

Large Scale Bioventing Degradation Rates of Petroleum Hydrocarbons and Determination of Scale-up Factors

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Abstract

Bioventing is a cutting edge, non-destructive treatment method that uses indigenous soil microorganisms in-situ to remediate petroleum hydrocarbons in the unsaturated soil zone. Transferring the application of this technology to a field environment still has some uncertainties due to scale-up challenges. In order to identify the scale-up factor, a 80 kg soil reactor system was developed, consisting of a custom made reactor, climate chamber, low flow venting system and an off gas capture device. Sandy and clayey soils were tested with known concentrations of spiked synthetic gasoline. Various environmental conditions were monitored which included: moisture levels, pH, microbial levels, nutrient and oxygen levels. Results show a second stage degradation rate similar to the degradation rate obtained from research conducted with a 4 kg reactor, giving an average scale-up factor of 2.3 ± 0.4 . The completed research shows that working with a 80 kg laboratory reactor is feasible, yet not always necessary for the development of scale-up factors. A complimentary study with aged soil contaminants was preformed and yielded degradation rates that were significantly reduced.

Keywords: Bioventing, Biodegradation Rate, Bioremediation, Soil Remediation, Scale-up Factor, Gasoline, TPH

INTRODUCTION

Soil contaminated with petroleum hydrocarbons is an environmental issue that poses a threat to human health. Humans can come in contact with contamination through exposure to contaminated surface soils, as well as through drinking contaminated groundwater. In Canada, under the Federally Contaminated Site Action Plan (FCSAP), there are over 5,800 active contaminated sites pending action and over 2,500 suspected contaminated sites that have yet to undergo further assessment (TBCS, 2015). Approximately half of these sites are contaminated with petroleum hydrocarbons (TBCS, 2015). FCSAP is a 15-year, \$3.5 billion dollar program that was established in 2005 by the Government of Canada to reduce environmental and human health risks from known federally contaminated sites (Government of Canada, 2014). Site remediation is expensive and government agencies are always looking for cost effective, yet environmentally responsible methods to clean up these sites.

Total petroleum hydrocarbons (TPHCs) are classified as a non-aqueous phase liquid (NAPL) and are released into the environment from chemical spills among other releases. The problem with large NAPL releases into the soil subsurface, is that a percentage of the release will reach the groundwater, causing groundwater contamination by the NAPL. In order to prevent groundwater contamination, it is necessary to remediate the NAPL quickly. Managing these contaminated sites carries significant costs for the assessment, remediation and long term monitoring that is required. Some of these contaminated sites are in isolated areas and are difficult to access and therefore difficult to assess and remediate.

A method that is frequently used to remediate a site is the excavation of the contaminated material from the site, with the void filled by clean fill. This process is commonly referred to as “dig and dump”. Dig and dump is quick and very effective, but transports the contamination to

another area and changes the natural soil structure of the area, causing a disruption to the local eco-system (Khan *et al.*, 2004). Another remediation method is soil vapour extraction (SVE) that can effectively remove petroleum hydrocarbons from soil in the unsaturated zone. SVE requires the placing of dry wells into the contaminated soil regions and using a pump to extract air out of the unsaturated soil region. This induced vapour flow allows for the faster removal of petroleum hydrocarbon contaminants from the unsaturated soil zone (Khan *et al.*, 2004). However, research has shown that mass transfer limitations develop over time, making it difficult to completely remediate a site. This problem is called tailing and is a significant challenge as the soil still has contaminant levels in excess of the cleanup criteria (Gidda *et al.*, 2006).

Bioventing is a type of in-situ soil remediation similar to the traditional SVE practices but there is no venting of volatilized organic hydrocarbons, only carbon dioxide and clean air. Additionally, there is no tailing of contaminants in the soil as the contaminants are broken down by petroleum-degrading bacteria. Bioventing is a natural biological process that is stimulated by the addition of nutrients, making it an ideal in-situ treatment technology for the remediation of petroleum hydrocarbons. There is a significant amount of evidence on the positive potential of bioventing. However, there is limited information on the decay rate of petroleum hydrocarbons based on site conditions, which leads to a difficulty in predicting the appropriate timeframe for remediation efforts (Frutos *et al.*, 2010).

In terms of bioventing, the biodegradation rates of petroleum hydrocarbons are known to vary with both environmental conditions and soil type. Studies conducted by Harper *et al.* (1998) and Gidda *et al.* (1999) suggest that excess soil water limits the mass transfer for SVE processes, yet this same water is beneficial to the microbial environment for bioventing. Biodegradation rates are greatly impacted by the types of microorganisms present in the soil (Franzluebbers, 2004).

Sufficient nutrient levels within the soil, particularly nitrogen, are required for soil microorganisms and insufficient nitrogen levels reduce the effectiveness of biodegradation (Shewfelt *et al.*, 2005). Shewfelt looked at different forms of nitrogen and concluded that degradation occurred at a rate five times faster than in the control group, when optimum nitrogen levels were created in the soil. Bioventing thus refers to the process of accelerating the biodegrading process by stimulating the microorganisms to increase their populations and activity. Stimulation is done through the addition of nitrogen into the soil. Shewfelt *et al.* (2005) found that the optimum carbon to nitrogen ratio was 10:1 using ammonium chloride as the nitrogen source.

Bioventing soil remediation work and studies have predominantly been conducted in the laboratory environment. Through bioventing experiments that have been conducted in the laboratory at the micro-scale and meso-scale level, scale-up factors have been established comparing the results of one scale to another to better predict the results that occur at the larger scale, like the macro-scale (field) level. Scale-up issues arise from the scale-dependent phenomena that influence the bioremediation process at the micro-scale (respirometers), meso-scale (soil columns and reactors) and macro-scale (field implementation) levels. The heterogeneity of the soil, reactor shape, age, and contaminant bio-availability were found to be major factors that influenced the scale-up factors. The study conducted by Ko (2007) suggests that field scale biodegradation is slower than the results found in the laboratory, partially due to decreased chemical diffusion in the soil, which in turn makes the contaminant less accessible to the biodegraders.

Eyvazi and Zytner (2009) developed a degradation rate correlation based on the type of soil grain, water content and organic matter in the soil. This correlation was developed based on laboratory research and applied to the seven classes of soil that Eyvazi and Zytner (2009) studied,

with each reactor holding 200 g of soil. Khan and Zytner (2013) used the correlation developed by Eyvazi and Zytner (2009) and conducted meso-scale experiments using 4 kg reactors which allowed for the development of a scale-up factor between the two experiments. The resulting scale-up factor was 1.8 ± 0.5 . In addition, a further refinement of the correlation developed by Eyvazi and Zytner (2009) came through the completion of Khan and Zytner's (2013) work on the 4 kg reactor bioventing soil remediation project.

Past research has led to the development of scale-up factors between experiments using different size reactors (Eyvazi and Zytner, 2009; Khan et al., 2015). However, it is unclear if these scale-up factors can be directly applied to scaling up an experimental design and applying it to the data in the field. Ideally field experiments should be done, but there is less control of the various variables. As such, additional work should be completed with larger reactors to determine if the scale-up factors change. Moreover, the degradation behaviour of aged soil contaminants is very different than that of freshly contaminated soil and can significantly impact the remediation process. Sutton et al. (2013) found that ageing of the contamination in the soil can lead to a reduction in the bioavailability due to the length of time that the contaminant has been present in the soil. In their experiment examining aged diesel fuel contamination, it was found that some of the diesel was partially degraded within the soil. Understanding the differences in the degradation rates between freshly and aged contaminated soils is critical for the development of proper site remediation closure times.

Review of the literature shows that there has been no significant bioventing research done at the larger laboratory scale (80-100 kg). Developing an apparatus at this scale would allow the effective evaluation of bioventing performance and provide improved scale-up data. It could also help determine if it is necessary to complete large scale (80 kg) bioventing laboratory experiments

or if micro-scale (200 g) experiments are sufficient. As such, this research project focused on the development of a technique to conduct bioventing research with a 80 kg reactor in the laboratory under optimal environmental conditions. The measured biodegradation rates would be used to revise scale-up factors by comparing the results to previously determined degradation values. A complimentary study was conducted to examine the effect of aging on the degradation rate.

METHODOLOGY

Soil Collection

The study was conducted in a walk-in fume hood laboratory at the School of Engineering, University of Guelph, Canada. For this research project two different types of soil were used. Soil from the Delhi Research Station, Delhi, Canada and the Elora Research Station, Elora, Canada were selected as they had been previously used in the bioventing soil vapour studies by Eyvazi and Zytner (2009) and Khan and Zytner (2013). Using the same soils allowed for a more accurate development of scale-up factors for bioventing SVE using the larger 80 kg reactors.

Soil collected from the field was dried in the laboratory prior to storage. Drying the soil allows the microorganism populations to go dormant until reactivated when water was added to the soil prior to starting the experiments based on the protocols established by (Eyvazi and Zytner, 2009; Khan and Zytner, 2013). In addition to drying the soil, the soil was sifted to remove any large rocks or large pieces of organic matter that were unintentionally collected from the field. A USCS Number 4 sieve with a 4.75 mm mesh size was used to remove all large particles.

Soil Mixing

Throughout the experiment it was necessary to ensure sufficient and uniform mixing of the soil and working with 80 kg of soil for the purposes of a research project was often quite challenging. For mixing the soil with water and synthetic gasoline, 1-gallon (US) paint cans were

chosen. It was calculated that 16 1-gallon cans would be required for each experiment in order to sufficiently fill the 80 kg reactor. Since the cans needed to be mixed twice for about 20 minutes each time, it was not feasible to mix the cans by hand due to physical limitations and the requirement for uniform mixing. As such, a mechanical mixer was designed to hold 4 1-gallon cans and tumble the soil top over bottom. The mixing carrousel was powered by a 1/9th HP low speed, high torque electric motor.

Synthetic Gasoline

Synthetic gasoline was used for spiking throughout the experiment consisting of five compounds: Isooctane (24.9 wt.%), Naphthalene (3.2 wt.%), 1,3,5- Trimethylbenzene (11.9 wt.%), m-Xylene (23.9 wt.%) and Toluene (36.0 wt. %). These five compounds represent the Canada Wide Standards for petroleum hydrocarbons. This mixture was derived from previous experimental work with Imperial Oil Ltd. (Shewfelt *et al.*, 2005) and provide a good representation of the components in gasoline fuels.

Gas chromatography was used to analyze the concentration of petroleum hydrocarbons in the soil taken from the 80 kg soil reactor and GAC off gas samples collected. The Gas Chromatograph (GC) (Hewlett-Packard 5890 Series II) was equipped with a Flame Ionization Detector (FID) as well as an auto sampler (Hewlett-Packard 7673). For the analysis of synthetic gasoline samples a J&W scientific DB5 column was used. This column had previously used by Khan and Zytner, (2013) and Eyvazi and Zytner, (2010). The column was 30 m long with inner diameter of 0.32 mm and a film thickness of 1 µm. Helium (grade: 5.0) was used selected as a carrier gas and the split column pressure was set at 82.74 kpa (12 psi).

In order to determine the concentrations of gasoline present in the soil samples, standard solutions with various concentrations of the synthetic gasoline blend were created to develop a calibration curve. Methylene Chloride (CH_2Cl_2) was the solvent used in preparation of the samples and extraction of the synthetic gasoline out of the soil. Typical solvent extraction efficiencies using the process similar to the one used by Khan and Zytner (2013) achieved an extraction efficiency of approximately 75%. Establishing this extraction efficiency was done by spiking 5 soil samples with known concentrations of synthetic gasoline and then taking an average spiked sample and completing the methylene chloride extraction process.

Air Flow

For the 80 kg soil reactor, the flow rates needed for the soil volume were calculated using the two methods: USACE (US Army Corps of Engineers, 2002) and stoichiometric methodology. Using the porosity of the Delhi soil (0.5) and the bulk density of the soil at 1550 kg/m^3 , the USACE method recommended 0.5 pore volume exchanges per day to minimize volatilization. It was determined that approximately 12.9 L of air would be required per day during the operation of the 80 kg reactor, which is equivalent to 8.9 mL/min as the required flow rate. The stoichiometric method produced a similar result.

In order to provide the desired flow rate of air for the reactor system, a Gilian-Sensidyne (LFS-113DC) low flow pump was used. Monitoring of the air flow rate drawn from the soil reactor was done with an Omega (FMA-5606ST) Micro Flow Meter. This flow rate was comparable in scale to the flow rate used by Khan and Zytner (2013) during work with the 4 kg reactors.

Soil Water Content

Changes in soil water content can impact the microbial activity as well as the air flow through the reactor system. The optimum water content for microorganism growth is approximately 50-77% of the soils field capacity, which equates to about 15 and 20 m³/m³ (Harper *et al.*, 2003). Working with an 80 kg reactor in a highly ventilated laboratory at room temperature (21-23°C) posed the risk for rapid moisture losses. Soil water content in the reactor was monitored using two HOBO soil moisture probes (S-SMC-M005) connected to a HOBO Micro-Data Logger Station (H21-002). Each of the soil moisture probe tips were capable of measuring the soil water content of a 0.3 litre volume of soil as per the specification from Onset, the probe manufacturer (Onset, 2016). Probes were calibrated by comparing representative soil samples that were tested with a standard gravimetric approach.

Several soil water experiments were conducted prior to contaminating the soil with the synthetic petroleum hydrocarbons to test the effectiveness of the moisture control system. The moisture control system consisted of a Plexiglas® enclosure surrounding the reactor and a moisture bubbler column. Humidification of the incoming air was done with a 120 cm tall, 12 cm diameter bubbler column that held approximately 10 L of water. Bubbling was done with a TopFin Air-8000 aquarium bubbler paired with an aquarium stone capable of bubbling air into the column at a rate of 10 L/min. The column was connected to the left side of the Plexiglas® enclosure. The air, then saturated with water vapour, entered the Plexiglas® enclosure and provided humidified air for the bioventing system.

Microbial Activity

It was important to check for the presence of soil microorganisms to ensure colonies were available in the soil. Therefore, microbial sampling was done for each of the experimental tests

for the duration of the experiments. Two particular types of soil microorganisms were of interest, total heterotrophic bacteria (THB) and petroleum-degrading bacteria (PDB). It was out of the scope of this research study to examine the specific microbial colonies present in the soil. The microbial testing carried out was to simply provide an indicator that both THB and PDB microorganisms were present in the soil during bioventing. Using a spread plating technique provided by the manufacturer, two different media were selected to grow the two different types of bacteria. Tryptic Soy Agar (TSA) was used for THB and Bushnell-Hass (BH) medium was used to culture the PDB. The PDB were stimulated in the BH medium by adding synthetic gasoline at 4 g/L as the carbon source directly to the BH-medium just before pouring it into the Petri dishes(Difco, 2015).

Running the Experiment

A nitrogen based additive is required to stimulate the microorganisms in the soil in order for bioventing to be effective. Previous research (Shewfelt and Zytner, 2005; Eyvazi and Zytner, 2009) showed that optimal nitrogen concentrations in the soil needed to stimulate the microorganisms follows the C:N ratio of 10:1. A stoichiometry calculation was used to determine the appropriate amount of ammonium chloride to use. Based on the initial concentration of synthetic gasoline in the soil reactor estimated at 2000 mg/kg, 14.10 grams of nitrogen was required. Nitrogen was in the form of ammonium chloride (NH_4Cl). Ammonium chloride is 26.19 % by wt nitrogen and a total of 53.84 grams was required for each experiment. For simplification of measuring, 54 grams of NH_4Cl was added to 800mL of water and mixed. After mixing, 50mL of the nutrient additive water was added to each of the 16 1-gallon cans. The remaining amount of water was then added to each of the cans to bring the soil to the optimum water content level; cans

were then capped and sealed with lids. The 1-gallon cans were then mixed in groups of 4 for 20 minutes using the mechanical mixer.

After the soil was mixed with the water and the NH_4Cl , it was stored in the laboratory at room temperature (21-23°C) for at least 5 days. This incubation period allowed for germination and reemergence of the natural soil microorganisms required to degrade the petroleum hydrocarbons in the soil. After the minimum 5 day incubation period, all of the 16 cans were placed in the refrigerator at approximately 4°C for approximately 18-24 hours in order to allow the soil to cool. This was done in order to limit volatilization of the synthetic gasoline prior to transfer to the 80 kg reactor.

Using the previously prepared synthetic gasoline stored in the freezer, 25 mL (20.05 g) was pipetted into each of the 16 1-gallon cans that were recently removed from the refrigerator. For the Delhi and Elora experiments, 25 mL or 20.05 g of synthetic gasoline equates to an approximate concentration of 4040 mg/kg in each of the 16 cans. For the aged Delhi soil experiment, the soil was spiked to an initial concentration of 8080 mg/kg, which equates to 50 mL or 40.1 g of synthetic gasoline per paint can. This higher concentration was used to account for some volatilization during the long term storage of the soil. The lids of the cans were then installed and the cans were mixed in groups of 4 for 30 minutes using the mechanical mixer designed for the research.

Loading the soil into the reactor needed to be completed in a prompt manner to limit volatilization of the synthetic gasoline from the soil. Immediately before the gasoline mixed soil was placed into the soil reactor, an approximate 5 gram sample was taken from each of the 16 cans and placed into a 40 mL amber glass vial for GC analysis. Immediately after the soil was loaded into the soil reactor, two soil additional samples were taken as time zero samples and the air pumps

turned on. These samples were referred to as the time zero samples and were the initial synthetic gasoline concentrations in the soil. Based on the practices that were used for this experiment, approximately 25% of the synthetic gasoline volatilized during the handling and packing of the reactor.

Six bioventing experiments were conducted as part of this research project. Two experiments were conducted with the Delhi soil, followed by two experiments with the Elora soils. These four experiments served as the main component of the research project. The fifth experiment was conducted using a combination of the Delhi and Elora soils, referred to as the “layered” test. The layered test stacked Delhi soil on top of Elora soil to create separate soil layers. The purpose of this test was to examine the biodegradation rates that occur in the two soils simultaneously when all conditions are the same. The results of this particular bioventing test were to help to confirm that biodegradation in soil is strongly dependent on the soil type. The final bioventing experiment studied aged contaminated soil. Delhi soil contaminated at an initial concentration of 8000 mg/kg in November 2015 was packed into an aluminum foil lined bin and stored until March 2016. The Delhi soil was then loaded into the reactor and the experiment was conducted using the same procedure used in previous experiments.

Sampling Soil

Using the customized soil sampler, cores were extracted from two different locations on the soil reactor. This procedure was done in order to ensure or test that biodegradation of the petroleum hydrocarbons was occurring consistently throughout the reactor. Each of the two samples of soil were quickly mixed and about 5 grams were placed into a pre-weighed 40 mL amber glass bottles and then reweighed. In each of the 40 mL amber bottles, 20 mL of methylene chloride was added; this solvent was used to extract the petroleum hydrocarbons from the soil.

After about 20 days of reactor operation, 15 mL of methylene chloride was used in order to get a more defined reading on the GC FID.

Once the methylene chloride was added to the vials, the vials were placed on a multi-wrist shaker on medium speed for 1 h then removed from the mixer and placed in the freezer overnight to allow for the settling of sediments. The next day, the samples were poured into 2 mL GC vials and prepared for analysis.

Sampling Off Gases

Sampling the off gases for the experiment was critical to characterize the amount of volatilization occurring in the soil reactor. It also provided a way to characterize the loss in the system. The off gases were captured online before reaching the vacuum pump using a combination of C4011-1 Target Vials and OBRO-30 tubes (National Scientific, 2016) with activated carbon. The tubes were designed to capture petroleum hydrocarbon off gasses at low flows (10-300 mL/min). Both the C4011-1 and the OBRO-30 tubes had two chambers, a front and back chamber separated by a porous synthetic fibre. Each chamber required separate analysis by the GC FID. The C4011-1 tubes were larger in size and were able to capture higher off gas concentrations which were present during the initial stages of the experiment. The OBRO-30 tubes were smaller and were more suitable during the latter phases of the experiment when off gas concentrations were lower.

Methylene chloride was used as the solvent to extract the petroleum hydrocarbons from the GAC. The 40 mL amber vials were placed on a multi-wrist shaker for 1 hour at a medium speed then removed and placed in the freezer overnight. The next day 2 mL of each vial solution was transferred to GC vials and capped in preparation for GC analysis.

Monitoring Oxygen Levels

Monitoring oxygen levels throughout the experiment was essential as it was a good indicator if the soil microorganisms were consuming oxygen to break down the synthetic gasoline and producing carbon dioxide. Ideally, the experiment should have measured carbon dioxide levels throughout, but, this was not feasible. If the reactor operated properly, oxygen levels immediately dropped at the beginning of the experiment and then slowly increased back to ambient lab conditions as the soil microorganisms degraded the synthetic gasoline in the soil.

Oxygen levels in the air leaving the bioventing reactor travelled through the sampling line to the pump and were flowed through a 6 L glass jar. The lid of the glass jar had three small holes: an inlet for the air, an outlet for the air and a hole for the Apogee Oxygen Meter (Apogee Instruments, 2016). Oxygen levels were recorded daily for each of the four experiment trials.

RESULTS and DISCUSSION

Prior to the commencement of the bioventing experiments, time was spent to refine the procedures. This included testing soil water content in the reactor, establishing calibration curves, soil mixing efficiencies and synthetic gasoline extraction efficiencies. Maintaining consistent soil water content was important for the experiment to better represent ideal and consistent environmental conditions. Mixing the spiked soil evenly ensured quality experimental results. Further discussion on these elements follows.

Soil Water Content

Significant water content losses, approximately 70% of initial levels were recorded throughout the 30 day experimental period prior to the development of Plexi-glas® enclosure.

Once the enclosure was developed, and used, water losses were minor as noted in Table 1 for all six of the experiments conducted. The losses measured did not pose a significant impact on biodegradation performance as the optimum moisture content for biodegradation was maintained. Slightly higher levels of water loss were recorded on the outside edge of the reactor for each of the experiments, which was expected as the air entry point. Losses along the edge did not appear to impact the internal soil moisture levels or impact the degradation results. Overall, the soil water content levels did not vary significantly and were still within the optimal range for the bioventing experiment, at roughly 50% of the soils field capacity (Harper *et al.*, 2003).

Oxygen Levels

Oxygen levels were also measured and recorded daily throughout each experiment to ensure that the bacteria were actually breaking down the gasoline. Figure 1 shows the recorded oxygen readings from the Delhi 1 and 2 soil tests which were as expected, very similar in nature.

The initial drop in oxygen concentration in the exhaust off gases directly corresponded to the bacteria in the soil beginning to degrade the synthetic gasoline. As the soil bacteria break down the petroleum hydrocarbons they consume oxygen and produce carbon dioxide and water. It would be expected that the oxygen levels leaving the soil reactor would be lower as some of the oxygen would have been used for the degradation process. Similar results were reported by Khan and Zytner (2013) and Eyvazi and Zytner (2009) when they conducted their bioventing experiments. The general trend of an immediate drop in oxygen levels followed by a steady incline over the life of the experiment was noted in all the experimental trials.

Off Gas Monitoring

The off gasses captured from the air drawn from the venting well were measured and documented as a part of this experiment. It was found that each of the experiments lost between 1-

2% of the initial amount of gasoline over the course of the approximate 30 day operation period through this volatilization process. This amount is considered a reasonable amount of loss for the scale of this experiment. Volatilization immediately spiked but then dropped off within the first 7-8 days. It is important to keep in mind that during the critical second stage of degradation, the off gasses captured were insignificant compared to the amount of synthetic gasoline in the soil reactor.

Microbial Activity

Microbial populations increased slightly as the experiment proceeded as shown in Figures 2 and 3. Stimulated heterotrophic and petroleum-degrading bacteria thrive in environments where there is a constant food source, such as gasoline. Once the gasoline concentrations decreased, the microbial populations subsided due to a lack of food source. The microbial populations, both heterotrophic and petroleum degrading bacteria, were higher in the Elora soil than in the Delhi soil which can be explained by the higher levels of organic matter in the Elora soil. These results were consistent in both trends, as well as approximate CFU/g counts reported by Khan and Zytner (2013). Overall, microbial work was completed to confirm the presence of these microbial species in the soil.

Degradation

A total of six studies were run for approximately 30 days each. Two tests were carried out with each of the Delhi and Elora soils which formed the framework for the research. A layered soil experiment was conducted as a complementary study to prove the concept that bioventing can occur radial to the venting column and is strongly dependent on soil type when environmental conditions are the same. Finally, an aged soil experiment was conducted to determine the impact that aged contaminants would have on the biodegradation rate.

One of the major objectives for this experiment was to obtain degradation rates using the 80 kg reactors for both the Delhi and Elora Soils. Bio-degradation occurred under optimal conditions for this experiment where moisture, nutrient and oxygen levels were maintained for ideal biodegradation. This means that overall it is expected that in the field environment, degradation would be slower as conditions would not be as optimal.

In order to determine the overall degradation rate of the petroleum hydrocarbon in the soil, the results obtained from the gas chromatography analysis were plotted. The results of the experiment showed that the concentration of the petroleum hydrocarbons degraded in an exponential form as noted in Equation 1:

$$\frac{C}{C_0} = e^{-kt} \quad (1)$$

where:

C = concentration of synthetic gasoline in soil, mg/kg

C₀ = initial concentration of synthetic gasoline in soil, mg/kg

k = decay rate, 1/d

T = time, d

This degradation pattern was previously noted in previous bioventing experiments conducted at a smaller scale (Khan and Zytner, 2013; Eyvazi and Zytner, 2009). It is important to note that the results depicted in the figures are the sample averages for each sample day, based on two soil samples collected on a daily basis throughout the entire experiment.

The Delhi experiments were the first in the series of six experiments conducted and as such the Delhi 1 experimental results are a bit cruder than the other experiments performed. Essentially, the Delhi 1 experiment was used to refine the procedure for sampling and working with a

bioventing experiment of this size in a laboratory environment. Microsoft Excel was used to plot the daily degradation results of the experiment having a strong coloration to an exponential degradation model. Figure 4 shows that the R^2 value for the first Delhi experiment was 0.77 compared to the R^2 value for the second Delhi experiment at 0.92. However, when the results of the second Delhi soil experiment were overlaid on the same graph, it could be seen that the degradation behaviour was very similar. To statistically check this difference, a Student's T-Test (McBean, 2014) was conducted on the degradation rates comparing the results of the Delhi experiments 1 and 2. The analysis showed that there was no statistical significant difference in the degradation results. This means that based on the two independent tests completed, the degradation rates were not statistical different.

Similar to the Delhi soil experiment, the Elora results are shown in Figure 5. The first Elora experiment had an initial spiking concentration of 4040 mg/kg of synthetic gasoline which was the same concentration used for both the Delhi soil experiments. The second Elora soil test was spiked with an initial concentration of 2500 mg/kg in order to determine if the contaminant concentration level impacted the two stage degradation rate.

The results of the second Elora experiment shown in Figure 5 indicate there was no noticeable visual difference in the degradation rate. The second stage degradation rate for the first experimental test was 0.071 d^{-1} , compared to 0.084 d^{-1} for the second experiment. In order to quantify this difference, a Student's T-Test was again conducted on the degradation rates comparing the results of the Elora experiments 1 and 2. It was found that there was not a significant difference in the degradation results. This means that based on the two experiments completed, the degradation rates were not statistical different.

Review of the Delhi and Elora degradation results in Figures 4 and 5 indicate that there was a clear change in the degradation rates occurring on the 7-9 day period after the beginning of the experiment. For comparison purposes, Day 8 was selected as the pivotal point where degradation rates seemed to change and the exponential degradation curves seemed to make the best fit based on the R^2 values. It should be noted that this is the same point of inflection of the two-stage degradation as noted by Khan and Zytner, (2013), who also did statistical confirmation. In the first stage, gasoline readily available in the soil water or lightly bonded to the soil particles, was easily broken down by the microorganisms. Of most value to the research was the second stage of the degradation (period starting at Day 8 running to the end of the experiment), as they can be used to develop closure times for remediation. At Day 8 on each of the experimental runs there was a clear transition on the rate of degradation (change in slope of the degradation curve). In fitting the collected data to the exponential degradation relationship there was an evident transition at the 8 day mark of each experiment.

When conducting the two Delhi and two Elora soil bioventing experiments, an effort was made to keep the environmental conditions constant throughout the approximate 30 day operation of the trial. It was important to ensure consistent conditions such as soil moisture, air flow and bacterial levels in order to quantify the differences in degradation of the petroleum hydrocarbons in the two soil types. To extend this examination, an additional experiment was conducted to investigate if the degradation was dependent on soil composition when all environmental conditions were the same. This was checked by conducting a layered soil experiment by placing a layer of Delhi soil in the bottom of the reactor followed by Elora soil on top in the reactor. Based on the results of the layered experiment depicted in Figure 6 and detailed in Table 2, it can be

concluded that degradation is significantly impacted by the type of soil present when environmental conditions are the same.

Figure 6 shows that the second stage degradation rates for the Delhi and Elora soils tests were quite different from each other even from a visual inspection. The Elora soil, which has a large silt and clay component, has a slow second stage degradation rate of 0.072 d^{-1} , when compared to the Delhi soil which is dominated by sandy loam with the second stage degradation rate of 0.112 d^{-1} which are statistically different from each other as shown by the Student's T-test.

Further comparison of the degradation rates was completed by comparing the 80 kg results with the previously determined 4 kg results (Khan and Zytner, 2013). Through statistical analysis it was determined that the degradation rate from the meso-scale (4 kg) experiments were similar to the large 80 kg experiments when the same soil was used. This similarity indicates that there was no scale-up between both sizes, showing that there was no significant advantage in completing bioventing experiments at the larger (80 kg) scale. Completing bioventing soil remediation experiments using 4 kg soil reactors would be easier and provide researchers with a degradation correlation that could be applied to field applications.

A summary of the degradation rates can be found in Table 2, which shows that the second stage degradation rates were lower in Elora soil than the Delhi soil for each of the experimental runs. However, when the layered Elora and Delhi results were compared to the second stage degradation rates from the first four experiments, all degradation rates were similar. After a statistical comparison of the degradation rates for the two different soil types, it was concluded that there was a statistical difference. This is consistent with what previous research found: that soil type is a major determining factor in degradation (Eyvazi and Zytner, 2009; Khan and Zytner, 2013).

Aged Contaminants

An aged contaminant experiment was conducted to better understand the biodegradation rates of soil contaminated with petroleum hydrocarbons for an extended period of time. It is known that when gasoline is spilled onto soil and is in contact with the soil for an extended period of time, the bonds between the gasoline and the soil particles strengthen (Jain, 1992). This increased bond strength becomes a limiting factor for biodegradation as microorganisms are unable to easily breakdown the petroleum hydrocarbons. The reasoning behind this increased bond strength is not well documented and could be a future area of study. Based on the extraction process used for this experiment, extraction efficiency for the synthetic gasoline from the soil was approximately 75% of the initially spike concentration.

The soil used for the aging experiment was contaminated to about 8000 mg/kg and stored in a refrigerated sealed container for 4 months prior to use in this experiment. The initial concentration in the soil after the 4 months in storage was measured to be approximately 2500 mg/kg based on the GC analysis, which after accounting for the 75% extraction efficiency using the methylene chloride and accounting for some volatilization losses, the concentration was 4000 mg/L. The results presented in Figure 7 show the concentrations measured after extraction without correction to show the day over day comparison to provide a degradation coefficient. It should be noted that when the aged soil was removed from aging container, it was stimulated with ammonium chloride on day 0 and immediately loaded into the reactor.

In the aged experiment it was noted the gasoline concentration did not drop immediately as the soil was stimulated at the start time rather than 5 days previous like the other bioventing experiments conducted. This was done since the soil was spiked with synthetic gasoline and sat in refrigerated storage for a period of four months prior to use in the experiment. Based on research

conducted by Jain (1992) and through analysis of the results after the 4 d period, the stimulated soil microorganisms dissolved the readily available gasoline in the soil moisture. In the second stage, degradation slowed and the process became more limited as the microorganisms began to degrade the petroleum hydrocarbons that were strongly bonded to the soil particles. In comparison to the biodegradation results from the Delhi soil experiment where the soil was freshly spiked, it is evident that the degradation rate is significantly slower, by a factor of two, in soils that had aged contamination. It was also noted that in the aged soil experiment there were no two distinct stages of degradation. This is explained by the lack of loosely bonded petroleum hydrocarbons present in the soil. The degradation rate for the aged contaminant experiment averaged 0.053 d^{-1} compared to the average second stage degradation rate for the freshly contaminated Delhi soil of approximately 0.118 d^{-1} .

Several studies have been completed indicating that a strong bond exists between soil particles and petroleum hydrocarbons, which can have a limiting factor on biodegradation (Chang *et al.*, 2011 and Sutton *et al.*, 2013). In freshly contaminated soil, the bonds between the soil particles and the petroleum hydrocarbons are weaker and can be dissolved back into the soil water when a concentration gradient exists (Jain *et al.*, 1992). Not only does this make the contamination harder to bio-remediate but impacts the solvent extraction efficiency. Studies have shown that extraction efficiency can drop to almost 50% for some types of petroleum hydrocarbons after a period of 28 days (Jain *et al.*, 1992). Aged contaminants require the soil microorganisms to first breakdown the petroleum hydrocarbons from the soil particles by using the microorganisms' bio-surfactants. Once the petroleum hydrocarbons are removed from the soil, it can be continuously biodegraded by the soil microorganisms (Jain *et al.*, 1992). Further study into aged contaminants

and the effect they have on the biodegradation process may be appropriate as it will help researchers develop remediation closure times for sites with this specific issue.

Development of a Scale-up Factor

Scale-up factors were only developed for the second stage of degradation as they are required for the development of the site closure time. Table 3 shows the degradation rates for the experiments conducted along with the corresponding scale-up factors.

The scale-up factors for this experiment were developed using the average of the two degradation rates for the 80 kg experiment and comparing them to the degradation rate from the micro-scale experiment conducted by Eyvazi and Zytner (2009). For the Delhi soil a scale-up factor of 2.7 was determined as compared to a scale-up factor of 1.9 for the Elora soil. When comparing the scale-up factors from the micro to meso-scale (4 kg) and micro to meso-scale (80 kg), it was noted that they are the same. This is expected as noted earlier where the Student's T-Test showed there was no statistical difference in the degradation rates from the 4 kg to 80 kg bioventing experiments.

Soils with aged petroleum hydrocarbons take a significantly longer time to biodegrade than soils with fresh contamination. Aged contaminants, particularly petroleum hydrocarbons, develop very strong bonds with soil particles which limit petroleum degrading bacteria from breaking down the contaminant (Jain *et al.*, 1992). Results of the aged contaminant experiment with the Delhi soil produced a second stage degradation rate of 0.053 d^{-1} which was twice as slow as the degradation measured during the freshly contaminated experiments. Scale-up factors have not previously been estimated for biodegradation with aged soil contaminants as aged soil bioventing experiments with these particular soil types and methodology has not been studied.

CONCLUSION

The completed research project examined the use of bioventing soil remediation as a viable clean-up technology for the removal of petroleum hydrocarbons from unsaturated soil. The completed experiments developed a 80 kg soil bioventing reactor system consisting of a custom-made reactor, climate chamber, low flow venting system and an off gas capture device. Environmental conditions that were monitored included: soil water content levels, microbial levels, nutrient and oxygen levels, ensuring an accurate representation of natural soil environmental conditions. Gas chromatography was used to measure concentrations in the soil samples taken daily from the reactor.

Results from the study show that a two-stage degradation process exists and a scale-up factor similar to the results obtained from a previous study at the meso-scale (4 kg) conducted by Khan and Zytner, (2013). The Delhi (sandy loam) soil had a second stage degradation rate of 0.12 d^{-1} and the Elora (silt loam) soil had a degradation rate of 0.075 d^{-1} . The corresponding scale-up factor was 2.7 for the Delhi soil and 1.9 for the Elora soil, with an average value of 2.3 ± 0.4 . Through analysis it was determined that the degradation rate from the meso-scale (4 kg) experiment showed no statistical difference from the degradation rate at the large (80 kg) scale when the same soil was used. This similar scale-up factor indicates that there is no significant advantage in completing bioventing experiments at a larger (80 kg) scale. As such, a degradation correlation that could be scaled up, could be applied to field applications.

An aged soil experiment was conducted to test the impact that aged contaminants have on the degradation process. Aged contaminants, particularly petroleum hydrocarbons, develop very strong bonds with soil particles which limit petroleum degrading bacteria from breaking down the contaminant. Results of the aged contaminant experiment with the Delhi soil produced a second stage degradation rate of 0.053 d^{-1} which was twice as slow as the degradation measured during

the freshly contaminated experiments. Overall, it is possible to complete bioventing soil remediation experiments that mimic field conditions and provide degradation correlations and scale-up factors that can be applied to field applications. However, there are some limitations to this, as soils with aged petroleum hydrocarbons take a significantly longer time to biodegrade than soils with fresh contaminants. Aged contaminants, particularly petroleum hydrocarbons, develop very strong bonds with soil particles which limit petroleum degrading bacteria from breaking down the contaminant.

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Table 1: Soil water losses recorded for each of the experiments.

Experiment	Location	Initial (m³/m³)	Final (m³/m³)	Total Loss (%)
Delhi 1	Inner	0.239	0.228	7.4
	Outer	0.254	0.238	12.7
Delhi 2	Inner	0.224	probe error	-
	Outer	0.196	0.192	2.0
Elora 1	Inner	0.152	0.139	6.9
	Outer	0.169	0.153	8.0
Elora 2	Inner	0.219	0.214	2.5
	Outer	0.176	0.164	6.0
Layered	Inner	0.207	0.175	15.1
	Outer	0.209	0.173	17.2
Aged	Inner	0.195	0.176	9.4
	Outer	0.207	0.171	17.2

Note: Initial and Final m³/m³ are direct output values taken from the HOBO Loggers. Total Loss takes into account the difference soil density and represents the total loss of moisture.

Table 2: Degradation rates for 80 kg large scale bioventing experiment for both of the Delhi and Elora soils as well as the Layered soil experiment.

Soil Type	Duration	80 kg Exp. 1	80 kg Exp. 2	Layered
Delhi	0-8	0.218	0.341	0.251
	8-30	0.141	0.101	0.112
Elora	0-8	0.389	0.384	0.362
	8-30	0.068	0.081	0.072

Note: Calculated using the average degradation rate constant for each 80 kg experiment.

Table 3: Degradation rates for the micro-scale, meso-scale and large scale bioventing experiments

Soil Type	Duration	Micro-scale	Meso-scale	Scale Up Micro to Meso	80 kg Exp. 1	80 kg Exp. 2	Layered Soil	Scale Up Micro to 80 kg
Delhi	0-8		0.598		0.218	0.341	0.25	
	8-30	0.045	0.123	2.7	0.141	0.101	0.11	2.7*
Elora	0-8		0.460		0.389	0.384	0.36	
	8-30	0.040	0.075	1.9	0.068	0.081	0.072	1.9*

Notes:

Calculated using the average degradation rate constant for each 80kg experiment.

Micro-scale (Eyvazi and Zytner, 2009)

Meso-scale (Khan and Zytner, 2013)

Scale-up factors are calculated using the reference Micro-scale (Eyvazi and Zytner, 2009) experiment

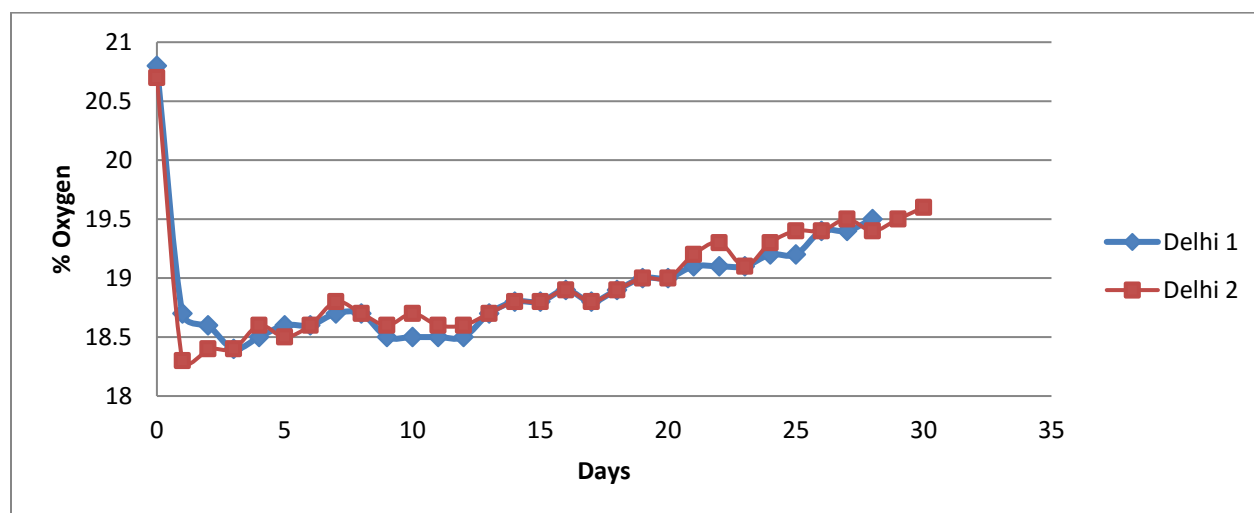


Figure 1: Oxygen readings from Delhi soil

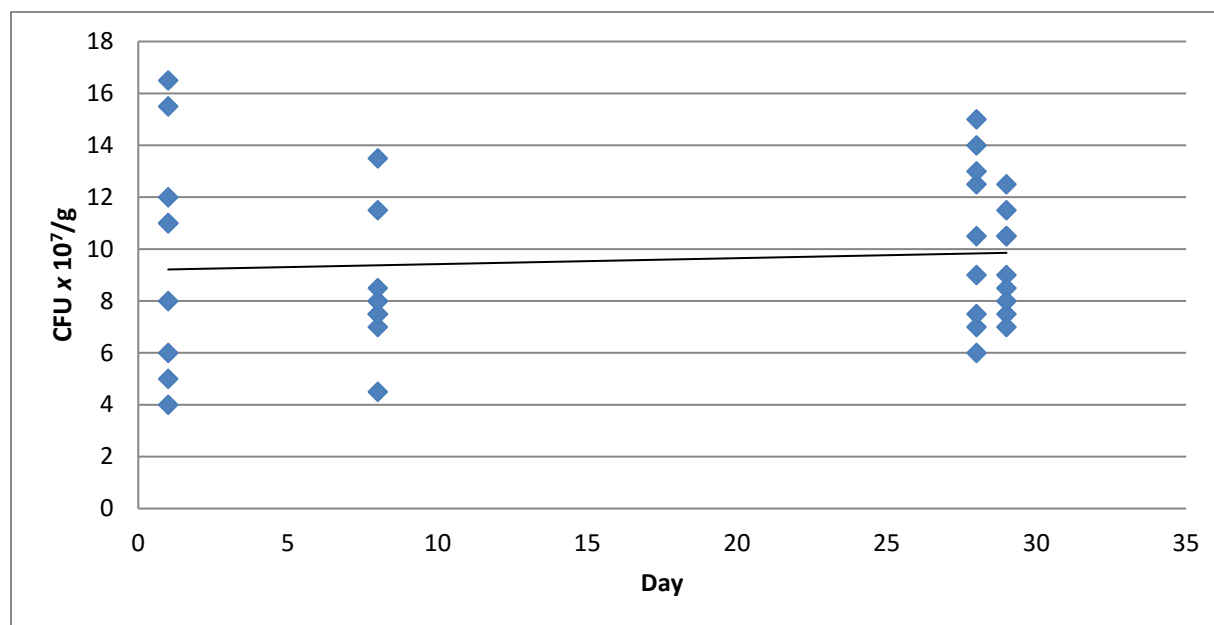


Figure 2: Total Heterotrophic Bacteria for Delhi soil

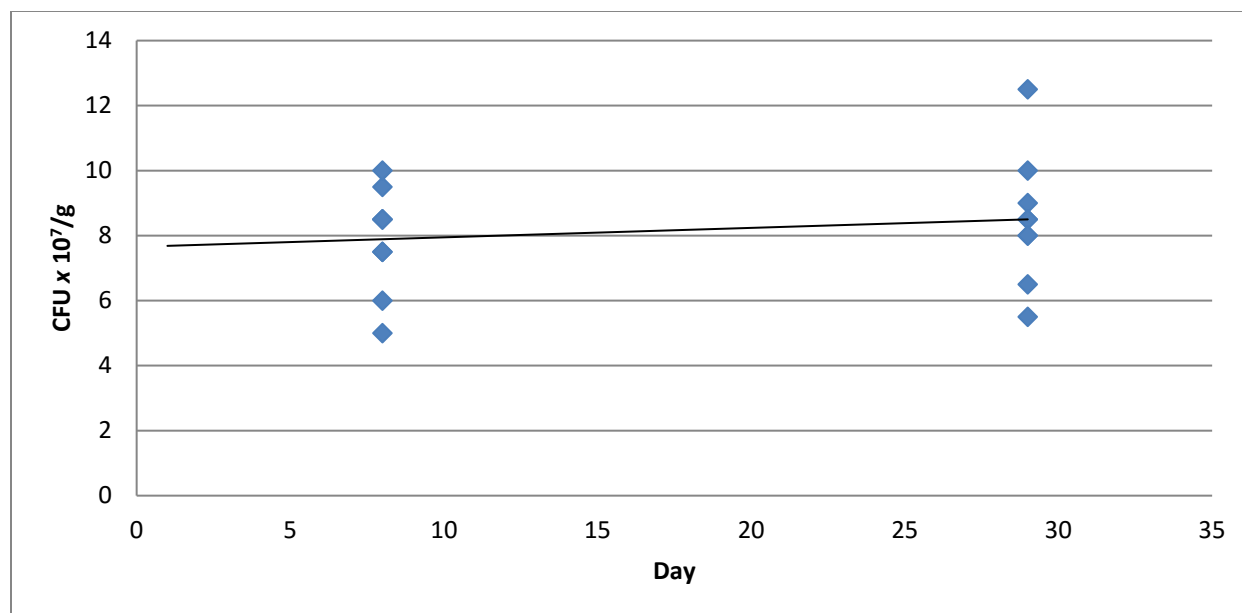


Figure 3: Total petroleum degrading bacteria for Delhi soil

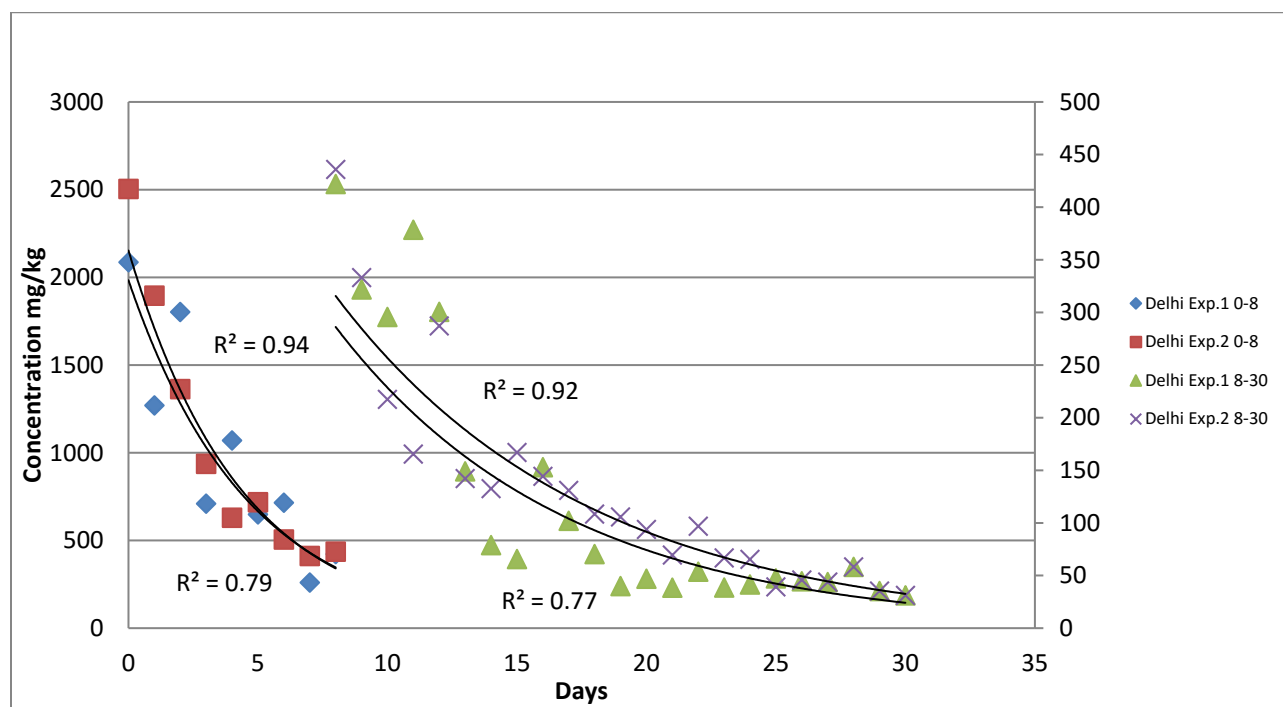


Figure 4: Two stage exponential decay for Delhi soil

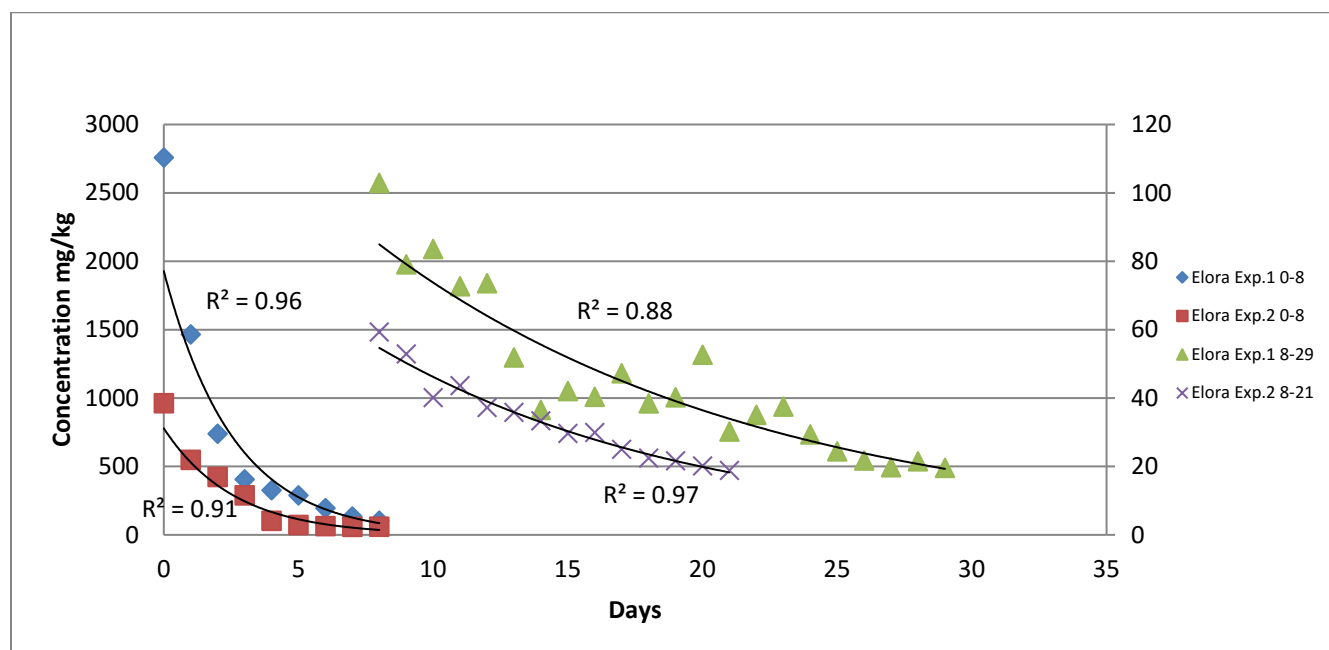


Figure 5: Two stage exponential decay for Elora soil

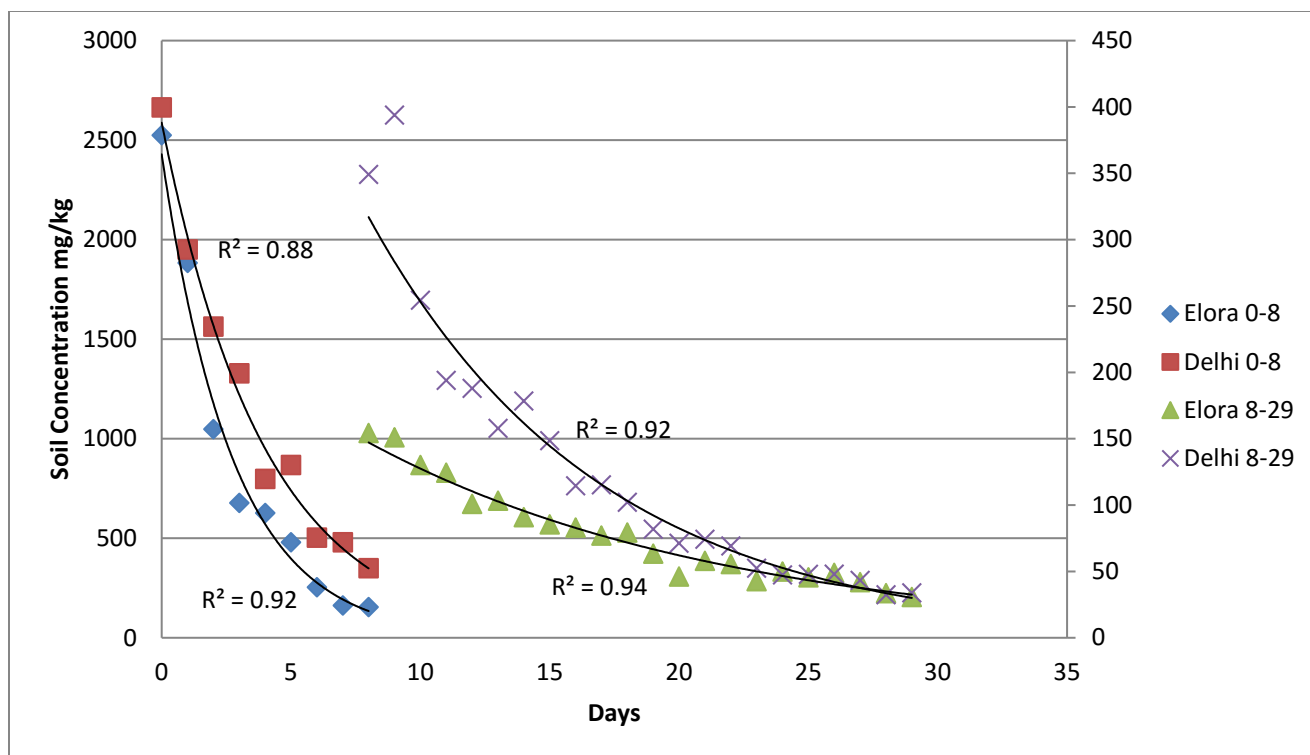


Figure 6: Biodegradation trends for layered soil bioventing experiments

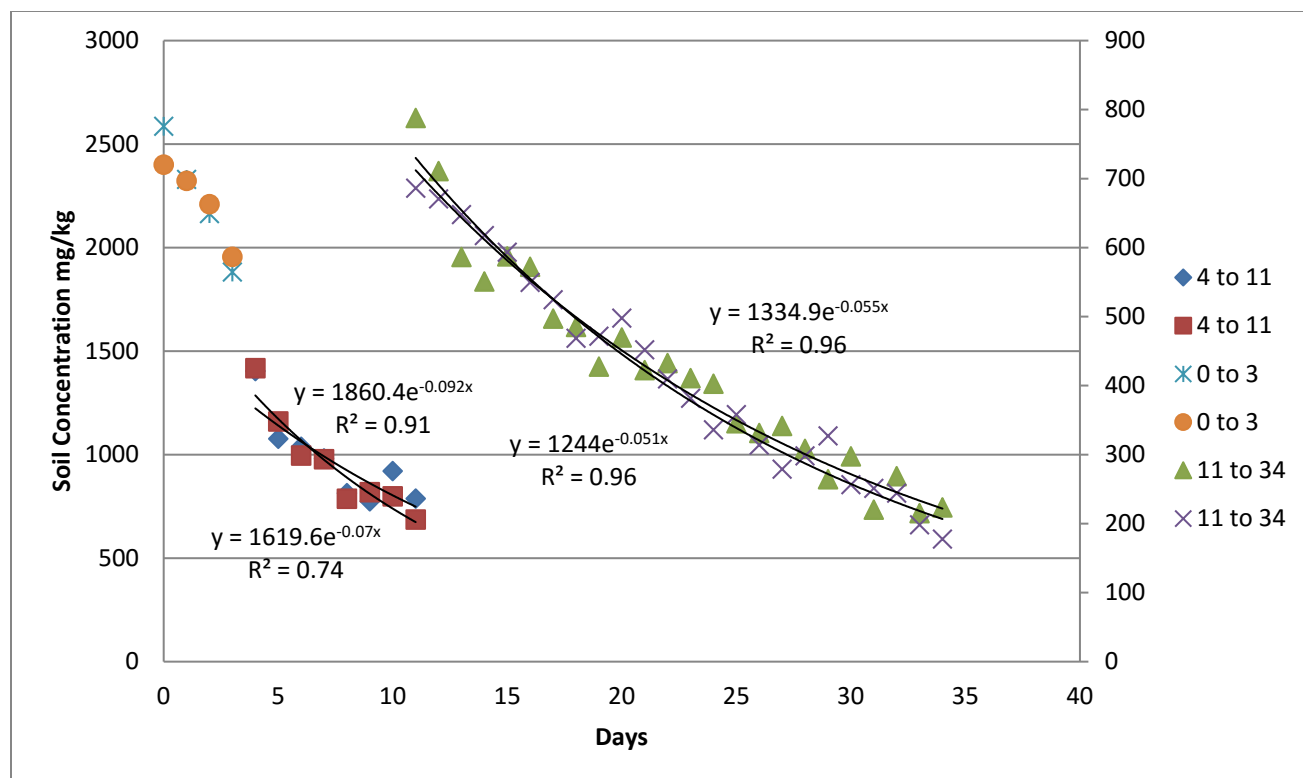


Figure 7: Aged soil bioventing in Delhi soil