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Assessing the Potential of Flow Bioreactors to Minimise Environmental Impacts of Landfill Leachate

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Abstract: A novel flow bioreactor design was used in this study to minimise the toxic effect of leachate obtained from a landfill site. The bioreactor used comprised sand inoculated with microbial hydrocarbon degraders to degrade the various contaminants in the landfill leachate. Prior to bioreactor testing, optimal leachate test concentrations were identified as 10 and 20% by toxicity screening with a *lux* bacterial biosensor. The bioreactor performance was assessed by carrying out different types of physical, chemical and biological measurements. In conclusion, the bioreactor designed in this study used was effective in treating the leachate in terms of hydrocarbon degradation. Conductivity and pH showed to have no significant effect on the bioreactor performance. This may indicate that the effect of some environmental factors might be site dependent.

Key words: Landfill leachate, human health, bioreactor, biodegradation, biosensor, toxicity

INTRODUCTION

Landfilling of waste has become a key strategy used to minimise the impact of both domestic and industrial wastes on human and environmental health. Consequently, different legislations have been adapted to regulate the landfill of waste. The main issue with respect to containment of waste in the disposal facility takes account of the quantity of waste material in leachate which will be released over a given period of time and the environmental impact from that discharge (Daniel, 1993).

Leachate from a landfill comprises water which is contaminated by wastes when it passes through the waste material. The composition of leachate is influenced by several factors such as, the type of waste, the local climate and the manner in which the facility is operated (Daniel, 1993; Kjeldsen *et al*., 2002). Leachate usually comprises a wide range of organic and inorganic constitutes (Hjelmar *et al*., 2000). The major potential environmental impacts relating to landfill leachate are contamination of surface and ground waters. The potential impacts of a leachate release to surface waters are expected to be oxygen depletion, changes in the stream bed fauna and flora and ammonia toxicity. In contrast, groundwater contamination probably represents the most severe environmental impact from landfill leachate. This is because that most old landfills were built without engineered liners and leachate collection systems (Kjeldsen *et al*., 2002). Therefore, many countries have required the installation of liners and leachate collection systems as well as developing a strategy to treat leachate (Hjelmar *et al*., 2000).

There are various *in situ* and *ex situ* remediation methods available for the treatment of contaminated landfill leachate. Some of these methods can be employed as a single option of treatment, whereas others can be combined together. The main methods employed for remediation are: bioreactors, biofilter, resin treatment, air stripping, pump and treat, barriers, biobarriers and biological technologies (Alexander, 1999).

Bioreactor technology is a closed system in which optimal conditions, for microbial growth and activity, are provided in order to accelerate the rate of hydrocarbon biodegradation (Daugulis, 1997). Bioreactors have been employed for the treatment of contaminated landfill leachate, liquids (e.g., pumped groundwater), solid materials and vapours such as factory air (Mueller *et al*., 1996; Vidali, 2001). Microbial degraders used in bioreactors may be derived either from a contaminant source or from an inoculum of organisms specific to a target contaminant (Alexander, 1999). In addition, microbial degraders from activated sludge culture can be

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used as an inoculum to landfill leachate (Kargi and Pamukoglu, 2003). Furthermore, nutrients are often added to the bioreactors to support the growth of microorganisms (Alamri, 2006).

The main advantages of bioreactors can be the rapid degradation rate of contaminants compared to other bioremediation techniques, lower waste toxicity and degradation of most hydrocarbon forms (Thassitou and Arvanitoyannis, 2001). However, the cost of these methods is relatively high and thus, implementation of this technology is currently limited (Mueller *et al*., 1996). The aim of this investigation was to assess the potential of a novel flow bioreactor design, utilising inoculated microbes as hydrocarbon degraders on a sand filter, for remediating landfill leachate.

MATERIALS AND METHODS

Leachate source: The landfill leachate used in this study was obtained from Peterhead landfill site, Aberdeen city, UK, in May 2007. The leachate contained a wide range of industrial and domestic wastes. The leachate was diluted in deionized water to produce a range of dilutions from 100% down to 0.1%. In order to assess the toxicity associated with particulate and non-particulate fractions in the samples, the leachate was filtered through a 0.45 μm plastic filter. Chemical and toxicological characterisations of the leachate (including filtered and non-filtered samples) were achieved by carrying out chemical analysis and toxicity measurements.

Chemical analysis: Assessment of salinity before and after remediation experiments was conducted by taking 10 mL from each sample, in triplicate and then the conductivity was measured using a HANNA instruments conductivity meter. Furthermore, the hydrogen ion concentration in each sample before and after the remediation process was measured using a JENWAY 3020 pH meter.

Toxicity measurement: The toxicity of the leachate samples was determined using a non-specific, *lux*-based bacterial biosensor *E. coli* HB101 pUCD607 (Rattray *et al*., 1990). The bacterial biosensor used was obtained from freeze-dried cultures which had been previously prepared using laboratory protocols described by Sousa *et al.* (1998). The freeze-dried cells were resuscitated in 10 mL of 0.1 M sterile KCl and incubated for 1 h at 25°C in an orbital shaking incubator at 200 rpm. Following incubation, 0.9 mL of each sample, in triplicate, was mixed with 0.1 mL cell suspension in luminometer cuvettes at 15 sec intervals between each sample. After 15 min exposure to the sample, light output was measured using a JADE luminometer (Labtech Instrumental, UK). The results were calculated as percentage luminescence relative to deionised water as control. Weekly toxicity tests (with a gradient dilutions starting from 0 to 100%) were carried out in order to monitor the presence of volatile compounds in the leachate samples throughout the study and to ensure a consistent toxicity controls pre and post the remediation process.

Assessment of microbial growth: The inoculated sand used in this study for remediating the leachates was supplied by Remedios Ltd. Assessment of microbial growth was carried out as follows.

Quantification of microbial biomass carbon: Determination of soil microbial biomass was modified from that described by Joergensen (1995). Chloroform fumigation of hydrocarbon contaminated soil samples was carried out in order to assess the quantity of carbon (C) assimilated as biomass. Twenty five gram inoculated sand and builder's sand was weighed out for each sample and was then transferred into a 100 mL conical flask. Six replicates were weighed out (3 fumigated and 3 not fumigated). Tissue paper was moistened with deionised water and placed in the bottom of the desiccator with the 100 mL conical flasks containing the samples to be fumigated and an empty 100 mL conical flask (considered as a blank).

One hundred milliliter conical flask containing approximately 50 mL acid-washed chloroform was added to the desiccator, which was evacuated until the chloroform had started to boil. Evacuation was continued for a further 2 min before the desiccator was detached from the pump. After 24 h of chloroform fumigation, the desiccator was evacuated again until the chloroform became undetectable by smell. The fumigated samples were then removed and transferred to glass universal bottles and 30 mL of 0.5 M potassium sulphate was added. The samples were then placed on a rotary shaker for 1 h. Soil extracts were then filtered using Whatman 1 filter paper. The soil extracts obtained were used to calculate microbial biomass-C, using an aqueous carbon analyser (LABTOC, Pollution and Process Monitoring) equipped with UV digestion and an infra red detector. The difference between the C values obtained from the non-fumigated and fumigated sand was used to determine the total microbial biomass. The calculation of soil microbial biomass-C was adapted from that described by Vance *et al.* (1987), where the K_{EC} factor used was 0.45 for the sands.

Determination of microbial number: Enumeration of microbial population including heterotrophic microorganisms and hydrocarbon were carried out using Luria Bertani and Bushnell Hass Media.

Enumeration of total microbial heterotrophs, bacterial and fungi using Luria Bertani broth (LB) medium: The plate count method for determination of microbial heterotrophs was modified from that described by Jørgensen *et al.* (2000). Enumeration of heterotrophic microbes was carried out by dissolving 20 g of LB in 1 L deionised water. Fifteen gram of bacteriological agar was added and then the mixture autoclaved for 15 min at 121°C. Ten millilitre of quarter strength Ringer's solution was added to 1 g dry weight samples in Wheaton vials and the samples were vortexed for 30 sec. The samples were sonicated for 1 min and then left to settle for about 2 min. Culturable cell counts were carried out by serially diluting a one hundred microlitre of the cell suspension in quarter strength Ringer's solution. Ten microlitre aliquots of the appropriate dilution were pipetted, in triplicate, onto drop plates and allowed to dry thoroughly. The plates prepared were incubated for 48 h and the number of colony forming units (CFU's) per 10 μL aliquot was counted and the number of colony forming units per gram oven dry weight of the initial cell suspension was calculated. In order to enumerate the total number of bacteria in the sample, 50 mg L^{-1} sterile filtered cycloheximide antibiotic was added to the LB media. In other LB media, 50 mg L^{-1} sterile filtered streptomycin antibiotic was added in order to enumerate the total number of fungi in the sample.

Enumeration of total heterotrophic, bacterial and fungal hydrocarbon degraders using Bushnell Hass (BH) medium: Enumeration of microbial degraders, including total microbial heterotrophs, bacterial and fungi were carried out as described in the previous section, except that BH was used as a growth medium for hydrocarbon microbial degraders. To prepare the medium, 3.2 g of BH broth medium was dissolved in 1 L deionised water. Fifteen gram of bacteriological agar was added and then the mixture was autoclaved for 15 min at 121°C. The autoclaved medium was allowed to cool to about 50°C and then 0.1% diesel was added as the only source of carbon (Alamri, 2006). The samples were sonicated for 1 min and then allowed to settle for 2 min. Culturable cell counts, including bacterial and fungal hydrocarbon degraders, were carried out as described previously.

Bioreactor design: Each microcosm consisted of a plastic column with a height of 25 cm and an internal diameter of 10 cm. The base of each column was covered with a fine plastic mesh with 0.2 mm in diameter, Whatman No. 42 (15 cm) filter paper and double layers of perforated plastic paper in order to control the flow rate of the leachate sample throughout the column. To provide aerobic conditions inside the column, a small hole was made on the surface of each column. Subsequently, a plastic tube was sealed by silicone from one end and connected to an air pump from the other end. This tube was passed through that hole inside the column. To allow air diffusion all the way through the column, the plastic tube was perforated by a fine bore needle. The columns were aerated during each run. The column base comprised glass wool to support the sand. At the top of that, 3 cm of builder's sand was topped with 3 cm inoculated sand, which contained the microbial degraders and this was packed by 3 cm builder's sand. Finally, 3 cm layer of glass beads was placed on the top of the packed column to assist the dispersal of the sample through the column (Fig. 1).

Experimental set-up: Two batches of leachate samples were tested using the above bioreactor. In the first batch, the leachate was diluted to 20% in deionised water. Two groups of columns were used, one with inoculated sand and the other was the control column. The control column comprises the same layers as described above, except that builder's sand was used instead of inoculated sand. Triplicates were tested for each treatment. In the second batch, the leachate was diluted to 10% in deionised water and three columns were used for each treatment. The first column comprised inoculated sand, whereas the second contained builder's sand. The third column was the control, which was used for normalisation, comprised builder's sand with deionised water run through it instead of leachate. The columns were set up and held by a leaching shelf. They were placed on funnels to channel the leachate into 250 mL Duran flasks which were covered with aluminium foil to reduce photolysis (Fig. 1). At the beginning of each run, the columns were wetted with 250 mL quarter strength Ringer's solution.

Assessment of bioreactor performance: The leachate samples from the columns were collected in 250 mL Duran flasks and after each cycle, 10 mL from each flask was removed for microbiological and chemical analysis. The remainder was recycled through the reactor and the process was repeated up to 5 cycles (each cycle took about 18 h). The chemical analysis included conductivity measurement, pH and Total Organic Carbon (TOC) using a LABTOC analyser, was carried out as described previously. For biological analysis, toxicity tests were

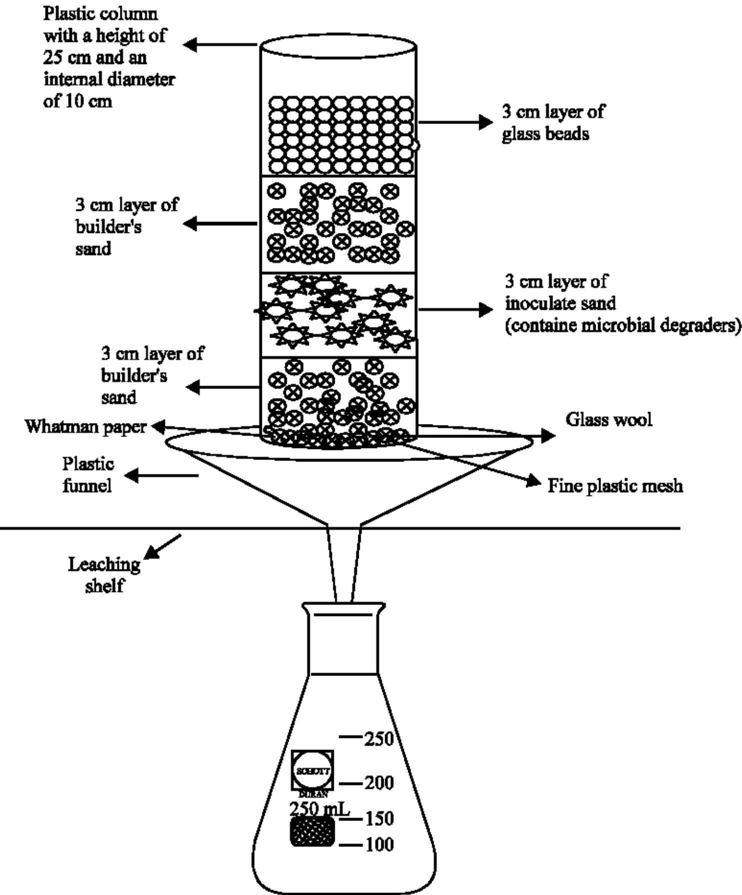

Fig. 1: Schematic representation of the flow-through bioreactor tested in this study for the bioremediation of landfill leachate

carried out for each sample throughout the five cycles using the microbial biosensor *E. coli* HB101 pUCD607 as described previously. The results of the inoculated and non-inoculated columns were calculated as percentages relative to the deionised water columns in the second batch.

Statistical analysis: Analysis of variance (one-way ANOVA) was carried out using the Minitab for Windows software package, version 13.1. If the data and residuals were not normally distributed or did not have equal variance, even after transformation, then the Kruskal-Wallis test was used. All significance was quoted at the 95% confidence level ($p \leq 0.05$).

RESULTS AND DISCUSSION

Preliminary leachate characterisation

Chemical characterisation: Landfill leachate can be characterized as a water-based solution of four groups of pollutants (Table 1). These pollutants include dissolved organic matter, inorganic macro-components (e.g., chloride, sulfate and hydrogen carbonate), heavy metals (e.g., cadmium, chromium and mercury) and organic compounds (e.g., aromatic and aliphatic hydrocarbons, phenols and chlorinated compounds) (Christensen *et al.*, 1994). The chemical analysis showed a relatively high concentration of metals in the landfill leachate which may suggest a negative effect of such concentrations on the performance of the bioreactor tested.

Total petroleum hydrocarbon (TPH) concentration was noticeably high in the landfill leachate. The concentration of TPH in landfill leachate is expected to decrease over time, depending on the rate of degradability of each compound in the landfill and its volatilization with landfill gas (Christensen *et al.*, 2001). In this study, loss of these volatile compounds caused a significant difference in leachate toxicity over the time, as confirmed by the EC_{50} values. The conductivity of the leachate was relatively low, indicating that the leachate had low salinity.

Table 1: Chemical analysis of peterhead landfill leachate (mg L^{-1})

Analyte	Concentration (mg L^{-1})
Arsenic	2.50
Barium	30.30
Cadmium	0.30
Chromium	3.50
Lead	1.80
Mercury	0.16
Iron	18.30
Phenol	19.00
Cyanide	12.30
Benzene	3.90
Carbon tetrachloride	0.35
Chlorobenzene	18.40
Chloroform	0.80
m-cresol	9.60
o-cresol	11.50
p-cresol	22.60
4 dichlorobenzene	4.45
2 dichloroethane	0.37
1 dichloroethylene	0.49
4 dinitrotoluene	0.09
Trichloroethylene	13.20
Vinyl chloride	0.12
TPH	3760.00
Sulfate	4200.00
pH	6.80
Conductivity (mS)	3.40

Consequently, osmotic stress was not considered to adversely affect the metabolic activities of the microbial degraders.

Toxicological characterisation: The toxicity of the leachate was expressed as a percentage of maximum bioluminescence, being the maximum calculated against a blank of double-deionized water at pH 5.5 (Sousa *et al*., 1998). The results obtained showed no significant difference between the filtered and non-filtered samples (data not shown). This suggests that the toxicity associated with suspended solids did not differ in the filtered and non-filtered samples. Consequently, the small particles which were present in the leachate had no effect on the sample toxicity.

The response curve showed a peak of bioluminescence with initial stimulation at low concentrations of the leachat (Fig. 2). This was followed by a sharp decline in bioluminescence, with increasing leachate concentration, up to 25% RLUs when the leachate concentrations were 20%. This suggests that contaminant concentrations higher than 20% would be sub-optimal concentrations for the bioreactor design in term of toxicity and the success of the remedial strategies applied. Thus, 10 and 20% concentrations were considered as ideal concentrations for the microbial degraders in this study, any concentration above that might have led to ineffective remediation.

The EC_{50} values were calculated from the toxicity response curve of the leachate and then analysis of variance was carried out to investigate the difference in the EC_{50} values over time (Fig. 3). The results obtained showed a significant difference in the toxicity level for the leachate over the period of bioremediation carried out in this study. The significant reduction in the toxicity of the leachate over time was more likely due to the presence of volatile chemicals in sample, as shown in Table 1.

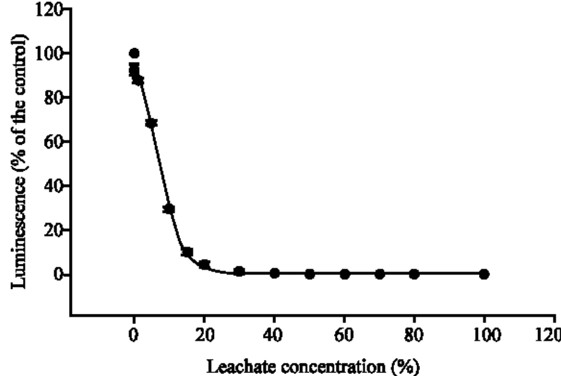

Fig. 2: Toxicity response curve for a wide range of the leachate concentrations. EC_{50} was calculated from the equation of the curve as 7.21 mg L^{-1}

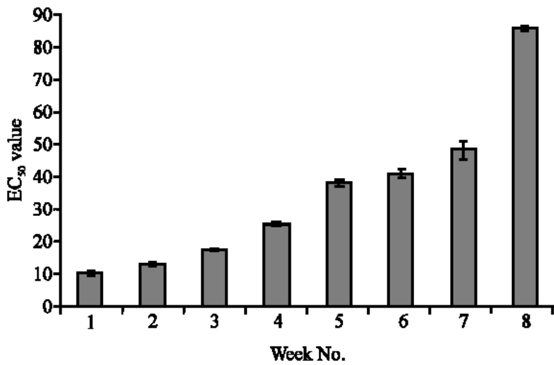

Fig. 3: EC_{50} values assessed during the period of bioremediation

Determination of microbial growth: The results showed that the inoculated sand had the higher number of microbial degraders (Table 2). This might due to the fact that the inoculated sand was obtained from a mixed hydrocarbon tank farm absorbed several hydrocarbon spill events over a long period of time. Although there has been a controversy among researchers on the effectiveness of bioaugmentation of hydrocarbon contaminated soil (Jørgensen *et al*., 2000; Rubertoa *et al*., 2003), the use of adapted microbial degraders was effective and reduced the overall time required for the remediation in this study.

Table 2: Plate counts and soil microbial biomass measurements for the inoculated and builder's (control) sands.

Plate count method and microbial biomass	Inoculated sand (cfu g^{-1} dry weight soil±SE)	Builders sand (cfu g^{-1} dry weight soil±SE)
Heterotrophs	$28.91 \pm 0.57 \times 10^7$	$12.97 \pm 0.72 \times 10^3$
Bacteria on LB medium	$35.69 \pm 1.60 \times 10^7$	$10.94 \pm 0.86 \times 10^3$
Fungi on LB medium	$99.81 \pm 1.67 \times 10^7$	No growth
HC degraders	$36.52 \pm 0.91 \times 10^7$	$0.56 \pm 0.34 \times 10^3$
Bacteria on BH medium	$27.80 \pm 1.30 \times 10^7$	No growth
Fungi on BH medium	$10.71 \pm 2.08 \times 10^7$	No growth
Microbial biomass (mg kg^{-1})	220	30

Table 3: Changes in mean conductivity (mS) during 5 cycles of passage through the bioreactor

	10% leachate concentration		20% leachate concentration	
Cycle No.	Non-inoculated sand	Inoculated sand	Non-inoculated sand	Inoculated sand
1	3.4	3.4	3.2	3.4
2	2.7	2.5	2.6	3.1
3	2.8	2.7	3.2	2.9
4	2.7	2.9	2.0	2.1
5	1.7	2.8	4.1	4.2

Test of bioreactor performance

Conductivity: In batch 1, the conductivity of the inoculated columns did not change during cycle 1. Following cycle 1, the conductivity decrease slightly from 3.40 mS down to 2.02 mS in cycle 4. However, it increased again up to 4.25 mS in cycle 5. The situation was broadly similar in the non-inoculated columns (the control), which showed no significant difference comparing with the inoculated columns. In contrast, there was a significant difference in the conductivity results of each treatment over the 5 cycles (Table 3).

Batch 2 showed a decrease in conductivity for all the three treatments. Leachate with inoculated sand showed a reduction from 3.4 to 2.8 mS. Samples with non-inoculated sand demonstrated a decline from 3.4 to 1.7 mS. There was a relatively small drop in the control columns with deionised water from 2.5 to 2.0 mS. The statistical analysis showed a significant difference between the treatments. In addition, each different treatment showed significant changes between conductivity results over the 5 cycles.

The increase in the conductivity was probably due to that the leachate washed the excess salts out of the sand in the column. On the other hand, the decrease in the conductivity shown in batch 2 was probably due to the salt levels in the column no longer being in excess and an equilibrium status being reached (Killham, University of Aberdeen, UK, personal communication). Consequently, some salts would have been remained on the column and less washed through with the leachate. Although there was a slight fluctuation in the conductivity results throughout the 5 cycles in each batch, the conductivity remained within an acceptable level in terms of salinity and possible effects on the growth and activities of the microbial degraders. As a result, the fluctuations in conductivity over the bioreactor trial seemed to have no effects on the hydrocarbon degraders and did not show a marked difference compared to the initial conductivities of the leachate. Similar observation was also reported by Galinski and Truper (1994).

pH assessment: Leachate pH remained relatively constant throughout the experimental period (data not shown). It was measured after each cycle and no significant change was recorded during each cycle in the two batches. Although previous studies (e.g., Atlas and Bartha, 1993) showed that the pH was a significantly factor affecting microbial activity, it did not show any effect on the rate of hydrocarbon biodegradation during this experiment. This might due to the pH levels monitored during this experiment were closed to neutral, which is the optimal pH value supporting the highest growth rate for most heterotrophic microorganisms (Moretto *et al*., 2005). Moreover, the rate of hydrocarbon biodegradation has been reported to be optimal across the pH range 6 to 8 (Eweis *et al*., 1998), which were the pH values recorded in the bioreactor.

Total organic carbon: Landfill leachates with high organic carbon contents are a pollution problem because they exert excessive oxygen demands to receiving water bodies and may cause disruption to ecosystems (Christensen *et al*., 1994). One of the three ways to measure the organic content of an effluent stream is TOC, which measures the total organic carbons present (Christensen *et al*., 1994). The TOC results for 20% leachate concentration showed a considerable reduction in the total organic carbon from 78.60 to 32.90 mg L^{-1} for samples with inoculated sand and from 78.60 to 48.77 mg L^{-1} for leachate with non-inoculated sand throughout the 5 cycles. This illustrated the efficiency of the remedial strategy used in this experiment, which provided optimal conditions for maximum microbial activity and thus leading to a rapid contaminant reduction. Statistically, there was a significant difference between the TOC throughout the 5 cycles with $p = 0.024$ for samples with inoculated sand and $p = 0.028$ for samples with non-inoculated sand (Table 4).

In batch 2, the results (Table 4) showed an increase in the TOC concentration in cycle 2, comparing with cycle 1. Following cycle 2, the TOC concentration declined slightly throughout the rest of cycles. The reason for the initial increase in the TOC was probably

Table 4: Changes in mean TOC concentration (mg L^{-1}) during 5 cycles of passage through the bioreactor

	10% leachate concentration		20% leachate concentration	
Cycle No.	Non-inoculated sand	Inoculated sand	Non-inoculated sand	Inoculated sand
0	20.16	20.16	78.60	78.60
1	24.60	23.50	54.25	40.97
2	21.16	18.12	61.08	62.35
3	18.04	15.80	49.90	51.90
4	17.08	14.10	47.01	40.91
5	17.72	12.90	48.76	32.90

due to the release of intrinsic carbon from the inoculated and builder's sands throughout the system, leading to a sudden increase in the TOC during cycle 2. Afterwards, there was a reduction in the TOC concentrations throughout the rest of the cycles, confirming the effectiveness of the novel flow-through bioreactor design tested in this study.

CONCLUSION

The present study showed that the novel flow-through bioreactor tested in this study was highly effective for the removal of hydrocarbon contaminants from landfill leachate. In addition, the toxicity data, assessed in this study, were highly valuable as they enabled optimisation of the concentration of leachate fed into the bioreactor. Conductivity and pH had no effect on the performance of bioreactor in this investigation, indicating that such constraints may only be site dependent.

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