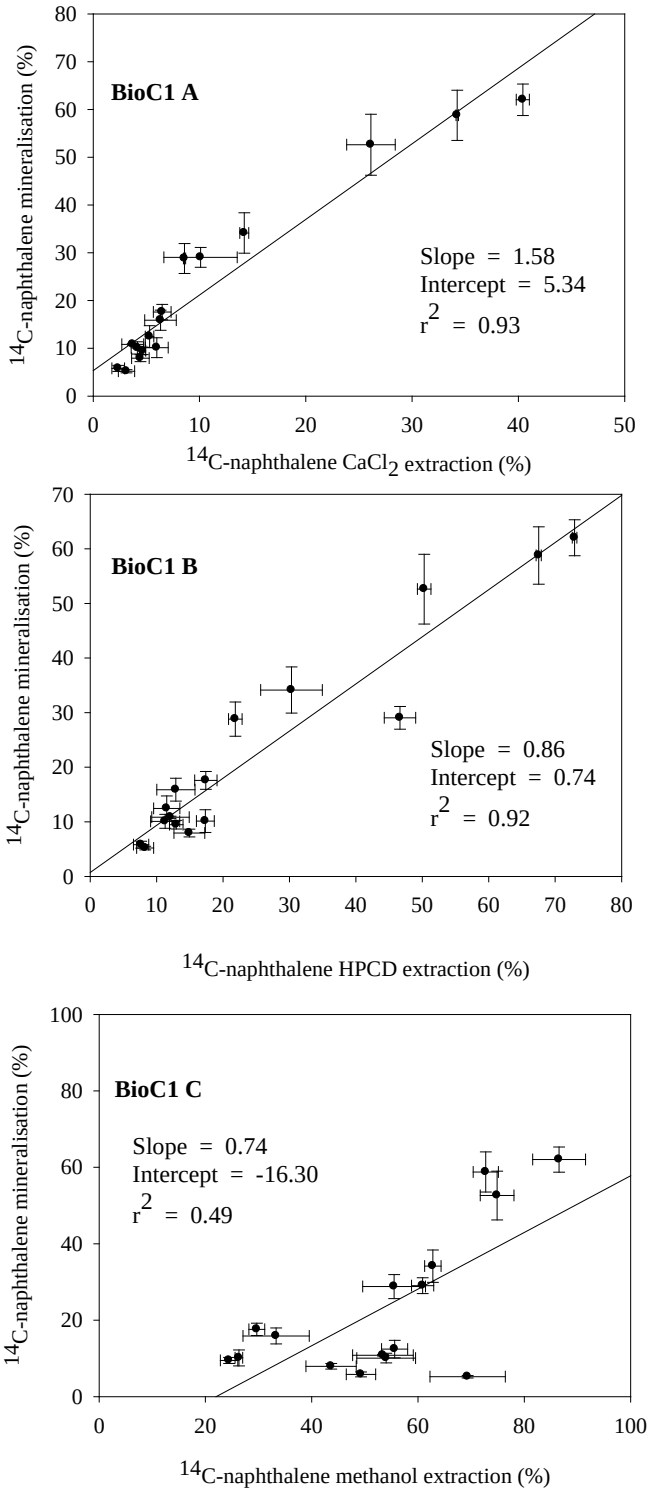

Figure 5 Correlation between extent of ^{14}C-naphthalene mineralised and ^{14}C-naphthalene extracted with (A) $CaCl_2$ (B) HPCD (C) methanol after 24 h with BioC1 amendment.

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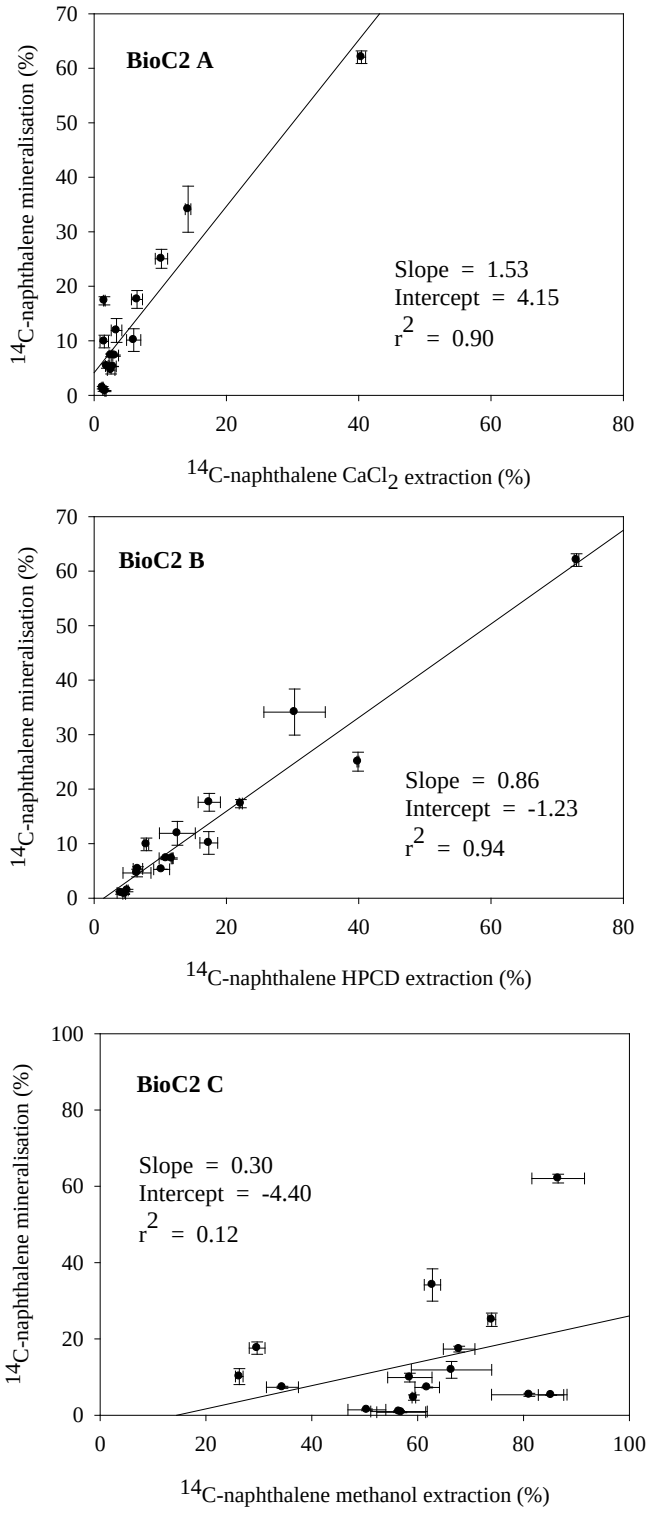

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805 Figure 6 Correlation between extent of ^{14}C-naphthalene mineralised and ^{14}C-
806 naphthalene extracted with (A) $CaCl_2$ (B) HPCD (C) methanol after 24 h with BioC2
807 amendment.