ABSTRACT

Bioventing can be effective for the remediation of soil contaminated with petroleum hydrocarbons. However, implementing laboratory results in field scenarios is difficult due to the lack of scale-up factors. Accordingly, laboratory bioventing experiments were undertaken at the meso-scale and then compared to previously completed micro-scale tests to evaluate the important scale-up factor. The developed meso-scale system holds 4 kg of soil, with bioventing conditions controlled from a nutrient, airflow and water content perspective. Three soils were tested, and categorised as sandy, clayey, and loam soil. Results over a thirty-day period showed a two-stage degradation pattern that encompassed first-order degradation rates as compared to the single-stage first-order degradation rate determined in the micro-scale study. For the first stage (0 to 8 d), the degradation rate for sandy soil was 0.598 d⁻¹, with the clayey soil at 0.460 d⁻¹, and mixed soil at 0.477 d⁻¹. After 8 days, the degradation rate constant for the sandy soil dropped to 0.123 d⁻¹, with the clayey soil dropping to 0.075 d⁻¹, and the degradation rate for the mixed soil dropping to 0.093 d⁻¹. Comparison of the measured degradation rate values with the results from the micro-scale experiments gave scale-up factors varying from 1.9 to 2.7 for the types of soil considered in the current study. These differences in degradation rates between the two scales show the importance of scale-up factors when transferring feasibility study results to the field.

Key words: TPH, gasoline, soil remediation, biodegradation rate, bioremediation, scale up factor
INTRODUCTION

Soil contamination is a worldwide environmental problem that is placing human and environmental health at risk. Health issues arise primarily through both direct and indirect contact with contaminated soil and from secondary contamination through water supplies and air pollution.

The unintentional release of petroleum hydrocarbons to the soil environment is typically related to industrial spills and leaking underground storage tank systems in the vadose zone (USEPA, 2004). The major concern at most sites is the risk of groundwater pollution due to hydrocarbons (Grathwohl and Klenk, 2000), followed by the residual contamination remaining in the soil. The magnitude of the problem is seen by the U.S. Environmental Protection Agency’s national priorities list that indentifies 1245 sites that must be remediated (USEPA, 2007).

Remediation of soils can be accomplished in a variety of ways, including dig and dump, soil flushing, soil vapour extraction (SVE), and bioventing. Dig and dump is popular due to the speed of completion, even though the contamination is transferred from one site to another. Soil vapour extraction is a popular in-situ soil remediation technology, despite the phenomenon of tailing. Tailing represents the progressively slow decline of gasoline removal from the soil and also results in lengthy soil remediation time. Several laboratory and field studies also report, that when tailing occurs, the contaminant concentration eventually stabilizes at a level that still exceed the clean up goals (Zakharchenko et al., 2011).

Tailing is associated with: i) hiding of contaminants in the intricate shapes of the soil particles, ii) reduced air flow in the lower permeable finer textured soil, iii) high soil water content, iv) sorption of contaminant to organic matter, v) preferential paths of airflow in heterogeneous soils, and vi) contaminants which restrict the growth of the microorganisms (Barakat & Zytner, 2005). Once tailing sets in, further clean-up attempts with SVE result in increasing costs with a
very slim chance of additional remediation. Use of a suitable bioremediation technology can help overcome these hindrances.

Bioventing is an in situ bioremediation technology that uses indigenous microorganisms to biodegrade the petroleum hydrocarbons present in the unsaturated zone, and can be used to further reduce the contamination left behind by other technologies. In bioventing, the activity of indigenous microorganisms is stimulated by inducing air (or oxygen) flow into the unsaturated zone and if necessary, by adding nutrients (Magalhães et al., 2009; Margesin and Schinner, 2001). Moreover, removal of produced gaseous components such as carbon dioxide and volatilised gasoline are also imperative as this allows better diffusion of oxygen to the microorganisms (Rathfelder et al., 2000; Coffey et al., 2012).

The challenge with bioventing is that the airflow needed to provide the oxygen can also induce volatilization (Magalhães, 2009) and in the process dry out the soil, reducing water content to less than 50% of field capacity, the level needed for microbial stimulation (Hinchee, 1994). Similarly, the development of preferential pathways can result in non-uniform oxygen delivery to the bulk of the soil. Uneven distribution of air can result in non-uniform soil remediation (Suthersan, 1997; USACE, 2002).

These challenges with bioventing involve understanding and integration of the biological, chemical and physical phenomena operating at scales ranging from that of the microbial cell (10⁻⁶ m) to that of the geological site (10-1000 m). By necessity, bioventing laboratory investigations are performed at a small scale (Sturman et al, 1995). Unfortunately, the optimum conditions necessary for bioventing that have been identified from micro-scale studies do not work at the field sites. Literature also reveals that micro-scale biodegradation rates correspond well with different contamination types while somewhat over estimating field rates (Davis et al., 2003; Aichberger et al., 2005).
Intermediate scale experiments, also referred to as meso-scale, are frequently used as a link between microcosms and a pilot or field-scale system. Intermediate-scale systems retain much of the control possible in bench-scale experiments, but allow observation of mass transport and interfacial transfer effects on biodegradation rates and contaminant movement (Sturman et al., 1995). Review of the literature shows that bioventing research at medium or large scale is sporadic (Woo et al. 1999; Patros, 2009; Khan and Zytner, 2011; Chang et al., 2011).

Rectangular tanks were used by Chevalier et al. (1999), Cort et al. (2001) and most recently Chang et al. (2011). Similarly, cylindrical columns were used by Angley et al. (1992), Malina et al. (1998), Widrig et al. (1995), Woo et al. (1999), and Patros (2009). From the different shapes of reactors tried, the cylindrical column seems a better representation of the site remediation scenario due to the radial flow of air in the contaminated soil.

The application of micro-scale results at field scale bioremediation sites is a challenge due to mass transport mechanisms and limitations, presence of multiple phase contaminants, competing microorganisms, spatial heterogeneities and factors that inhibit bacterial growth. One of the ways to account for the challenges in transferring micro-scale data to field settings is calculating a scale-up factor (Khan and Zytner, 2011; Ko et al., 2007). The scale-up factor would be a number that encompasses all the factors. The origin of scaling theory leans on the typical work of Miller and Miller (1955). The importance of developing a scale-up factor with biological processes was identified by Paerl and Steppe (2003).

Overall, limited work has been done on determining the hydrocarbon degradation rate constant at the larger scale. Likewise, the literature is silent about scale up factors. Therefore, the objective of this research was to determine the hydrocarbon degradation rate at the meso-scale level using specially designed reactors. The developed meso-scale system holds 4 kg of soil, with bioventing conditions controlled from a nutrient, airflow and water content perspective.
Three soils were tested, categorised as loamy sand, silt loam, and sandy loam soil. The collected degradation results were compared to the first-order degradation rates determined in a previously completed micro-scale study (Evyazi and Zytner, 2009) to evaluate scale-up factors. Work was also done on evaluating the appropriate degradation model, as Shewfelt et al. (2005) and Zytner et al. (2006) reported that a single stage first order degradation might not be the best model for all scales of soil remediation.

METHODOLOGY

The study was conducted in the environmental engineering laboratories at the University of Guelph, Canada. Two different types of soils were collected from the Elora research station (County of Wellington, Ontario) and Delhi research farm (Delhi, Ontario). These soils were distinct due to the sandy composition of the Delhi soil, and the clayey composition of the Elora soil. Both soils were dried under the sun during the summer season and passed through a 2 mm sieve to remove gravel and other debris. The third soil was prepared by mixing Delhi (sandy) and Elora (clayey) soil in equal mass and it was named Mixed soil. Six replicates were carried out using the Delhi and Elora soils. Among them, four experiments with the original version of the meso-scale reactor, while two replicates were completed after reactor modification by wrapping glass wool around the reactors. Only three replicates were done using Mixed soil and all were done with glass wool wrapped reactors.

The soils were tested by the University of Guelph Laboratory Services for their particle size fraction, organic matter content, pH, cation exchange capacity (CEC), and ammonium content. Table 1 lists the properties of these soils.

Soil stored in the laboratory was in the dry form, with the microbes expected to go dormant. Microbial activity was enhanced by amending the soil with nutrients and water prior to the start of
each experiment. Ammonium Chloride (NH$_4$Cl) was used as the source of nitrogen with the petroleum hydrocarbon as the carbon source to achieve a C:N ratio of 10:1 (Eyvazi and Zytner, 2009).

The required amount of NH$_4$Cl was dissolved in water and the solution was uniformly mixed with the soil. The amended soil was kept at room temperature (22-24$^\circ$C) for at least 5 days, with the moisture content in the soil maintained at 50-70% of the field capacity for the soil in question. This incubation period allowed germination and emergence of the microorganism from the spores prior to starting the experiment.

The soil was then spiked with synthetic gasoline to a concentration of 4000 mg/kg of soil. Approximately 40-50% evaporation was expected during mixing and handling of the soil. The measured amount of synthetic gasoline was added to the 4 kg soil in five equal amounts, with care taken to handle/store soil in air tight bins to minimize volatilization. To assist with homogeneous mixing of hydrocarbon and soil, shaking of the bins was done manually for about fifteen to twenty minutes. The composition of the synthetic gasoline used in the treatments is shown in the Table 2

Experiments were carried out at a medium scale, defined as meso-scale. The meso-scale reactor (MSR) used in this study was originally designed and developed by Patros (2009) and could hold 4 kg of soil. The cells of the reactor were made from sheets of perforated 316 SS with dimensions of approximately 15.9 cm × 15.4 cm × 17.8 cm [O.D. × I.D. × H], respectively.

Plain woven mesh 316 SS, with a 200 × 200 opening was used to wrap the inside and outside of the cell. The cell sandwiched between two layers of the mesh sheets was fastened with 316 SS screws. The mesh sheets not only acted as a shield for the soil and keep small particles within the reactor, but also provided support in distributing airflow evenly and radially into the soil column. The central well had slotted narrow openings to draw air from outside the cell and laterally through the soil column. Uniform circulation of air (oxygen) was needed in the soil to maintain
microbial activity. To assist with the placement of the well, Coffey et al. (2012) used computational fluid dynamics (CFD) modeling techniques to check the effect of well opening on mass flow rate tendencies on air movement through the soil column. The modeling results suggested that restricting air passage to the lower two third parts of the suction well minimized short circuiting of the air. Accordingly, the upper one-third part of the suction well was sealed. Modelling also showed that this configuration provided uniform air flow in the soil profile. Figure 1 provides a schematic of the reactor system.

After mixing, the soil was placed in the reactor in lifts to attain the desired density. Once all the soil was placed, a sample was taken from the reactor to determine the initial gasoline concentration. Typical parameters monitored during the experiment were air flow rates, concentration of volatile hydrocarbon and O₂ in the soil by monitoring off gases. Characteristics of the soil reactor that were monitored included, concentration of nutrients, concentration of total petroleum hydrocarbon (TPH), soil pH, soil water content, room temperature and population of petroleum utilizing bacteria.

Microbial concentration in soil was measured through the spread plating technique. Tryptic Soy Agar (TSA) was used for total heterotrophic bacteria (THB) and Bushnell-Hass (BH) medium was used to enumerate petroleum-degrading bacteria (PDB). The microbial enumeration method used was similar to the procedures adapted by Shewfelt (2005) and Eyvazi and Zytner, (2009). However, the standard recipe/directions suggested by supplier (Difco™) were adapted for cooking the media. Gasoline @ 4g/L as the carbon source was added directly to the BH-medium just before pouring it into Petri dishes. The colonies for PDB were counted after 7 and 14 days of incubation at 25°C ± 1°C, while colonies for THB were counted after 3 days of incubation.

In order to provide sufficient oxygen, and replace carbon dioxide and minimize volatilization, the correct air flow rate was needed. Stoichiometric analysis of the synthetic gasoline and pore volume replacement techniques indicated that a 0.4 mL/min air flow rate was
needed. This rate of air flow was well below the threshold for SVE conditions. Testing showed that the industrial hygiene pumps (Gilian, model: LFS-113DC) could provide an air flow rate of 0.75 mL/min to each reactor, where the suction port of the pump was attached to a manifold connecting two wells to draw fresh air through the outer screen. Calibration of pumps was done by using an Air Flow Meter (Gilmont Micro Flow Meter, Model: GF-9760). A subsequent study by Coffey et al. (2012) showed that wrapping glass wool around the reactors also provided for uniform entry of air into the system and could further reduce the flow rate to 0.40 mL/min. Hence the reactors were wrapped with glass wool.

The oxygen level in off-gases from the reactors was frequently measured by using the “flow through” option of the Apogee Oxygen Meter, Model: MO-200 Series. The flow through head of the oxygen meter was installed at the suction well of soil reactor in series with the suction pump.

Volatilization of hydrocarbons was monitored by capturing hydrocarbon vapors through carbon sorbent tubes (Supelco ORBO® 32 Small). These tubes were installed in the suction line and were replaced at 4 h intervals during the first seven days, and after that changed on 12 h intervals until day 30. The captured hydrocarbon was extracted by using 10 mL of methylene chloride as solvent.

Biotic and abiotic control reactors were run parallel to experimental reactors. Soil was sterilized through a dry heat method (Trevors, 1996). Incubated/stabilized soil was placed into the oven at 200°C for 24 h (Alef et al., 1995). Special care was exercised and the soil was placed in 1.5–2.0 cm deep layer in aluminum plates. After 24 h of heating in the oven, the soil was rewetted using autoclaved water, bringing the water content up to 15%. The wet soil was placed again in the oven at 200°C for an additional 24 h period. Microbial enumeration was done for assessing the success of sterilization process.
Soil samples were collected daily for the first seven days and then every alternate day up to thirty days. Soil and sorbent tubes samples were tested using a gas chromatograph equipped with a flame ionization detector.

The observed soil hydrocarbon data was plotted to determine the appropriate degradation rate constant. This degradation rate was determined from the semi-log plot of concentration versus time, with the slope of the line taken as the degradation rate constant. Analysis revealed that the degradation was a two stage process, giving \( k_1 \) and \( k_2 \) values.

For comparison purposes with similar soils and contamination, and the evaluation of scale-up factors, the micro-scale degradation rate constant as estimated by the correlation developed by Eyvazi and Zytner (2009) was used:

\[
\ln(k) = 2.803 \times PDP + 0.21 \times Sand + 4.886 \times OM - 0.094 \times Clay - 0.004 \times (SW \times Sand) - 0.021 \\
\times (Sand \times PDP) - 0.632 \times (OM \times PDP) + 0.004 \times (SW \times Clay) - 23.5
\]  \[1\]

where,

\[
\begin{align*}
  k & = \text{first order degradation rate, d}^{-1} \\
  PDP & = \text{initial population of petroleum degrader microorganisms in soil, log colony-forming units [CFU]/g} \\
  Sand & = \text{sand content, \%} \\
  Clay & = \text{clay content, \%} \\
  SW & = \text{soil water content, \%} \\
  OM & = \text{organic matter content, \%}
\end{align*}
\]

Soil properties and values of observed parameters were inserted into Equation 1 to estimate \( k \). The ratio of \( k \) and \( k_2 \) (i.e., \( k:k_2 \)) gives the estimated value of scale-up factor for each type of soil.
RESULTS AND DISCUSSION

The meso-scale reactor (MSR) used in this study made it possible to simulate radial flow in an unsaturated environment as shown in Figure 1. The porous mesh at the edge of the soil profile allowed the industrial hygiene pump to easily draw air in under laboratory conditions. This was confirmed by the smoke test that was completed, where a smoke stick was placed 100 mm from the reactor wall. The smoke was drawn towards the reactor, which easily showed the intended airflow pattern. The pump remained functional for low, medium, and moderately high water levels, while it failed to operate at fully saturated conditions, a situation that does not occur when using bioventing. Overall, the selected industrial hygiene pump suitably maintained bioventing conditions in the MSR.

Concern with the initial induced air flow of 0.75 mL/min was volatilization of the synthetic gasoline and drying out of the soil. Through the bioventing process, some volatilization is unavoidable due to the air flow needed to provide oxygen to the bacterial population (Rathfelder et al., 2000). In order to determine the magnitude of the synthetic gasoline volatilization, sorbent tubes were used to sample the off gas leaving the MSR. Figure 2 shows the typical volatilization losses measured through the suction line and it can be seen that the losses were elevated at the beginning of the experiments and then dropped to very low levels after the initial 5 d.

Analysis of the sorbent tubes showed that about 55-60 mg gasoline/kg of soil was lost over the 30 d, which translates to 2.5% of the initial concentration of synthetic gasoline. This was quite low on relative terms considering the volatility of the synthetic gasoline. A similar value of volatilization (2.5% of initial concentration) was observed by (Mao and Yue, 2010) for a biodegradation experiment with diesel in a closed vessel at aeration rate of 200 mL/min. Accordingly, the observed 2.5% cumulative loss of hydrocarbon during 30 d in the meso-reactors at an air flow rate of 0.75 mL/min was considered acceptable for the 4 kg of spiked soil used.
However, at the 0.75 mL/min flow rate, it was observed that drying of soil was occurring. To reduce the water loss, glass wool was wrapped around the reactors based on the work of Coffey et al. (2012). The glass wool provided additional resistance to airflow, which in turn reduces volatilization as air movement is the biggest cause of moisture loss when the air is not humidified (not possible in current experimental configuration). Simultaneously, the glass wool also provided for uniform entry of air into the soil. Water content observations post wrapping showed that good water levels were maintained in the soil, promoting ideal conditions for optimum biodegradation.

Airflow measurements showed that the air flow resistance reduced the aeration rate per reactor to 0.40 mL/min. Measurements of synthetic gasoline volatilization showed that the loss was reduced to only 0.03 % of the initial concentration of gasoline. Accordingly, the volatilization loss of hydrocarbons was ignored in subsequent hydrocarbon degradation calculations.

The off-gas line was also used to continuously monitor the oxygen level using the Apogee oxygen meter. The oxygen meter had two options for usage: measurement by diffusion or flow through. The diffusion method required 75 mm of soil cover from all sides which was not possible in the MSR due to the existence of suction well in the centre of the reactor. As such, the flow through option of the oxygen meter was installed, in series, between the suction well and pumps, allowing the oxygen level to be measured regularly. The results showed that the oxygen level was in the range of 19 to 21 % throughout the experiment, confirming that oxygen was not limiting for aerobic degradation, even with glass wool wrapping where the flow rate was reduced to the minimum of 0.40 mL/min.

Hydrocarbon Degraders
The growth rate of the microbes within the reactors was an important indication of microbial activity. The populations of total heterotrophic bacteria (THB) and petroleum-degrading bacteria (PDB) were measured at different intervals during the thirty days of treatment.

For the abiotic control reactors, the microorganisms were inactivated by the dry heat method of autoclaving. Accordingly, the initial population of both heterotrophic bacteria and petroleum degraders in the abiotic control was much lower than that of the biotic control. However, it was not zero, indicating partial success of the autoclaving and the difficulty with sterilizing soil. This was consistent with the challenges and methods reported for soil sterilization (Trevors, 1996; Wolf and Skipper, 1994; Eyvazi and Zytner, 2009). The microbial counts in the abiotic control of Mixed soil at the beginning of the experiment was very small (log 3.95 cfu/g soil) when compared to the amended soil microbial count (log 5.81 cfu/g soil). Similar results were seen for abiotic controls of Delhi and Elora soil. The same trends were seen in the replicates, which consisted of at least three repeats with each soil. Even though the procedure for sterilization in this study was different from the one used by Eyvazi and Zytner (2009), the results were similar.

Figure 3 shows the typical changes in total heterotrophic and petroleum degrading bacterial population over the course of experiment for Delhi, Elora, and Mixed soils. Solid lines and solid markers indicate the total heterotrophic bacteria count, while open markers with broken lines represent petroleum-degrading bacterial population in the soil.

The microbial results were also used to compare the microbial growth in the MSR with and without wrapping of glass wool. The letters, “gw” in the legend of Figure 3 represents the results for the reactors wrapped with glass wool.

The solid lines distinctly running above the broken lines display a higher population of total heterotrophic bacteria (THB) as compared to petroleum degrading bacteria (PDB) and this was expected. The average population of PDB for Elora soil remained 79-84% and for Delhi it was 80-85% of THB population. Overall, the microbial population number for the MSR is slightly higher
than the findings of Eyvazi and Zytner (2009) who reported PDB population hovering at 80% of the total heterotrophic population on average in the micro scale reactors. This suggests that the degradation potential in the MSRs was slightly better or in other words more like optimum conditions.

The increase in the population of microbes during the early days of the experiment makes sense due to the good environment and abundant food in the form of hydrocarbon, plus sufficient nitrogen. Eyvazi and Zytner (2009) and Ka et al. (2001) observed a slight decline in the number of petroleum-degrading bacteria at the beginning of micro-scale experiments, and then increase thereafter. This decline was related to the bacterial adaptation period to the petroleum hydrocarbon. However, this hump or decline has not been observed in the current study.

An interesting observation was the 11.5% higher population of PDB in the reactors with glass wool wrapping. This difference can be related to the fact that the glass wool wrapped reactor helped maintain higher water contents in the soil, providing an ideal environmental condition.

Overall, the consistent increase in the population of petroleum degraders in all the experiments confirmed the biodegrading process in the meso-scale experimental system.

Degradation of Total Petroleum Hydrocarbons

Review of the data showed that the decrease in concentration of petroleum hydrocarbons in the soil was a logarithmic process. This first order degradation process was consistent with other published data concerning the degradation of hydrocarbons in soil (Rathfelder et al., 2000; Shewfelt et al., 2005; Eyvazi and Zytner, 2009). However, close review of all the data showed that one curve could not be used to fit all the data simultaneously. This fitting situation suggested
that the data should be broken into two groups or analyzed with a more complex model (Shewfelt et al., 2005; Zytner et al., 2006; Maletić et al., 2009). Based on the trends showed by the data, it was broken into two stages. Stage 1 was 0 to 8 d, while Stage 2 was 8 to 30 d. The breakpoint for splitting the data was determined by regressing the data to get the best coefficient of determination ($r^2$).

The first eight days showed a higher first order degradation rate, while the last 22 days had a reduced degradation rate. During the first stage of degradation, the dormant microorganisms became highly active due to plenty of food and nutrients present. One could say an ideal condition. However, after this initial burst, the degradation trend approached a more realistic condition. Increases in the population of petroleum degraders showed similar behaviour.

Figure 4 confirms the two stage trend as seen by the results of the Elora soil on one graph. The two stage trend is easy to see, with reasonable $r^2$ values considering the difficulty in working with soils. Similar two stage trends were seen with the other two soils, along with the consistency among the replicates.

Figures 5, 6 and 7 show the resulting two stage fit for Delhi, Elora and Mixed soils on an individual basis for improved clarity. All three graphs show reasonable $r^2$ values with outliers at the transition point at the beginning of Stage 2. The transition point is referred to as 8-11 day value where the degradation goes from Stage 1 to Stage 2. Further research is required to identify if day 8 is the appropriate transition point. The lower $r^2$ value for Elora soil can be attributed to the difficulty of mixing synthetic gasoline with the higher clay fraction present in the Elora soil.

The individual first order degradation rate constants for Stage 1 and Stage 2 for the six experiments with Delhi soil were 0.598 d$^{-1}$ and 0.123 d$^{-1}$, respectively. Likewise, the rate constants for the Elora soil for Stage 1 was 0.460 d$^{-1}$ and for Stage 2, 0.075 d$^{-1}$. Only five experiments are shown due to experimental error with one of the experiments. The degradation rates for the three treatments of Mixed soil ranged from 0.477 for Stage 1 and 0.093 for Stage 2.
Comparison to the micro-scale results showed that the degradation rate constant \( (k_1) \) for Elora (silt loam) soil was lower than the \( k_1 \) of Delhi (loamy sand). As expected, the \( k_1 \) value for the Mixed soil during Stage 1 was between both Elora and Delhi soil. A similar trend was observed when comparing \( k_2 \) values from Stage 2.

The use of a two stage degradation model shows agreement with the findings of Zytner et al. (2006). The majority of degradation occurred in the first 10 days, suggesting that a single stage first order model may not be the appropriate model for a degradation period longer than 10 days when nutrient limited. On the other hand, there was no limitation of nutrients in the current study. The two stage degradation concept also agrees with the findings of Shewfelt et al. (2005). Shewfelt reported that the majority of the degradation occurred by approximately day 6, with very minor removal during second interval up to total 30 d incubation.

However, the literature is mixed on the need for more complex kinetics or two stage degradation models (Kampbell and Wilson, 1991; Moeller et al., 1996; Zytner et al., 2001; Brook et al., 2001; Pala et al., 2006; Lee et al 2006; Eyvazi and Zytner, 2009; Maletić et al, 2009) Further work is required to see if the larger scale used in this work causes the more complex two stage degradation.

In a micro-scale study, where Eyvazi and Zytner (2009) used 150 g of soil in a closed respirometer, the degradation rate constant varied from 0.01 d\(^{-1}\) to 0.1 d\(^{-1}\) for similar soils and the same synthetic gasoline. These degradation rates are smaller than the first stage of the current study, but comparable to the second stage of degradation. Similarly, Hallman et al. (2003) reported a degradation rate of 0.077 using 4 kg of soil and this is comparable with the second stage while lower than the first stage. Furthermore, Song et al. (1990) reported a degradation rate for unleaded gasoline of 0.30-0.46 d\(^{-1}\), which is comparable with the range of the first stage but higher than the degradation rate for the second stage of this study. Likewise, Onwurah and Nwuke (2004) determined the degradation rate for crude oil at 0.085-0.28 d\(^{-1}\), and this range is closer to
the second stage results of the current study. Aichberger et al. (2005) used 0.01 kg for small scale and 90 kg for column scale and found the degradation rate constant in the range of 0.02 to 0.06 d\(^{-1}\). This degradation rate is comparable with the second stage rate, while lower than the first stage value of the current study.

Chang et al. (2011) conducted a study using 200 kg soil (large scale) and determined first-order biodegradation rate constants in the range of 0.004 to 0.024 d\(^{-1}\). In comparison, the current meso-scale study showed higher rates of petroleum hydrocarbon degradation. This discrepancy could be due to the difference in experimental conditions, with this study possibly having more optimum conditions.

Furthermore, Chang et al. (2011) utilized field aged contaminated soil, while in this study the soil was freshly spiked with synthetic gasoline. The field aged contamination causes a significant impact on the efficiency of contaminant degradation in soil because of increased residence time which translates into decreasing contaminant accessibility (Travis and Macinnis, 1992). The petroleum hydrocarbon fraction used in the current study was C6-C10 while Chang used C10-C36. Smaller fractions of hydrocarbon degrade at faster rate than larger molecules of gasoline (Haddadi et al., 2009; Wang et al., 2010; Coulon et al., 2005).

The mass of tested soil might have also created a discrepancy as Chang used 200 kg of soil, while the soil used in MSR was 4 kg. Finally, in the MSR, a perforated screen on the wall and central well to draw air ensured the radial flow of air for homogeneous dispersion. This configuration of reactor is different from Chang’s set up, who packed soil in a stainless steel tank and supplied compressed air axially from the headspace of the tanks. Literature also relates the variation in rate of hydrocarbon degradation with efficient diffusion of oxygen from outside or within system (Barker et al. 1987; Sturman 1995; and Davis et al 2003). Similarly a small diffusion length might have increased the diffusion rates of fresh air in the MSR as compared to the closed vessel in Chang et al. (2011), thus supporting the higher degradation rates in the current study.
Circulation and uniform dispersion of oxygen in the soil can help remove produced gases and increase aerobic microbial activity. Coffey et al. (2012) from the modelling studies indicated that the configuration of suction and delivery lines of air have the dominant effect of creating a homogeneous condition on the soil perspective. This is further evidence that the design of the reactor caused the difference in results between Chang’s work and the current study.

**Scale up factors**

Using Equation 1, the predicted k values are 0.045 $d^{-1}$ for Delhi, 0.040 $d^{-1}$ for Elora, and 0.043 $d^{-1}$ for Mixed. These rates are slower than the Stage 1 degradation rates reported in this study, but are in the same order of magnitude as the Stage 2 degradation rates, which is the expected long term degradation that develops at a remediation site. Accordingly, scale-up factors were determined by taking the ratio of the k values to the $k_2$ values determined in this study. Table 3 summarizes the values related to the degradation rate and scale-up factors.

As previously discussed, the experimental conditions successfully stimulated the microbial population to degrade the synthetic gasoline and at the same time had minimal volatilization. Accordingly, it is believed that the difference in degradation rates can mainly be attributed to the scale-up factor, which includes factors like microorganism population, availability of nutrients and substrate, easy access to oxygen, adsorption/desorption, interphase transfer, soil type, soil heterogeneity, water content and weathering of contaminant. Similar observations were noted by Ko et al. (2007). Comparing decay rates from Equation 1 to the experimental values of Stage 2, it was seen that scaling up the size of the reactor increased the biodegradation rate. The value of the Stage 2 scale up factor for the Delhi soil was 2.7, Elora had a value of 1.9, while the Mixed soil had a value of 2.2. This trend is as expected since the Mixed soil properties fall between Delhi and Elora soils. The increase in scale-up factor values suggest that for this study the degradation
conditions were more optimum as compared to the conditions implemented in Eyvazi and Zytner (2009), highlighting the need of correctly adjusting respirometer data from degradation assessment studies before transferring the results to the field. Similar observations were noted by Kao et al. (2010).

Typically the bioremediation rate decreases when the reactor size increases (Sturman et al., 1995; Davis et al., 2003; Wang et al., 2008). However, when the results of this study are compared to the respirometer values of Eyvazi and Zytner (2009), where the environmental conditions were similar, the degradation rate increased. It is expected that the larger volume of soil/surface area ratio provides for improved oxygen transfer in the soil, as well as better access by the microorganisms to both carbon and nitrogen sources of food resulting in a higher degradation rate for all soils. Therefore, the higher degradation rate can be attributed to a better environment for microbes. Consequently, it is important that further work be done at a larger scale to evaluate the scale-up factor needed to transfer respirometer laboratory results to the field (Ko et al., 2007). From a practicality perspective, respirometer studies are easier to complete, but if scale-up factors are not available, transferring the results to the field will be difficult. It is also suggested that the Eyvazi and Zytner (2009) correlation be revised to incorporate scale-up affects to better estimate the k value needed in the field.

Review of the individual synthetic compounds shows that Isooctane, at 24% proportion in synthetic gasoline, was observed to degrade at the fastest rate during starting 8-10 d, while naphthalene remained the last choice for the hydrocarbon degraders. Naphthalene maintained its initial concentration until isoctane, toluene, trimethyl benzene were prominently degraded. This indicates that the smaller molecules were easier accessible and degraded by the microorganisms. The observed degradation trend agrees with studies related to desorption kinetics or degradation of hydrocarbon. Haddadi et al. (2009), Wang et al. (2010) and Coulon et al. (2005) has similar findings while studying naphthalenes, dibenzothiophenes, phenanthrenes,
and pyrenes, and showed a clear decrease in their degradation rate as a function of the size of the PAH molecule.

The smaller fractions of the synthetic gasoline were the favourite chemicals for the microbes and showed faster decline in the soil during starting eight to ten days of incubation. Faster degradation of the smaller molecules could be responsible for the two stage degradation trend that was observed. This corresponds with the slow decline of the larger molecules in the second stage of degradation and supports the two stage degradation pattern.

CONCLUSION

Completion of the project has demonstrated that the meso-scale reactor system was a dependable apparatus for determining the degradation rate constant. Volatilization of the synthetic gasoline was minimal and measured at only 0.03% when glass wool was wrapped around the reactors. A healthy degradation environment was observed, with hydrocarbon degraders present in all three soils. The measured hydrocarbon degrader population was approximately 80% of the total heterotrophic population.

The completed study showed a two-stage degradation pattern as compared to the single-stage first-order degradation rate determined in the micro-scale study. The higher degradation rate was observed during starting eight days of experiment (Stage 1) as compared to last 22 d (Stage 2). The smaller fractions of hydrocarbon remained the favourite carbon source for the bugs and degraded at faster rate than larger fractions during the starting eight days of incubation. This preferred action of microbes was the basis on which to consider the two stage degradation processes. Overall, the degradation rates for the loamy sand (Delhi) soil were higher than the silt loam or mixed soils, which is consistent from a soil type perspective. Similarly, for Delhi, Elora and Mixed soils the degradation rates were higher for the meso-scale reactor as compared to the
small scale system resulting is range of scale-up values: 2.7 for Delhi soil, 1.9 for Elora soil and 2.2 for Mixed soil. The higher degradation rate demonstrated that the meso-scale reactors were providing an optimum degradation environment for the microbes. As such, it is important that appropriate scale-up factors be determined so that results from degradation assessment studies, typically done with respirometers, can be properly transferred to the field.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the support of the NSERC of Canada in the form of a Discovery Grant.

REFERENCES


Figure 1: Sketch showing Arrangement of the System with Movement of Air within Meso Scale Reactors
Figure 2: Volatilization of Gasoline Captured by Sorbent Tubes
Figure 3: Typical Pattern of Microbial Population during Thirty Days Experiment.
Stage 1
\[ \ln C = -0.463 \times t + 7.17 \]
\[ R^2 = 0.75 \]

Stage 2
\[ \ln C = -0.075 \times t + 3.04 \]
\[ R^2 = 0.52 \]

Figure 4: Two-Stage Degradation Trend of Five Experiments for Elora Soil
Figure 5: Degradation Trend of Synthetic Gasoline for Delhi soil
(Stage 1: 0-8d and Stage 2: 8-30d)
Figure 6: Degradation Trend of Synthetic Gasoline for Elora soil
(Stage 1: 0-8d and Stage 2: 8-30d)
Figure 7: Degradation Trend of Synthetic Gasoline for Mixed soil (Stage 1: 0-8d and Stage 2: 8-30d)

Stage 1:
- Concentration formula: \( C = 1643e^{-0.477 \times t} \)
- \( R^2 = 0.92 \)

Stage 2:
- Concentration formula: \( C = 64.1e^{-0.093 \times t} \)
- \( R^2 = 0.78 \)
Table 1: Physical and chemical properties of soil

<table>
<thead>
<tr>
<th>Soil Name</th>
<th>Sand (%)</th>
<th>Silt (%)</th>
<th>Clay (%)</th>
<th>Soil texture</th>
<th>PDB (CFU/g soil)</th>
<th>OM (%)</th>
<th>CEC (cmol/kg)</th>
<th>WHC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delhi</td>
<td>86.5</td>
<td>9</td>
<td>4.5</td>
<td>Loamy sand</td>
<td>6.04</td>
<td>1.2</td>
<td>8.27</td>
<td>25</td>
</tr>
<tr>
<td>Elora</td>
<td>34</td>
<td>50.1</td>
<td>15.9</td>
<td>Silt loam</td>
<td>6.26</td>
<td>3.1</td>
<td>18.9</td>
<td>30</td>
</tr>
<tr>
<td>Mixed</td>
<td>55.9</td>
<td>31.3</td>
<td>12.9</td>
<td>Sandy loam</td>
<td>6.11</td>
<td>2.4</td>
<td>14.2</td>
<td>27</td>
</tr>
</tbody>
</table>

Where
PDB = Initial Population of Petroleum Degrading Bacteria
OM = Organic Matter
CEC = Cation Exchange Capacity
WHC = Water Holding Capacity
CFU = Colony Forming Units
Table 2: Composition of Synthetic Gasoline and Vapour Pressure of the Constituents

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Chemical formula</th>
<th>Vapour Pressure mmHg/torr at 20-25 °C</th>
<th>Mass %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>C\textsubscript{10}H\textsubscript{8}</td>
<td>0.05</td>
<td>3.2</td>
</tr>
<tr>
<td>1,3,5-Trimethylbenzene</td>
<td>C\textsubscript{9}H\textsubscript{12}</td>
<td>1.86</td>
<td>11.9</td>
</tr>
<tr>
<td>m-Xylene</td>
<td>C\textsubscript{8}H\textsubscript{10}</td>
<td>7.9</td>
<td>23.9</td>
</tr>
<tr>
<td>Toluene</td>
<td>C\textsubscript{7}H\textsubscript{8}</td>
<td>28.5</td>
<td>36.0</td>
</tr>
<tr>
<td>Isooctane</td>
<td>C\textsubscript{8}H\textsubscript{18}</td>
<td>41</td>
<td>24.9</td>
</tr>
</tbody>
</table>

Composition previously used by Shewfelt et al. (2005)
Table 3: Degradation rates and Scale-up Factors

<table>
<thead>
<tr>
<th>Soil type</th>
<th>Duration (days)</th>
<th>Degradation rate constant, (d⁻¹)</th>
<th>Second stage scale up factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Meso-scale (k₂)</td>
<td>Micro-scale (k)</td>
</tr>
<tr>
<td>Delhi</td>
<td>0-8</td>
<td>0.598</td>
<td>0.045</td>
</tr>
<tr>
<td></td>
<td>8-30</td>
<td>0.123</td>
<td></td>
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<tr>
<td>Mixed</td>
<td>0-8</td>
<td>0.477</td>
<td>0.043</td>
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<tr>
<td></td>
<td>8-30</td>
<td>0.093</td>
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<tr>
<td>Elora</td>
<td>0-8</td>
<td>0.460</td>
<td>0.040</td>
</tr>
<tr>
<td></td>
<td>8-30</td>
<td>0.075</td>
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</table>