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IMPACT OF AGED CONTAMINATION ON BIOVENTING PERFORMANCE

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Abstract: Bioventing can be an effective in situ remediation method to clean-up a site contaminated with petroleum hydrocarbons. Significant work has been completed on determining the optimum conditions needed to stimulate the native microorganisms so that native bacteria degrade the petroleum hydrocarbons. Understanding on scale-up factors has improved so that smaller laboratory experiments can be run, and then the degradation rate adjusted to represent what would take place at a larger scale. However, much needs to be learned on the impact of aging. Aging happens when petroleum hydrocarbons are released into the soil and stay in contact with the soil for an extended period of time, causing a bond to develop between the contaminant and the soil particles. This bond makes it difficult for the microorganisms to access the petroleum hydrocarbons.

Experiments were completed by spiking loamy sand to about 8000 mg/kg and storing the mixture four to ten months in the refrigerator, followed by testing in an 80 kg bioventing reactor. Two conditions were tested, dry soil storage and wet soil storage. Additional experiments were completed by spiking the soil, aging the wet mixture and then conducting bioventing tests at regular intervals using 150 g respirometers to determine if the time of aging had an impact on degradation. The respirometers showed an increase in degradation rate for the wet loamy sand, starting at 0.079 d^{-1} for freshly contaminated soil to 0.171 d^{-1} for soil aged up to 300 days. In contrast, the 80 kg reactor results showed no change in degradation rate of the aged experiments for the loamy sand soil under similar aging conditions. However, for the dry-aged conditions, the degradation rate was half that of the freshly contaminated reactor test after only four months of aging; 0.053 d^{-1} as compared to 0.118 d^{-1}. In all cases, a sharp reduction in extractable contaminants was observed due to an increase in aging. These results show that the bonding that happens through aging impacts the bioavailability and the rates of biodegradation differently under different aging conditions and soil types, which ultimately increases the time required to attain site closure in large-scale experiments.

1 INTRODUCTION

Sites contaminated with petroleum hydrocarbons are a challenge worldwide. The contamination negatively impacts the soil and water environment, increasing the exposure to all forms of life, with an ultimate impact on humans. In Canada it is estimated that are approximately 22,000 federally contaminated sites, with an estimated liability of $4.9 billion (OAGC, 2012). The Federally Contaminated Site Action Plan (FCSAP) in Canada indicates that there are an additional 2,500 suspected sites that still have to undergo assessment (TBCS, 2015). As a result, site remediation is a big business and governments are always looking for cost effective, yet environmentally responsible methods to clean up these sites.

A popular remediation technology is bioventing. In this process the native microorganisms are stimulated by adding sufficient nutrients, water and oxygen, which allows the microorganisms to environmentally

breakdown the contamination into harmless by-products (Zytner et al., 2001). Bioventing is a non-destructive approach that is cost effective and can address the remnants of other technologies like soil vapour extraction that cease to be effective due to mass transfer limitations that develop.

Significant effort has been spent on determining the rate coefficients of the bioventing process as a function of soil types. These rate coefficients help estimate the time of remediation (Frutos et al., 2010; Eyvazi and Zytner, 2009). Sutton et al. (2013), Chien et al. (2009) and Kao et al. (2010) noted that the results and correlations developed in typical laboratory respirometers do not always represent what occurs in the field due to the impact of heterogeneity and aging of contaminants. As a result, Khan et al. (2015) and Mosco and Zytner (2017) determined scale-up factors to assist with the transfer of lab results to the field, using 4 kg and 80 kg reactors respectively with different soils, with the degradation results compared to the results from 150 g respirometers (Eyvazi and Zytner, 2009) for similar soils. The scale-up factors in both sets of experiments were found to be approximately 2 in both cases, where the 4 kg and 80 kg degradation rate coefficients were larger than the 150 g rates.

Determining the scale-up factors showed that the 150 g degradation rates need to be adjusted when estimating bioventing performance. The work by Mosco and Zytner (2017) also showed that experiments with the largest reactor (80 kg) are not needed, as the 4 kg size reactors are easier to use and provide enough degradation information. However, these scale-up experiments did not consider the aging affect. Jain et al (1992) reported that aging causes chemical bonds between soil particles and petroleum hydrocarbons, which becomes stronger with an increase in age. This is supported by the work of Sutton et al. (2013), where aged contamination lead to a reduction in the bioavailability of the hydrocarbons to the microbial population. As a result, remediation efforts would require additional time when using bioventing. Understanding the differences in the degradation rates between freshly and aged contaminated soils is important for evaluating remediation options. Consequently, large (80 kg) and small scale (150 g) experiments were completed to determine the degradation rates of aged contamination and compare it to fresh contaminated soil.

2 METHODOLOGY

The two soils used for the study. One was Delhi loamy sand, collected from the Delhi Research Station, in Delhi, ON, the other Elora silty loam obtained from the Elora Research Station. After collection, both soils were air dried and sieved to pass a 2 mm sieve opening. Khan and Zytner (2015), previously determined that the loamy sand had a porosity of 0.4 and an average bulk density of 1550 kg/m^3, while the silty loam had a porosity of 0.48 and a bulk density of 1350 kg/m^3.

After drying, the soil was spiked to an initial concentration of 8080 mg/kg of synthetic petroleum hydrocarbon in the mixing cans. No water was added to the soil and synthetic gasoline mixture. This higher concentration accounts for some volatilization during the long-term storage of the soil in the refrigerator. After mixing, the soil was placed into aluminum lined containers, sealed and placed in the refrigerator for approximately five months to minimize volatilization during the aging process.

Following five months of storage, the loamy sand soil was placed into mixing cans. Nitrogen in the form of NH4Cl was dissolved into sufficient water to achieve a 15 – 18 % soil water content by weight, which would provide the required 50 % water content of the soils field capacity. This provided the ideal water content and C:N ratio of 10:1 for optimum degradation (Eyvazi and Zytner, 2009). After mixing, the soil was packed in layers in the 80 kg reactor to a bulk density of 1550 kg/m^3. Speed was of the essence to minimize volatilization. The 80 kg reactor was then placed in an experimental chamber within a fumehood. The chamber made it possible to control the air flow rate at 8-10 mL/min. The air was humidified via a bubbler to ensure that the soil did not dry out, which would have stopped the degradation process. The reactor was also wrapped a moist cotton wrap to help minimize the loss of water from the reactor. The reactor also had a vapour sampling port where granular activated carbon sorption could be installed to capture any petroleum hydrocarbons. Analysis showed that volatilization was minimal as expected. Complete details are in Mosco and Zytner (2017).

In order to determine if water content played a role in the aging process, additional loamy sand was taken and spiked to a level of 4000 mg/kg of synthetic petroleum hydrocarbon. In addition to the synthetic gasoline, water was added at a soil water content of 15 % to test aging under wet conditions. The soil was then stored for approximately ten months in the refrigerator. After 10 months, ammonium chloride was added to the soil to stimulate the microbial population and then placed in the 80kg reactor to conditions similar to the dry soil conditions.

For both 80 kg reactor tests, the degradation rate was tracked by taking approximately 5 g of soil sample from the reactor at regular intervals using a specifically design coring device. The soil was placed in 40 mL amber vials, to which 20 mL of methylene chloride was added to extract the petroleum hydrocarbons. The vials were then placed on a shaker for 1 h, after which the vials were placed in a freezer overnight to allow the sediment to settle. The next morning, approximately 2 mL of solvent was removed from the supernatant and placed into gas chromatograph (GC) vials for analysis. Chemical analysis was done on a GG equipped with a Flame Ionization Detector.

Loamy sand soil was also prepared for respirometer testing, where it was wet aged as for the large reactors, but this time a portion of the soil was removed on regular intervals. The respirometers were prepared by placing 150 g of the stimulated soil in 1 L glass bottles that could be sealed. However, before sealing, a test tube of KOH was placed inside the respirometer to carbon dioxide produced by the microbial respiration (Eyvazi and Zytner, 2009). The respirometers were incubated at 25°C. Periodically, the respirometers were vented to ensure the oxygen concentration remained at an adequate level, but air flow through the soil is kept entirely passive. This procedure kept volatilization to a minimum. Every few days, a respirometer was sacrificed to extract soil samples to track degradation rates. The soil samples were analyzed using the same method as described above, for the 80kg reactor.

Extracted soil samples were also tested for the presence of soil microorganisms. The two groups of interest were total heterotrophic bacteria (THB) and petroleum-degrading bacteria (PDB). Gasoline was added to the PDB mixing solution at a rate of 4 g/L to provide the food source. Using a spread plating technique, 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. Serial dilution was used to plate 100 uL of solution on each batch of TSA and BH plates. The TSA plates were counted for colonies after 48 h and then reconfirmed after 72 h in the incubator. The BH plates were counted for colonies after 7 d and then reconfirmed after 14 d in the incubator. The results of the experiment were reported as colony forming units per gram of soil weight (CFU/g).

3 RESULTS

3.1 Large Reactor

The loamy sand soil used in the large reactor aging experiment was spiked to a concentration of about 8000 mg/kg in dry soil. Sampling the soil prior to the degradation experiment, showed that after 4 months of storage, the initial concentration had dropped to 2500 mg/kg. This was deemed acceptable based on all the handling and storage losses, mainly through volatilization, which was reduced due to the refrigerated storage. After sampling the soil, it was immediately stimulated with ammonium chloride and placed in the reactor, where bioventing took place at room temperature.

Figure 1 shows the aging data collected by Mosco and Zytner (2017). Two reactors were run in succession, with the data sets showing good agreement. Figure 1 shows that there is a two stage degradation process; Stage 1 - Day 0 to 11 with solid circles and open triangles; Stage 2 – Day 11 to 34 with open circles and solid triangles. However, the second stage was not as predominant as the non-aged experiments completed by Mosco and Zytner (2017). The difference in second stage degradation was attributed to the fact that the aged soil experiments needed to acclimate themselves first as nutrients were added to the soil just prior to placement into the reactor. For the non-aged soil, the nutrients were added 5 days prior to gasoline spiking. For the aged soil it was assumed that the 4 months of storage in the refrigerator would allow for sufficient acclimation. After the 4 d, the stimulated soil microorganisms dissolved the readily available gasoline in the soil water and the degradation pattern was similar to the non-aged experiments.

The first order degradation rate for the data given in Figure 1 averaged to 0.053 d^{-1} for both experiments. This rate is approximately half the degradation rate of the freshly contaminated Delhi loamy sand, which had a degradation rate of approximately 0.118 d^{-1}. The difference in degradation rates can be attributed to the increased bonds between the gasoline and the soil particles (Jain, 1992). This increased bond strength becomes a limiting factor for biodegradation as microorganisms are unable to easily breakdown the petroleum hydrocarbons. 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. 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.

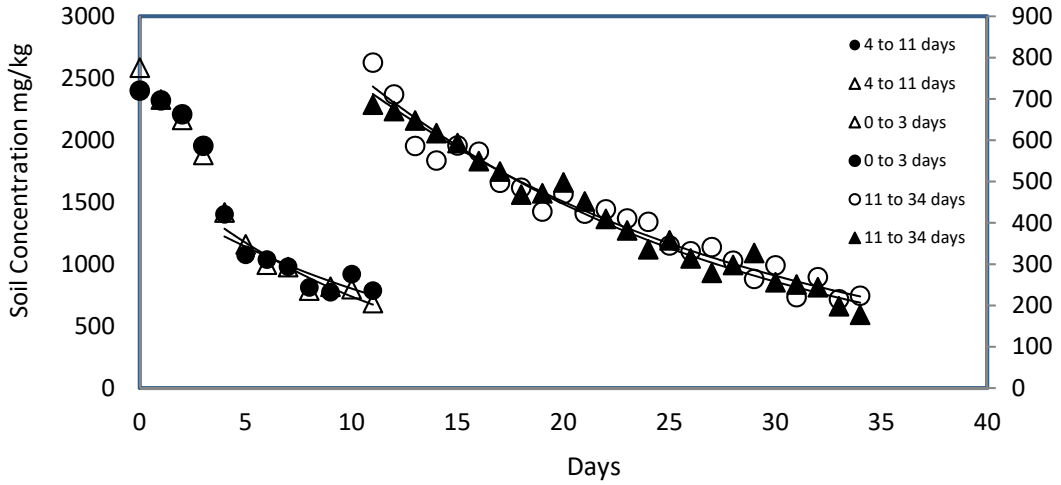

Figure 1: Degradation Trend of 80 kg Reactor – Delhi Loamy Sand

The degradation trend of the wet-aged loamy sand soil that was stored for approximately 10 months was seen to be a single-stage degradation rate. The first order degradation rate was measured at 0.129 d^{-1} in the one reactor that was run. This rate is statistically similar to the degradation rate of the freshly contaminated soil, at 0.118 d^{-1}, and twice that of the 4 month dry aging test. The increase in degradation rate for the wet aging was attributed to the difference in aging process, where wet-aging allowed for the microbial communities to better acclimatize to the contaminants present in the soil, providing for improved biodegradation of the petroleum. The microorganisms were not dormant like in the dry aged soil and were able to start breaking down the contamination once stimulated by the nutrients.

Review of the results for the Elora silty loam soil from the 80 kg reactor showed no measureable signs of degradation. This suggests that the long term aging increased the bond to such a state that the microorganisms were unable to access the synthetic hydrocarbons. Based on the trends observed for both soils, further study into the aging condition is needed. Why does dry aging have such an impact on the degradation rate? What are the governing soil and chemical interactions? The level of spiking may also have an impact. The wet-aging condition was spiked at a lower level and research is needed to determine if this was a governing condition.

As expected, the microbial testing showed that the stimulated heterotrophic and petroleum-degrading bacteria thrived in both soil environments as there was a constant food source such as gasoline. Overall, microbial work was completed to confirm the presence of these microbial species in the soil and that they had increased.

3.2 Respirometers

Respirometer studies were first completed with the loamy sand. Respirometer experiments with 150 g of soil that were exposed to different aging intervals were completed to determine if there was a difference in the degradation trend when compared to the 80 kg reactor. The initial spiking was at 4000 mg/kg, and then over the course of 10 months, bottles were sacrificed to provide the aged soil for the experiment without disturbing the aging process for the rest of the batch. Results showed that the starting concentration (post aging) decreased from around 2000 mg/kg, which account for the losses due to mixing and handling of the soil, to less than 100mg/kg after 9 months.

Figure 2 shows that there a general increase in the measured degradation rates with an increase in contaminant age for the loamy sand soil for wet aging. The degradation rates increase from 0.079 d^{-1} to 0.171 d^{-1} over the course of 10 months. The increase in degradation rate is likely due to the acclimatization of the bacteria during the aging process, creating colonies that thrive better in TPH-rich soil and that are better at the desorption of TPH once stimulated by nutrients. The presence of water also made it easier for the microorganisms to access the contamination. Taking the slope of the line in Figure 2 allows the calculation of the overall degradation for the wet-aged soil. The overall degradation rate was 0.146 d^{-1} at the 150 g scale, which is statistically similar to the 0.129 d^{-1} measured in the 80 kg reactor for the loamy sand. The equal degradation rate, respirometer vs 80 kg reactor for similarity aged soils is different from that found in freshly contaminated soil, where the larger reactors had a degradation rate that is approximately twice that of the respirometer (Mosco and Zytner, 2017).

Respirometer tests were also done with the Elora silty loam soil. As in the 80 kg reactor, no measureable signs of degradation were seen. This suggests that the long term aging increased the bond to such a state that the microorganisms were unable to get any access to the synthetic hydrocarbons.

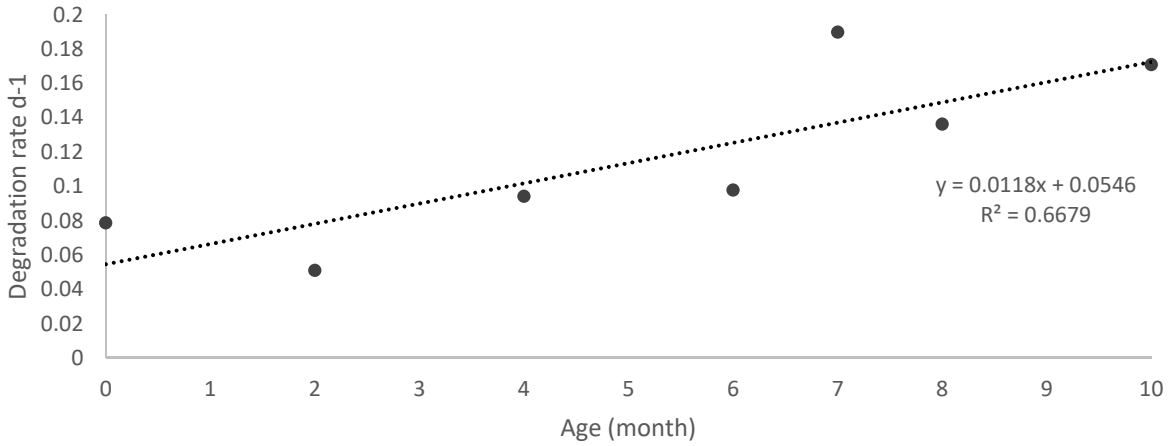

Figure 2: Degradation Rate Increase in 150g Reactor – Delhi Loamy Sand

The change in behaviour in degradation rates between fresh and aged soil highlights the differences between dry and wet-aging. The dry soil was air dried, while the wet-aged soil was at a 15% water content. At the higher water content aged soil there is an increase in degradation rate from both the 150 g experiments and at 80 kg, whereas the dry-aged soil showed a decrease in the degradation rate. It shows that the presence of water allows for better bacterial acclimatization and improved degradation. This behaviour needs to be investigated further for additional soils and conditions.

3.3 Application Implications

The remediation implications of this research suggest that spills in dry soils will take longer to remediate if they are allowed to age for an extended time period. First the microorganisms will have to be reactivated from the dormant state by adding sufficient water, and then stimulated with nutrients to ensure that they

degrade the spilled contamination. Conversely, it can be expected that an aged petroleum hydrocarbon spill in a wet unsaturated soil will remediate faster once the appropriate nutrients are added.

The type of soil also plays a role in remediation. It was observed that the loamy sand has a faster degradation rate than the silty loam. This is consistent with the findings of Eyvazi and Zytner (2009) and Khan and Zytner (2015) where the soils with a higher fraction of fine particles are more difficult to remediate. The main cause would be increased sorption, which impacts remediation for both fresh and aged spills. The result is a remediation process that would take longer to reach closure.

4 CONCLUSIONS

Bioventing experiments were conducted with a 150 g respirometer and an 80 kg bioventing reactor by spiking two types of soil four to ten months in advance and storing the mixture in a refrigerated sealed container. Additional experiments were conducted by spiking the soil and letting it age at different intervals to determine the time period where aging had an impact on degradation. The respirometers showed an increase in degradation rate for the loamy sand, from 0.079 d^{-1} for freshly contaminated soil to 0.171 d^{-1} for the wet aged soil that was aged up to 300 days. It was also seen that the degradation rate from the respirometers was statically similar to the degradation rates from the 80 kg reactor, suggesting no scale-up factor under wet aging conditions. The decrease in bioavailability was further seen by the silt loam soil test where no degradation was measured after 250 days of aging in both the respirometers and 80 kg reactors.

The results suggest that water content of the soil during aging is a factor. Dry soil aging minimized microbial acclimatization and the microorganisms most likely went dormant. Thus when remediation began, the microbes first had to be reactivated when stimulated. The result being a degradation rate for the dry aged soil that was half of the freshly contaminated reactor test after only four months of aging; 0.053 d^{-1} as compared to 0.118 d^{-1}. In all cases, a sharp reduction in extractable contaminants was observed due to an increase in aging. The tests and analysis showed that the petroleum contamination was sufficiently tightly bound that microorganisms could not access the petroleum hydrocarbon. These results show that aging impacts the bioavailability and the rates of biodegradation, and the complexities and differences between different aging methods, ultimately increasing the time required to attain site closure in large-scale experiments.

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