Journal of Hazardous Materials 403 (2021) 123676

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Simultaneous adsorption and biodegradation of trichloroethylene occurs in a biochar packed column treating contaminated landfill leachate

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ARTICLE INFO

Editor: R. Debora

Keywords:
Bioremediation
Trichloroethylene
Permeable Reactive Barrier
Biochar
Waste reuse

ABSTRACT

Trichloroethylene (TCE) is a human carcinogen that is commonly found in landfill leachate. Contaminated leachate plumes may be intercepted prior to reaching groundwater and treated *in situ* using permeable reactive barriers (PRB). This study used a packed column system containing herbal pomace and spruce biochar, previously shown to have TCE adsorptive capabilities. Influent containing raw or autoclaved landfill leachate was used to investigate the potential for environmental micro-organisms to establish a TCE-dechlorinating biofilm on the biochar, in order to prolong the operational life span of the system. TCE removal $\geq$ 99.7 % was observed by both biochars. No dichloroethylene (DCE) isomers were present in the column effluents, but *cis*-1,2 DCE was adsorbed to the biochar treating raw landfill leachate, indicating that dechlorination was occurring biologically in these columns. Known microbial species that are individually capable of complete dechlorination of TCE to ethene were not detected by 16S rRNA gene sequencing, but several species capable of partial TCE dechlorination (*Desulfitobacterium* spp., *Sulfurospirillium* spp. and *Desulfuromonas* spp) were present in the biofilms of the columns treating raw landfill leachate. These data demonstrate that biochar from waste material may be capable of supporting a dechlorinating biofilm to promote bioremediation of TCE.

1. Introduction

Trichloroethylene (TCE; CAS 79-01-6) is a Group 1 human carcinogen (IARC, 2012). It has had multiple historical and current industrial uses, including as a degreaser, a dry cleaning agent and an extractant for spices and caffeine. As a result, significant volumes of TCE have historically been disposed of in hazardous and municipal landfills, resulting in TCE being detected in landfill leachate at a high frequency (70% of 104 landfills tested) (Kjeldsen and Christophersen, 2001) and concentration (up to 750 μg l^{-1}) (Kjeldsen et al., 2002). Unlined or damaged landfills may allow contaminated leachate to move through the soil to groundwater sources and increase the risk of human exposure (Xu et al., 2018).

Treatment of point source plumes has been facilitated using permeable reactive barrier (PRB) technologies. This involves the placement of a vertical layer of permeable material in the path of a contaminant plume, which treats the contamination as the plume passes through. Such technology has been used successfully for treatment of TCE point source plumes, where the reactive material employed was typically zero valent iron (ZVI) (Phillips et al., 2000). The oxidation of $Fe^{\circ} \rightarrow Fe^{2}$ releases two electrons, which mediates the degradation of TCE. This reaction also increases the pH, leading to the formation of mineral precipitates that cause mineral fouling of the barrier, reducing resident time and effectiveness (Li and Benson, 2005).

Therefore, there is a need to identify an alternative material that may be used in PRBs for the treatment of point source TCE-contaminated plumes. Activated carbon is chemically stable, with effective adsorbant properties such as a high surface area and different types of surface functional groups (Thiruvenkatachari et al., 2008), and a documented adsorptive capacity for TCE (Erto et al., 2010a, 2010b). Biochar pyrolysed from waste plant feedstock displays many of the same adsorbant characteristics as activated carbon – high surface area, surface functional groups, high pore volume and large micropore size. In addition to the environmental benefits of recycling such material, biochar production costs are about 20 % of that of activated carbon (Huggins et al., 2016). In batch scale studies, plant-derived biochars have been shown to be capable of TCE adsorption, with buffalo weed biochar removing 88 % of TCE (Ahmad et al., 2014), and peanut shell and soybean stover

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https://doi.org/10.1016/j.jhazmat.2020.123676
Received 30 May 2020; Received in revised form 25 July 2020; Accepted 7 August 2020
Available online 14 August 2020

biochars removing 50 % of TCE (Ahmad et al., 2012a), from an initial TCE solution concentration of 20 mg l^{-1}. Our own studies have demonstrated > 99.5 % TCE removal by spruce and oak biochars, and 93 % removal by biochar produced from herbal pomace, from a 20 mg l^{-1} initial TCE solution (Siggins et al., 2020).

To the best of our knowledge, only one study has employed a column experiment to investigate biochar for TCE adsorption capacity, with small columns (diameter 18 mm, length 98 mm) operating downflow at a rate of 9 ml min^{-1} (Zhang et al. 2015). The columns were filled with 5 g of a soybean stover-derived biochar, pyrolysed at 300 °C (BC300) or 700 °C (BC700), to treat a TCE solution at a concentration of 100 mg l^{-1} (Zhang et al., 2015). They reported maximum adsorption capacities of the biochar at 35.9 and 515.1 mg g^{-1} for BC300 and BC700, respectively (Zhang et al., 2015). Although this study demonstrated the capacity for TCE adsorption by biochar in a column experiment, it also reported that the biochar became rapidly saturated, with exhaustion of the columns (defined as when the effluent TCE concentration was equal to the influent TCE concentration) occurring at 10.5 (BC500) and 66.3 h (BC700), from the start of operation (Zhang et al., 2015). This observation seriously hinders the advancement of these technologies. One potential solution to overcome media saturation is bioregeneration, where a biofilm established on the adsorbent material may be capable of degrading the contaminant, thereby prolonging the operational lifespan of the system (Simpson, 2008). Aerobic biodegradation of TCE adsorbed to granular activated carbon (GAC) has been investigated (Nakano et al., 2000), and only partial degradation was found to occur. In addition, the regenerated GAC demonstrated reduced adsorption capacity, potentially due to the presence of bacterial metabolites (Nakano et al., 2000). Bioregeneraton of media used to adsorb TCE under anaerobic conditions, representative of an *in situ* groundwater treatment system, remains however, unexplored. Furthermore, the use of biochar as the adsorbant media, with its associated carbon sequestration benefits, is an attractive option for a sustainable environmental treatment technology. Therefore, the aim of our study was to determine if an anaerobic microbial community associated with environmental TCE contamination could mediate TCE dechlorination within a biochar fixed bed column system.

2. Materials and Methods

2.1. Biochar characterisation and preparation

Two types of biochar, produced from waste herbal pomace (HPB) and spruce (SB), were used in this study. These were previously characterised (Siggins et al., 2020), where they were referenced as “Herbal Pomace Biochar 1” and “Spruce Biochar 2”. Briefly, both biochars were produced using the PYREG process (www.pyreg.de) at a pyrolysis temperature of 600 °C and a residence time of 30 min.. Biochar was sieved to 0.5 – 2 mm. The porosity of the biochars was determined experimentally by volumetric analysis of triplicate samples to be 0.73 ± std. dev 0.02 (HPB) and 0.64 ± std. dev 0.03 (SB).

2.2. Design of packed columns

Twelve stainless steel columns, [600 mm (length) x60 mm (diameter)], were clamped vertically (Fig. 1). The influent was pumped at the base to maintain anaerobic conditions within the packed bed. A mesh screen and bed of 4 mm diameter glass beads (depth 30 mm) at the base of the column was used to disperse the influent and prevent preferential flow pathways from developing. The columns were packed with HPB (6 columns) or SB (6 columns) to a depth of 500 mm. Columns were dry packed slowly, with regular tapping and tamping every 100 mm to encourage even settling of the biochar within the column, in order to prevent channelling or air pockets. The effluent port was shielded with a 25 mm layer of 4 mm-diameter glass beads. This resulted in a column length:diameter of ca. 8, greater than the minimum recommended ratio

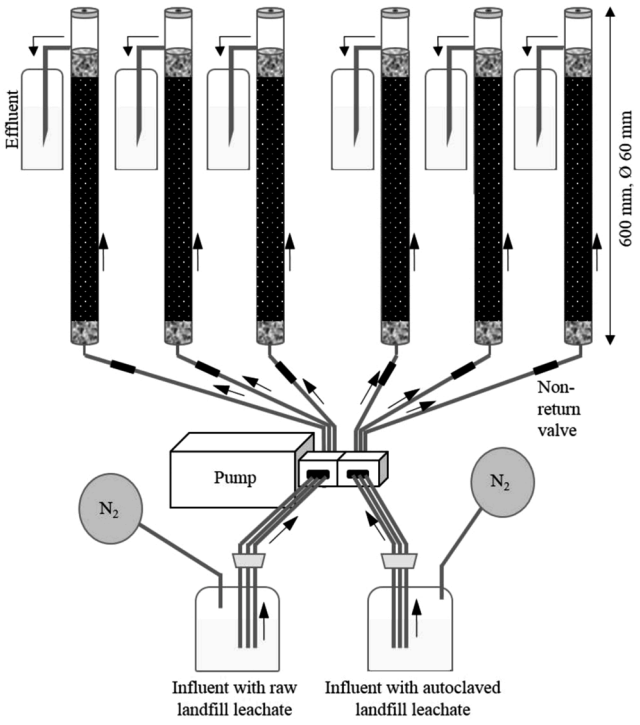

Fig. 1. Configuration of column experiment for one biochar (6 columns). An identical configuration was used for both biochars, resulting in a total of 12 columns. Each treatment was carried out in triplicate.

of 4, to ensure that the effective porosity is constant (Relyea, 1982). Filled columns were allowed to settle for 24 hours, then flushed for 10 pore volumes (PV) with bottled mineral water to remove fine particles and any compounds that may leach from the biochar. One PV was determined experimentally, by recording the volume of water required to fill triplicate columns packed with each biochar using the same packing procedure as for the experimental columns. Thus, the PV of the columns were determined to be between 780 and 800 ml, with 1 P V =800 ml used for subsequent influent flow calculations for all columns. Previous batch analyses showed that neither biochar leached detectable amounts of nitrate, but both demonstrated phosphate release, at 851 and 96 mg kg^{-1}, respectively (Siggins et al., 2020). Where possible, all construction materials for the column study were selected based on their compatibility with TCE to i) prevent corrosion or damage of the material and ii) to prevent sorption to any material other than the waste media being investigated. To this end, materials employed included Viton tubing, stainless steel columns, polyvinylidene fluoride (PVDF) connectors and influent storage, glass syringes, PTFE filters and septa.

2.3. Column influent

Two sets of influent were prepared for the column studies, each feeding triplicate units (Fig. 1). Both influents contained: landfill leachate (10 % v/v); TCE (approximately 35 mg l^{-1}); and sodium lactate as an electron donor (850 mg l^{-1}), which is over 10 times in excess of the stoichiometric electron equivalent required for complete TCE dechlorination. The TCE concentration of the influent was selected to be sufficient to challenge the column performance, but below the toxicity threshold of dechlorinating microorganisms of 1 mM (131.4 mg l^{-1}; Haest et al., 2010). The landfill leachate of one influent was autoclaved in order to assess the impact of the landfill leachate microbiome on TCE adsorption/degradation within the system. Influents were prepared using bottled mineral water (Comeragh Irish Water) throughout the study as due to technical issues, ultrapure water was unavailable. Landfill leachate was collected from the East Galway municipal landfill,

Ireland (53°19'11.4"N 8°25'56.3"W), and stored at 10 °C. The influent was prepared in glass bottles, fitted with a nitrogen filled Tedlar® gas bag to prevent TCE volatilisation and as anoxic storage of influent will facilitate anaerobic conditions within the column. TCE was allowed to solubilise for a minimum of 24 h prior to use (Ahmad et al., 2012b), and batches of influent were used within 72 h.

2.4. Operation of packed columns

Columns were operated at 10 °C, in triplicate, where each set of triplicates (1-3) was denoted by the biochar type (HPB/SB) and the treatment of autoclaved or raw (A/R) landfill leachate. This resulted in a nomenclature system for the columns of (HPB or SB)-(A or R)-(1 or 2 or 3).

At the commencement of the study, one PV of influent containing mineral water and autoclaved or raw landfill leachate were pumped into the columns to displace the mineral water, and acclimatised for 48 h to allow attachment of any microbes present to the biochar surface. Following this, influent was intermittently pumped to the columns, using a peristaltic pump, for a total of 13 PVs. Influent was pumped at an interstitial pore water velocity of 100 mm day^{-1}, which is at the higher end of groundwater migration rates in Irish soils (Healy et al., 2012), and achieved a hydraulic retention time of 5 days.

2.5. Sampling and analysis of influent and effluent

Column influent and effluent were sampled three times per week, aliquoted and prepared for analysis. Samples for volatile organic compound (VOC) analysis (TCE; 1,1 dichloroethylene (DCE); *cis*-1,2 DCE; *trans*-1,2 DCE) were filtered and stored in 22 ml amber glass vials following the dechlorination and preservation steps outlined in EPA Method 502.2 (Slater and Ho, 1995). Briefly, 2 mg sodium thiosulphate was added to the vial, the filtrate was added until overflow, acidified with 1 drop 1:1 HCl, and sealed with a PTFE-faced screw cap with no headspace. Vials were stored in the dark at 4 °C for a maximum of 14 days, before analysis by headspace GC-MS as per Siggins et al. (2020). The limit of quantification was 0.005 mg l^{-1}.

As biochar has been shown to be a suitable matrix for attachment and growth of microbial species (Cooney et al., 2016), it is likely that some of the microorganisms present in the landfill leachate may attach to the biochar within a PRB. Pathogens associated with this biofilm may therefore become dislodged sporadically and released. As such, the column influents and effluents were monitored weekly for any increase in *Escherichia coli*, an indicator organism for the presence of pathogens, and *Pseudomonas aeruginosa*, an opportunistic pathogen reported in landfill leachate (Grisey et al., 2010) that is capable of biofilm formation on biochar (Nabiul Afrooz and Boehm, 2016). *E. coli* were enumerated using the Colilert-18 test (IDEXX; Maine, USA) following the manufacturer's recommendations. *P. aeruginosa* was analysed by membrane filtration using a 0.45 µm mixed cellulose ester filter, which was placed on Pseudomonas Agar Base with CN Supplements and incubated at 36 ± 2 °C for 24 h.

2.6. Sampling and analysis of biochar

At the conclusion of the study, the columns were allowed to drain and the media were removed. Five x100 mm horizons were collected from each column, and labelled A-E, where A was the uppermost horizon and E was the lower horizon. Each biochar sample was thoroughly homogenised prior to subsampling for dry weight, VOC analysis and 16S rRNA sequencing analysis. For dry weight assessment, triplicate 1 g samples of wet biochar were dried at 105 °C overnight. For VOC analysis, the analyte was desorbed from the biochar following NIOSH protocol 1022 (Foley, 1994). Briefly, samples of biochar were gently washed with sterile Ultrapure water to remove un-adsorbed VOCs. One gram (wet weight) of biochar was mixed with 10 ml low benzene CS_2 containing 2 % v/v 2-phenoxyethanol, in a 22 ml amber vial. Vials were sealed with PTFE-coated caps and mixed on a shaker for 30 min.. The desorption solution was filtered through a 0.45 µm PTFE-hydrophilic syringe filter and analysed immediately by headspace GC-MS as per Siggins et al. (2020). A standard curve was prepared from a serial dilution of known standards of the target VOCs in CS_2 solution with 2% v/v phenoxyethanol. The extraction efficiency was determined following the method of (Foley, 1994). Briefly, known standards of VOCs in CS_2 solution with 2% v/v phenoxyethanol were added to clean biochar and incubated at room temperature overnight, before desorbing and filtering as described previously. The extraction efficiencies for TCE and the three DCE isomers were found to be within 1 % for each biochar, and were determined to be 96 % and 78% for HPB and SB, respectively. Statistical analysis of TCE and DCE values were determined using Data Analysis in MS Excel™.

Based on the detection of DCE in the lower horizon (E) of some columns, this horizon was selected for 16S rRNA analysis of the attached biofilm for all twelve columns. Samples were gently washed with sterile Ultrapure water to remove planktonic cells prior to freezing and storage at −20 °C. Samples were shipped on ice to RTL Genomics (Texas, US) for DNA extraction (using Qiagen PowerSoil kit), amplicon library preparation and sequencing. Amplicon library preparation was performed using the Parada et al. (2016) version of primers 515f/806 r originally developed by Caporaso et al. (2011) targeting the V4 16S rRNA region, followed by addition of sequencing adapters and dual-index barcodes, using a limited number of PCR cycles as per the Illumina protocol (Illumina, 2013). Paired-end sequencing was performed using the Illumina MiSeq platform with 2 × 300bp V3 kit chemistry, to a minimum depth of 10 K.

2.7. Sequence analysis

Processing of paired-end 16S rRNA reads was undertaken using Mothur software version 1.42.3 (Schloss et al., 2009) following the pipeline of Kozich et al. (2013). The SILVA database (v132) was used for alignment of sequences and sequences were classified against the Ribosomal Database (v.9). After quality filtering, samples contained an average of 25,200 reads and analyses were performed on a normalised dataset, subsampled to the lowest number of sequences (n = 10397). Alpha diversity metrics, namely Good's coverage, number of OTUs observed, Simpson's evenness and diversity, were calculated as implemented in Mothur, revealing a sample coverage (Good's coverage) above 98.4% for all samples. Differentially abundant taxa were determined using LEfSe analysis (Segata et al., 2011).

3. Results and Discussion

3.1. E. coli and P. aeruginosa analysis of column influent and effluent

Concentrations of *E. coli* in the prepared raw influents were approximately 10^2 MPN 100 ml^{-1} (Fig. 2). No increase was observed in the raw column effluent samples compared to the influent, indicating that growth of *E. coli* was not occurring within the system. In general, SB was capable of greater *E. coli* removal than HPB, with an average removal of 81 % [± standard (std.) error 2.3 (where std. error = std. deviation/$\sqrt{n}$, and n is the number of occurrences)] for SB compared to 66 % (± std error 3.5) for HPB. In addition, the removal of *E. coli* by SB was more consistent, ranging from 53 – 100 %, while removal by HPB ranged from 17 – 100 %. No *E. coli* were detected in the autoclaved influents or corresponding effluents.

Growth of *P. aeruginosa* on the membrane filter resulted in colonies that clumped together, even at low colony counts, making counting challenging and inaccurate. Results were therefore reported as not detected (-), low growth (+), moderate growth (++), or high growth (+++) (Table 1). *P. aeruginosa* was not detected in the autoclaved influent or corresponding effluents. It was, however, detected in the raw

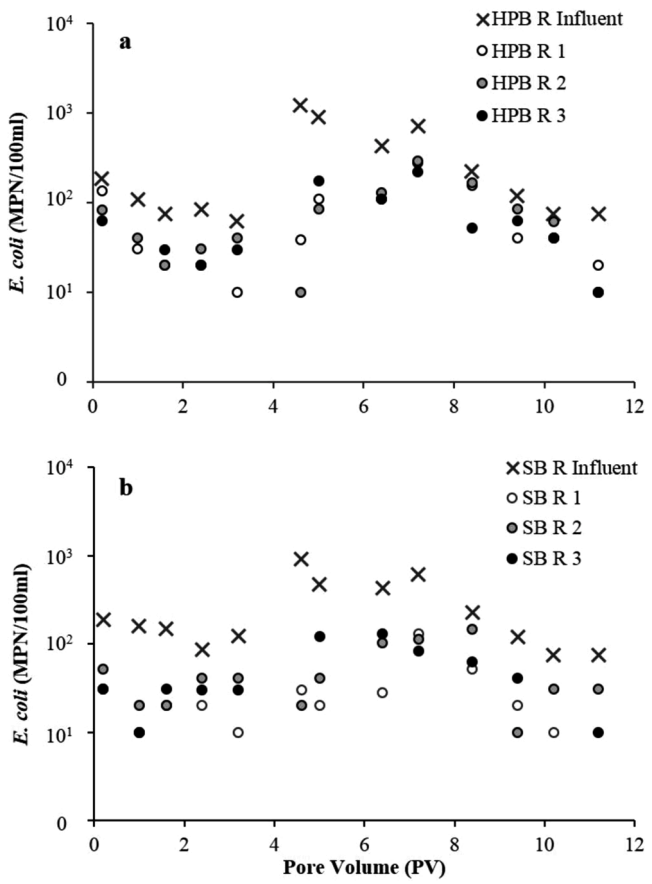

Fig. 2. Influent and effluent *E. coli* concentrations (MPN/100 ml), for the columns containing (a) Herbal pomace biochar (HPB) and (b) Spruce biochar (SB), with both sets treating influent containing raw (R) landfill leachate.

landfill leachate, in the raw influent, and in the majority of effluent samples from the columns treating raw influent (Table 1). In general, levels of *P. aeruginosa* were lower in the effluent samples than in the influent (Table 1), indicating that the use of biochar in such an application is unlikely to result in significant increases in this pathogen downstream of a PRB.

Biochar selected for use in a PRB must not cause a "pollution swapping" effect, whereby other contaminants are released into the environment downstream of the PRB. Our previous studies demonstrated that both biochars, HPB in particular, have the potential to release phosphate but not nitrate (Siggins et al., 2020). In addition to inorganic compounds, there is a concern that biochar may facilitate the growth of pathogenic organisms arising from the contaminant plume, although the data presented here indicated that this was not occurring for *P. aeruginosa* or *E. coli*. Nonetheless, landfill leachate is a complex matrix for analysis of microbial diversity, and advances in non-culture methodologies have shown that it can contain a number of indigenous microorganisms, particularly bacteria including the phyla Firmicutes, Bacteroidetes, and Proteobacteria, as well as Archaea and Fungi (Sekhohola-Dlamini and Tekere, 2019). As such, any potential growth and release of microorganisms from such a system should continue to be considered in future investigations.

Table 1
Presence of *Pseudomonas aeruginosa* from influent and effluent of replicated columns treating influent containing raw landfill leachate, where HPB is Herbal Pomace Biochar and SB is Spruce Biochar. "-"denotes no *P. aeruginosa* detected, and "+", "++" or "+++" denote density of growth on plates. No *P. aeruginosa* were detected in the influent or effluent for the columns treating autoclaved landfill leachate.

Pore	HPB R				SB R			
Volume	Influent	1	2	3	Influent	1	2	3
0	++	-	-	-	++	-	-	-
0.2	+	-	-	-	+	-	-	+
1	+	+	-	++	+	++	+	++
2.4	++	++	+	+	+++	-	-	+
3.2	++	+	+	+	++	-	+	-
4.6	+++	++	++	++	+++	-	+	+
5	++	++	++	++	++	++	++	++
6.4	+++	++	++	++	+++	++	++	++
7.2	+++	++	-	+	+++	+	+++	++
8.4	++	+	++	+	+	+	+	+
9.4	+	+	+	+	+	+	+	+
10.2	+	+	+	+	+	+	+	+
11.2	+	++	++	++	+	+	+	+

3.2. TCE and DCE concentrations in the column influent and effluent

The TCE concentrations of the influent were variable due to solubility effects of TCE. Statistically, this variability did not result in significant differences ($p > 0.05$) in the TCE concentration introduced to each column, therefore this was not considered to have impacted the experimental design. Influent concentrations at each sampling point are given in Supplementary Fig. 1.

The effluents of two columns, HPB-A-1 and SB-R-1, were consistently below the limit of quantification for TCE (0.005 mg l^{-1}; Fig. 3). TCE was sporadically detected from the effluents of the remaining ten columns, although concentrations were typically below 0.02 mg l^{-1} (Fig. 3), which is the WHO guideline limit for TCE in drinking water (WHO, 2005).

The highest concentration of TCE (0.36 mg l^{-1}) was detected in the effluent of SB-A-3, at 7.6 PV (Fig. 3c). At 8.7 PV, the TCE concentration of the effluent of SB-R-2 was 0.118 mg l^{-1} (Fig. 3d). These two occurrences still represented 99.0 and 99.6 % TCE removal, respectively, by the packed columns.

With the exception of these, all other observations of TCE in the effluents of the columns were below 0.1 mg l^{-1} (Fig. 3), which corresponded to 99.7 % TCE removal. None of the three DCE isomers were detected in the effluents of the twelve columns. DCE is produced as the first step in the biological reductive dechlorination pathway under anaerobic conditions (Freedman and Gossett, 1989). The absence of DCE in the effluent could be indicative of four potential scenarios: 1) TCE was not degraded, but was solely being removed by adsorption to the biochar medium; 2) TCE was undergoing dechlorination to DCE, but the resulting DCE(s) were also being adsorbed by the biochar within the system; 3) TCE was undergoing further partial or complete dechlorination to vinyl chloride or ethylene; 4) TCE was undergoing chemically driven double beta elimination to chloroacetylene or acetylene due to the presence of iron in the biochar. The biochars both contained iron, at concentrations of 1.8 and 3.6 g kg^{-1}, for HPB and SB, respectively. As such, we cannot conclusively rule out double beta elimination as a route for TCE degradation. However, the focus of our study was not to carry out a full mass balance for TCE, but to investigate if *biological* TCE degradation (i.e. reductive dechlorination via DCE intermediates) could be driven by the microbial biofilm within such a system. Simultaneous reductive dechlorination and beta elimination have been shown to occur in an iron based system (Campbell et al., 1997; Su and Puls, 1999), thereby it is unlikely that beta elimination would inhibit reductive dechlorination from occurring within our system. Therefore, analysis of the degradation products of beta elimination were not deemed to be relevant to this hypothesis, but may be of interest in future experimental designs. Within the remaining options, option three was unlikely as the dechlorination of TCE has been regularly observed to stall at the DCE → vinyl chloride or vinyl chloride → ethene steps of the sequential process in environmental ecosystems (Pant and Pant, 2010; Shukla et al., 2014). Incomplete dechlorination is an undesired outcome, as these intermediate products are also carcinogenic, therefore significant work must still be completed to ensure total dechlorination is occurring before this

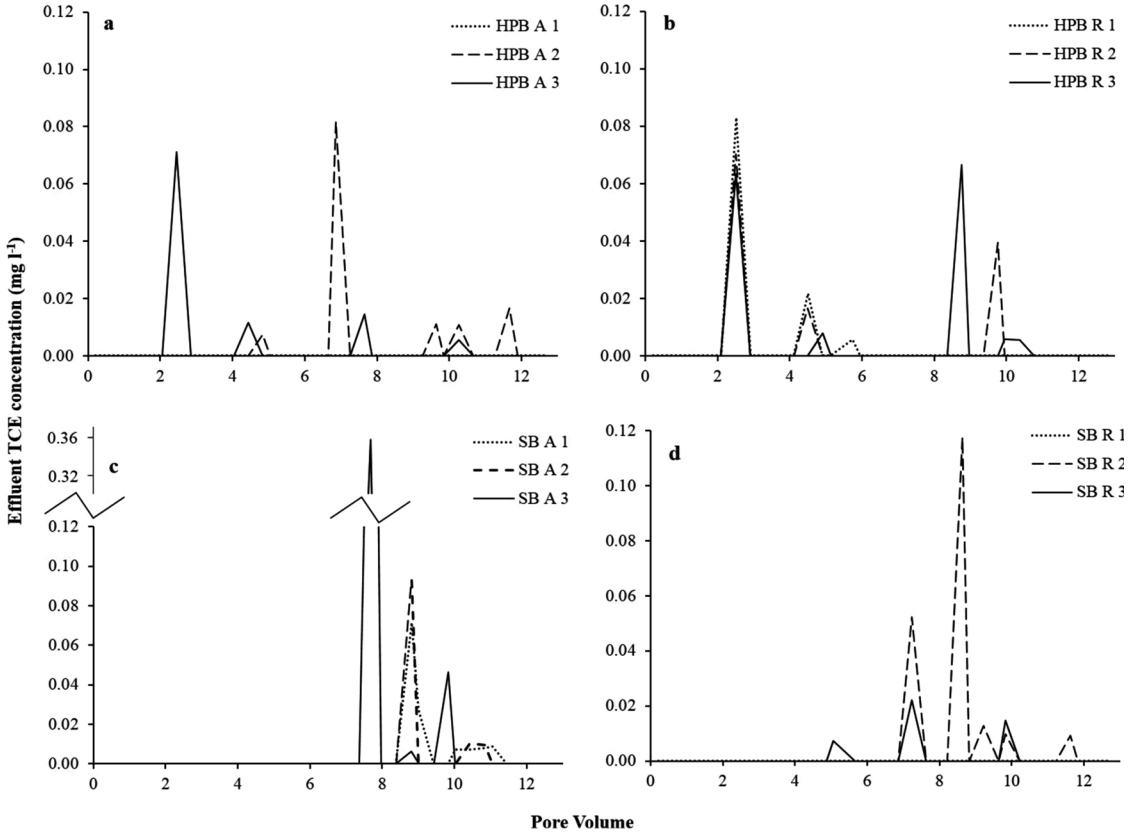

Fig. 3. Effluent TCE concentrations (mg l^{-1}) from the replicated (n = 3) columns over the course of the study, where a) is HPB A - Herbal Pomace Biochar treating autoclaved landfill leachate; b) is HPB R - Herbal Pomace Biochar treating raw landfill leachate; c) is SB A - Spruce Biochar treating autoclaved landfill leachate; d) is SB R - Spruce Biochar treating raw landfill leachate.

technology could proceed to field scale. A number of factors may be responsible for incomplete dechlorination, including the absence of specific microbes required to mediate the electron transfer or competition for electron donor compounds. Only *Dehalococcoides ethenogenes* (later redesignated *D. mccartyi*) has been reported to independently facilitate the complete dechlorination of TCE to ethene (Maymó-Gatell et al., 1997). Although a range of dehalorespiring microorganisms, including *Geobacter* sp. and *Desulfovibrio* sp., are capable of carrying out stages of the reductive dechlorination process (Loffler et al., 2003), a strong correlation between the presence of *Dehalococcoides* sp. and complete dechlorination of TCE in contaminated soil and groundwater has been reported (Hendrickson et al., 2002). The presence of organisms associated with other electron-requiring processes may also affect the reductive dechlorination of TCE, and competition for electrons by methanogens and homoacetogens have been reported in anaerobic environments (Siggins et al., 2011; Yang and Mccarty, 2000). The inclusion of excess sodium lactate in the influent should mitigate the effect of competition for electron donors, meaning that the capacity of the systems to degrade TCE is largely dependent on the presence of dechlorinating species within the landfill leachate component of the influent. The landfill leachate was tested for the presence of TCE and DCEs, and while no TCE was observed, *cis*-1,2 DCE was detected at 0.0052 mg l^{-1}. Environmental pollution by *cis*-1,2 DCE in Ireland is typically secondary, i.e. from degradation of TCE, rather than primary i.e. through its direct release (EPA, 2005). Therefore, the *cis*-1,2 DCE detected likely originated from dechlorination of TCE, indicating that some species capable of at least partial reductive dechlorination of TCE were present in the landfill leachate, and may be capable of colonising the biochar in the packed columns. Bioengineering of the microbial community, in order to ensure the presence of microorganisms capable of complete dechlorination of TCE, may be required for further advancement of this technology beyond this "proof of concept" study. Furthermore, the addition of sodium lactate as an electron source would be problematic in a field scale system. However, this study aimed to optimise conditions in order to determine if biological dechlorination by a biochar based biofilm was feasible. Further studies will be required in order to determine if the landfill leachate would be independently capable of acting as a sufficient source of electrons for TCE reductive dechlorination.

3.3. TCE and DCE concentrations adsorbed to the biochar

As the maximum capacity for biochar to adsorb TCE has been well reported (Ahmad et al., 2014, 2013; Siggins et al., 2020), destructive sampling of the twelve columns was carried out while the columns were efficiently removing TCE, in order to characterise the progression of adsorption and/or dechlorination of TCE through the packed bed. TCE was detected throughout the lower 200 mm of all twelve columns, at concentrations ranging from 6 – 1786 mg kg^{-1} dry weight of biochar (horizons D & E, Table 2). The migration of TCE throughout the column was observed for all treatments, with one of each replicate column containing TCE in the upper 400-500 mm horizon of biochar (A, Table 2). Statistical analysis by two factor Anova with replication showed that no significant difference ($p > 0.05$) in TCE adsorbed to the total biochar in the columns (including all horizons A-E) was observed between different landfill leachate types (autoclaved versus raw), either when data from the two biochars were considered separately (Table 3) or collectively. However, similar comparison of the two biochar types showed that SB significantly ($p < 0.05$) outperformed HPB in terms of total TCE adsorption, but only when the data from the autoclaved and raw columns were considered collectively. This is generally in agreement with batch test analysis of these materials, where, at an initial TCE concentration of 200 mg l^{-1}, SB and HPB demonstrated 88 % and 70 %

Table 2
Concentrations of TCE and *cis 1,2*-DCE adsorbed to the biochar on takedown of the columns, where HPB is Herbal Pomace Biochar, SB is Spruce Biochar, A is influent containing autoclaved landfill leachate and R is influent containing raw landfill leachate. Columns were analysed in 100 mm segments, labelled A-E with A being the upper horizon (near the outlet) and E the lower horizon (near the inlet). No *trans*-1,2 DCE or 1,1 DCE were detected in any samples. Values are expressed in mg kg^{-1} biochar (dry weight).

Horizon		HPB A			HPB R			SB A			SB R		
		1	2	3	1	2	3	1	2	3	1	2	3
TCE (mg kg^{-1})	**A**	2			3			6			3		
	B				5		2	20		4		13	11
	C		9		8	7	16		3	139	35	639	21
	D	6	60	25	118	98	109	101	257	216	378	957	487
	E	189	158	256	427	447	438	1786	1768	1236	200	559	264
***cis*-1,2 DCE (mg kg^{-1})**	**A**												
	B												
	C											50	56
	D										35	68	59
	E				13	19	11				28	20	27

Table 3
p values for TCE adsorbed to biochars, determined by single Anova analysis of individual column horizons (A, B, C, D, E), and two-factor Anova with replication for column segments analysed collectively (A-E). Statistically significant values ($p < 0.05$) are highlighted in grey.

	Horizon	HPB A	SB R
HPB R	**A**	0.952	0.963
	B	0.207	0.271
	C	0.145	0.338
	D	0.010	0.049
	E	0.001	0.434
	A-E	0.239	0.168
SB A	**A**	0.630	0.637
	B	0.271	0.998
	C	0.386	0.427
	D	0.310	0.086
	E	0.002	0.004
	A-E	0.091	0.509

TCE removal, respectively (Siggins et al., 2020). Comparison of the individual horizons (A-E) revealed that significant differences in TCE adsorption were primarily observed in the bottom 200 mm (horizons D and E) of the biochar bed. In the lowest horizon, E (0-100 mm), when comparing either treatment or biochar (Table 3), significant differences were observed in almost all cases. For that horizon, the average quantity of TCE adsorption per gram of biochar was typically in the order SB-A>HPB-R > SB-R>HPB-A (Table 2).

Analysis of the biochars showed that, while *trans*-1,2 DCE and 1,1 DCE were not detected in any samples, *cis*-1,2 DCE was present in the lower horizon (E; 0-100 mm) of all columns treating influent containing raw landfill leachate (Table 2). For HPB-filled columns, this was the only horizon where *cis*-1,2 DCE was detected, but for SB filled columns, *cis*-1,2 DCE was also detected in all columns in horizon D (100-200 mm) and in two columns in horizon C (200-300 mm; Table 2). This suggests that for columns treating influent with raw landfill leachate, TCE removal was occurring by a combination of adsorption and dechlorination, with subsequent adsorption of the dechlorination product. Statistical comparison of horizon E only (Single Factor Anova) or horizons C-E collectively (Two Factor Anova with Replication), shows that in all columns treating raw landfill leachate, the amount of *cis*-1,2 DCE adsorbed to biochar was significantly higher in the SB-filled columns than HPB ($p < 0.05$). This supports the trend observed previously, where SB has a greater adsorption capacity for TCE than HPB (Siggins et al., 2020). To the best of our knowledge, no studies have investigated the capacity for biochar to adsorb *cis*-1,2 DCE, with a small number of studies reporting adsorption by compounds such as raw mulches (Wei and Seo, 2010) and tyres (Lu et al., 2017). In those studies, the removal of TCE and *cis*-1,2 DCE from aqueous solutions was comparable at the higher adsorbent levels investigated. Another study investigated the dechlorination of TCE in microcosms containing either soil and sawdust or soil and peat (Mondal et al., 2016). That study found that complete dechlorination to ethene occurred in the peat/soil microcosms, but in the sawdust/soil microcosms dechlorination stalled at *cis*-1,2 DCE (Mondal et al., 2016). Peat has previously been reported to be an efficient electron donor for redox reactions (Kao et al., 2001), while wood-based compounds, including biochar, require pre-modification such as chemical reduction to be capable of facilitating complete dechlorination to ethene (Saquing et al., 2016). Supplementary sodium lactate was included in our influent, so electron donor limitations were unlikely to occur, with a lack of capable microbial species more likely to be the rate limiting factor. Nonetheless, it appears that TCE did not undergo complete dechlorination to ethene; instead, this process likely stalled at *cis*-1,2 DCE, which was then itself adsorbed to the biochar. This is supported by the absence of detectable levels of *cis*-1,2 DCE in the effluent streams from all columns. If this is the case, our data indicate that a greater level of dechlorination is occurring in the SB columns (Table 2). This is reflected not only in the quantity of *cis*-1,2 DCE adsorbed to the biochar, but also in the distance *cis*-1,2 DCE has migrated through the columns (Table 2). The differences observed in TCE adsorption between the two biochars treating raw influent may have been associated with difference in the colonisation of the biochar within the column, as well as different sorption processes (Siggins et al., 2020). This dechlorination was likely driven by the microbial community originating from the landfill leachate, as no DCE isomers, including *cis*-1,2 DCE, were present in any of the biochar samples from the six columns treating influent containing autoclaved landfill leachate (Table 2). For these columns, the absence of DCE in the biochar and effluent supports the rejection of the second potential scenario put forward in section 3.2: "*TCE was undergoing dechlorination to DCE, but the resulting DCE(s) were also being adsorbed by the biochar within the system*". Consequently, for autoclaved landfill leachate, the most likely fate of TCE is removal solely by adsorption to the biochar, without degradation occurring, due to the lack of dechlorinating species present in the system.

3.4. Microbial community structure of the biofilm colonising the biochar

The twelve biochar samples from horizon E of the columns all contained complex microbial communities, with a limited number of dominant species, as observed by low Simpsons evenness (Fig. 4a). This trend is common for environmental samples, and has previously been reported for environmental communities exposed to chlorinated compounds (Kotik et al., 2013). The biofilms from the six columns treating autoclaved landfill leachate likely originated from other materials within the system, such as the biochar, column, tubing or water. The aim of the study was not to ensure that these columns were entirely sterile, rather to assess the specific contribution of the landfill leachate microbiome to biochar colonisation and biological TCE degradation. Yield of

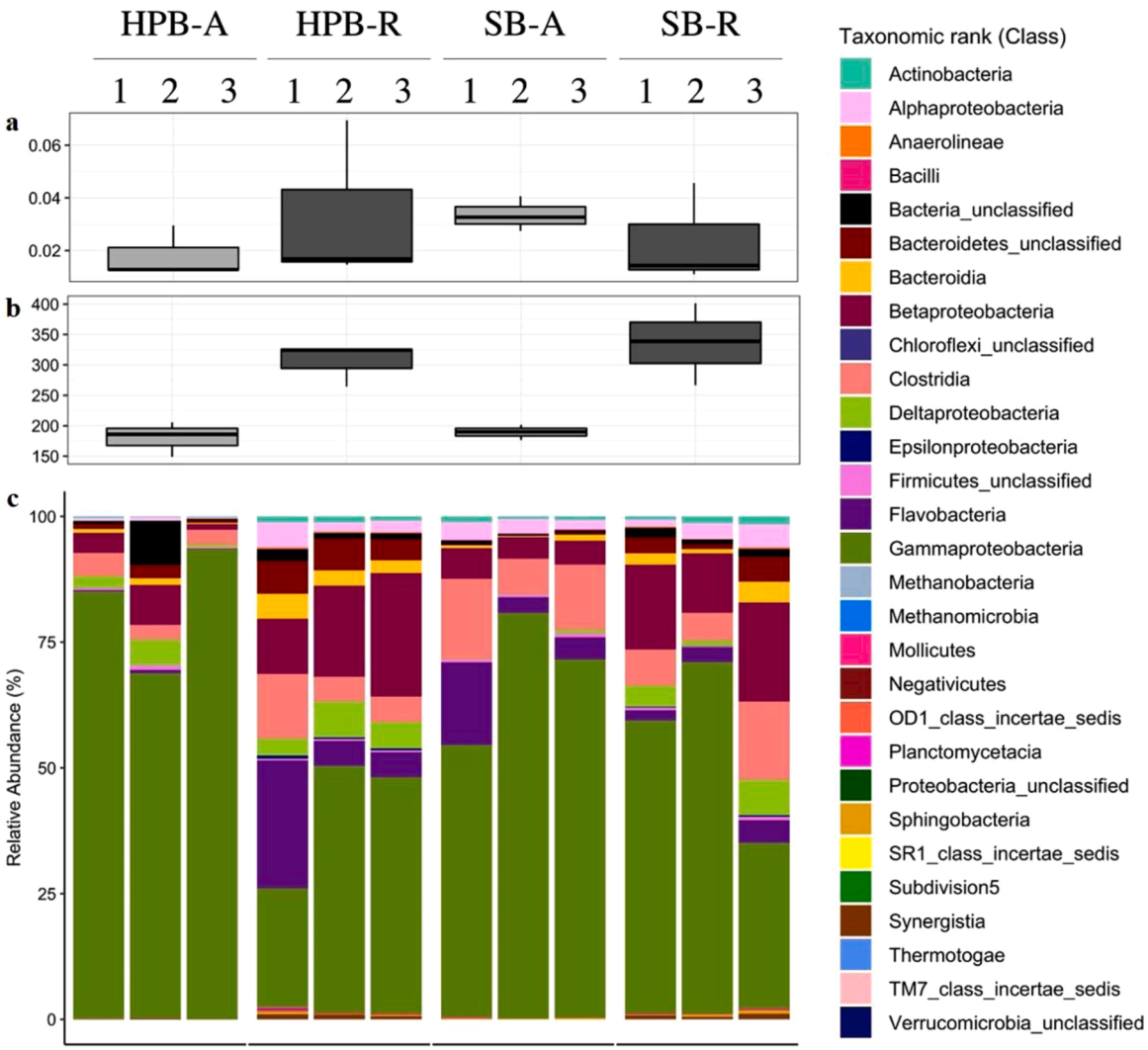

Fig. 4. Microbial community analysis of the biochar samples taken from horizon E (0-100 mm) of the twelve columns, where HPB is Herbal Pomace Biochar, SB is Spruce Biochar, A is autoclaved landfill leachate, R is raw landfill leachate. All column were operated in triplicate (1-3). (a) is Simpsons evenness (b) is the observed OTU richness and (c) is the community composition at class level.

DNA across all samples was in the range of 1.6 – 6.8 μg per gram dry weight biochar (Table 4). One-way Anova of the data showed that there were no significant differences in DNA yield as a function of biochar type, or as a function of influent type for HPB ($p > 0.05$). However, the DNA yield from SB treating autoclaved influent was significantly higher than that from SB treating raw influent ($p < 0.05$), indicating that the biochar biofilm originated from multiple sources within the treatment system, and not just the landfill leachate. Nonetheless, species within the microbial community originating from the landfill leachate were crucial for TCE degradation, which was not observed in the columns treating autoclaved landfill leachate, regardless of the quantity of biomass within the biofilm. The OTU richness was higher in the columns treating raw landfill leachate, regardless of biochar type (Fig. 4b), with the increased richness potentially resulting in the presence of species associated with TCE degradation that were absent in the lower diversity systems treating autoclaved leachate.

Table 4
DNA yield extracted from biochar samples, expressed as μg DNA/g sample (dry weight)

	HPB-A	HPB-R	SB-A	SB-R
1	2.89	4.75	5.19	1.64
2	1.71	2.43	6.75	3.15
3	5.78	3.38	4.15	2.33

The microbial community colonising the biochar in all twelve columns was dominated by Gammaproteobacteria, accounting for 22-94 % of all operational taxonomic units (OTUs) (Fig. 4b). Members of the class Gammaproteobacteria, particularly *Pseudomonas* spp. and *Acinetobacter* spp., have been identified as dominant organisms within a microbial community exposed to TCE (Futamata et al., 2005). For ten of the twelve columns, *Acinetobacter* was the dominant genus within the Gammaproteobacteria, with *Pseudomonas spp.* predominant in SB-A-3, and unknown members of the Bacteroidetes phylum most prevalent in HPB-R-1 (Fig. 4b). These organisms have been reported to be capable of co-metabolism of TCE in co-operation with certain members of the classes Alphaproteobacteria and Betaproteobacteria, including *Acidovorax* spp. (class Betaproteobacteria) (Adetutu et al., 2015). *Acidovorax* spp. were detected in the majority of the biochar samples ($n = 11$), and represented a higher proportion of the community colonising the biochar treating raw landfill leachate than the biochar treating autoclaved leachate ($p < 0.05$). The molecular detection of *Pseudomonas spp.* was supported by the culture based detection of *P. aeruginosa* discussed in section 3.1.

Individual species that have been reported to be capable of complete dechlorination of TCE to ethene under anaerobic conditions, including *Dehalococcoides spp.* (Maymó-Gatell et al., 1997) and *Propionibacterium*

spp. (Chang et al., 2011), were not detected in any of the twelve biochar samples (Fig. 4b). As such, reductive dechlorination of TCE within the columns would likely require the sequential and co-operative action of a number of microbial species that are capable of reducing different compounds along the degradation pathway. Under environmental conditions, without the provision of supplementary electron donors and in the absence of *Dehalococcoides* spp., complete dechlorination is unlikely, with the process typically stalling at vinyl chloride (Smits et al., 2004). Several microorganisms, including *Desulfitobacterium* spp. (from the class Clostridia), *Sulfurospirillum* spp. (class Epsilonproteobacteria), *Desulfuromonas* spp. and *Geobacter* spp. (both class Deltaproteobacteria), have been reported to be capable of dechlorination of TCE to DCE (Buttet et al., 2013; Duhamel and Edwards, 2007; Loffler et al., 2000; Smits et al., 2004), while *Dehalobacter* spp. (class Clostridia) can progress one step further to vinyl chloride (Grostern and Edwards, 2006). In our study, *Desulfitobacterium* spp., *Sulfurospirillium* spp. and *Desulfuromonas* spp. were not associated with any of the biochar samples taken from columns treating autoclaved landfill leachate, but were associated with the biochar from columns treating raw leachate, indicating that the landfill leachate was the source of these species (Fig. 4b). *Geobacter* spp. followed a similar trend, present in all biofilms treating raw leachate, but was observed at a low relative abundance (< 0.02 %) in one of the HPB biofilms treating autoclaved leachate (Fig. 4b). Interestingly, while *Sulfurospirillium* spp. and *Desulfuromonas* spp. were detected in the biofilms of both biochars treating raw landfill leachate, *Desulfitobacterium* spp. were only present in the biofilm colonising the SB, not HPB (Fig. 4b).

4. Conclusion

In this study, the mechanism of TCE removal from landfill leachate-contaminated groundwater by a biochar supporting biofilm growth was investigated. The relationship between adsorption and dechlorination was of particular interest in order to determine if biochar could support a biofilm that would degrade TCE, thus theoretically increasing the lifespan of an *in situ* bioremediation technology, such as a permeable reactive barrier. Our main findings are as follows:

- All columns were capable of $\geq$ 99.7 % TCE removal.
- No degradation derivatives were detected in the column effluents, but *cis*-1,2 DCE was detected adsorbed to biochar in columns treating raw landfill leachate, indicating that at least partial microbial degradation of TCE was occurring.
- The biofilms attached to the biochar in these columns contained microorganisms that have been reported to be capable of partial dechlorination of TCE to DCE, including *Desulfitobacterium* spp., *Sulfurospirillium* spp. and *Desulfuromonas* spp.

Overall, the use of such pyrolysed waste materials for use in a PRB treating TCE contamination is promising. However, to encourage full dechlorination to ethene, rather than production of more toxic intermediates such as DCE or vinyl chloride, further studies should investigate if the system performance would benefit from incorporation of known dechlorinating species into the biochar biofilm.

Credit Author Statement

Dr Alma Siggins is the MSCA-IF Fellow on this project. She co-designed the project, wrote the grant proposal, implemented the experimental work, carried out the sample collection and GC-MS analysis, wrote the initial draft of the manuscript and is the corresponding author.

Dr Camilla Thorn provided input to the 16S rRNA sequencing analysis, including carrying out the statistical analysis of the 16S rRNA data, generating the associated figure and contributing to the sequencing discussion section of the manuscript.

Professor Mark Healy hosted the MSCA-IF project and contributed significantly to the project design and manuscript preparation. He was particularly associated with engineering aspects of the study.

Dr Florence Abram was a project advisor. She contributed significantly to the project design and manuscript preparation. She was particularly associated with the Microbiological aspects of the study.

Declaration of Competing Interest

The authors report no declarations of interest.

Acknowledgements

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie Actions grant agreement No 748106. The authors would like to acknowledge assistance with column installation from Edward Kilcullen, NUI Galway; biochar contributions from Caspar von Ziegner, NovoCarbo, Germany and Timo Ferdinand, HerbaCarbo, Germany; and supply of landfill leachate from Barry Cormican, East Galway Landfill.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jhazmat.2020.123676.

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