IMPACT OF COLD TEMPERATURES ON BIODEGRADATION RATES
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ABSTRACT
The impact of cold temperatures on biodegradation rates is poorly understood and compounded by the lack of published data, particularly under field conditions. This paper presents a collection of case history data from monitoring and remediation projects completed at cold temperatures. The first data set is compiled from 60 case histories under anaerobic conditions (47 field and 13 laboratory) from sites where groundwater temperatures are typically less than 10°C. By normalizing the data to +5°C and +10°C, the scatter in rates for BTEX degradation in groundwater was dramatically reduced. The second data set collated information from 47 ex-situ soil remediation projects conducted in Northern Canada. Biopiles and landfarms were implemented at 66% and 28% of sites surveyed, respectively. Biodegradation rates ranged from 0.26 to 760 mg/kg/d. Common limiting factors included temperature, aeration, and amendment addition.

RÉSUMÉ
L’impact des températures froides sur des taux de biodégradation est mal compris et composé par le manque de données éditées, particulièrement sur le terrain. Cet article présente une collection de données d’antécédents des projets de surveillance et de remédiation accomplis aux températures froides. Le premier modém a compilé de 60 antécédents dans des conditions anaérobies (champ 47 et laboratoire 13) des emplacements où les températures d’eaux souterraines sont typiquement moins que 10°C. En normalisant les données au 5°C et 10°C, l'éparpillement dans les taux pour la dégradation de BTEX en eaux souterraines a été nettement réduit. Le deuxième modém a assemblé l'information de 47 projets ex-situ de remédiation de sol conduits au Canada nordique. Biopiles et landfarms ont été mis en application à 66% et 28% d'emplacements a examiné, respectivement. Les taux de biodégradation se sont étendus au terrain communal de 0.26 a 760 mg/kg/d. Facteurs limitant inclus la température, l'aération, et l'addition d'amendement.

1. INTRODUCTION
Bioremediation of organic contaminants, primarily petroleum hydrocarbons, has become a widely accepted cost-effective remediation method. In cold and arctic climates however, colder temperatures will slow the activity of the microbes. In addition, there is a paucity of data on biodegradation rates under cold temperatures, particularly for field applications. In response Alberta Environment and Environment Canada sponsored separate projects at the University of Alberta in 2004/2005 to collate case history data from monitoring and remediation projects for regulators and practitioners to utilize.

The first study was to develop a groundwater database on cold temperature anaerobic biodegradation rates and relevant contaminant and hydrogeological data that could be reviewed to choose appropriate rates for preliminary analysis. This paper provides a summary of the anaerobic first-order biodegradation rates for select petroleum hydrocarbon compounds in groundwater found in the database. These rates have been obtained from the literature or by back calculation based on data from case histories. The rates have also been normalized to +5°C and +10°C using the Q10 rule. Summary statistics have also been compiled for the data, and compared to rates published in Suarez and Rifai (1999), which does not include temperature details.

The second study involved compiling information regarding the application of bioremediation to petroleum hydrocarbon (PHC) impacted soils in the Arctic and other cold climates. Through correspondence and site visits with consulting firms, government agencies, and industries within Alberta, Alaska, Nunavut, the Northwest Territories, and the Yukon, a listing of unpublished reports has been identified and summarized. Information collected has been collated into a database which will aid in the development of an Environment Canada guidance document for bioremediation in the Canadian Arctic. The scope of the second study included review of full and pilot scale projects, including both in-situ and ex-situ treatments. The aim of this research was to provide a short description of each project entailing field conditions, remedial methodology, and rates of removal; the contents of which were examined to identify the most common and/or successful strategies employed.

2. METHOD

2.1 Groundwater Biodegradation Study
Data for the groundwater study was obtained from a number of sources that reported BTEX biodegradation in groundwater at various temperatures. Data sources included case histories and laboratory experiments obtained in the literature reporting: biodegradation rates; concentration versus distance data (analyzed using the Buscheck and Alcantar (1995) solution to estimate biodegradation rates); concentration versus time data (analyzed to calculate biodegradation rates); and field data obtained from file review of sites in Alberta and British Columbia that were included in the CORONA Plume-a-thon (Cross et al. 2001) (analyzed using the Buscheck and Alcantar (1995) solution to estimate biodegradation rates).

These rates were then adjusted using the $Q_{10}$ rule assuming that $Q_{10} = 2$ to normalize the rates to both $+5^\circ C$ and $+10^\circ C$. Using $Q_{10} = 2$ assumes that rates will approximately double for a temperature increase of $10^\circ C$ (Randall et al. 2002). This, then, provides an estimate of biodegradation rates that might be expected in groundwater at temperatures likely encountered in cold climates. Rate data was log-normally distributed when plotted. Therefore, biodegradation rates were converted to log(day$^{-1}$) units to attain a Gaussian distribution. Statistical analysis of the data was then completed to determine the mean, standard deviation and 95% confidence interval of the data. The 95% confidence interval for this study and for Suarez and Rifai’s data was then compared.

Recognising that the rates in the field are highly dependent on many local variables, the database contains a variety of pertinent site information. The complete database and sources are listed in detail in the final report issued to Alberta Environment which can be found at http://environment.gov.ab.ca/info/library/6684.pdf.

2.2 Soil Remediation Study

At the 2005 Assessment and Remediation of Contaminated Sites in Artic and Cold Climates (ARCSACC) conference in Edmonton (May 8-10), a survey requesting cold climate bioremediation data was circulated and interested participants were identified. From this point, correspondence with these parties led to the acquisition of site reports as well as additional contacts within consulting firms, government agencies, and industry. Agencies contacted are included in the reference section under soil remediation study. Between the period of July 3-9, 2005, visits were made to Yellowknife and Whitehorse to review reports on file with various organizations. In Yellowknife, personnel who were visited and provided related information include Ms. Lisa Dyer (DIAND), Mr. Rob Thom (Dept. of Transportation), and Mr. Harvey Gaukel (Environmental Protection Division, Government of NWT). In Whitehorse, those visited include Ms. Shannon Jensen (Contaminated Sites, Government of the Yukon) as well as Mr. Don Wilson (EBA).

Reports were reviewed and the data was summarized in a database based on climate, soil type, terrain, presence of permafrost (continuous or discontinuous), soil temperatures, ambient air temperatures, volume of spill/leak, volume/mass of soil treated, amendments, moisture content, initial concentration, final concentration, biodegradation rate, remediation criteria and source, timeframe, and cost estimates. The complete database and sources (also listed in the reference section under soil remediation study) are listed in detail in the final report issued to Environment Canada. For a copy of the report contact Ivy Stone at Environment Canada, Assessment and Monitoring, 5204-50th Avenue, Suite 301, Yellowknife, Northwest Territories, X1A 1E2.

3. RESULTS AND DISCUSSION

3.1 Groundwater Biodegradation Study

Table 1 provides a summary of biodegradation rates obtained from field and laboratory groundwater studies. Data from 60 case studies (47 field and 13 laboratory) were statistically analyzed to determine ranges of biodegradation rates for BTEX under different redox conditions. Iron reduction, sulphate reduction, and methanogenesis each comprised 31% of the TEAPs reported in the cases (for a total of 93%), while denitrification and manganese reduction made up the balance. This redox distribution is typical of intrinsic bioremediation as oxygen, nitrate and manganese are readily consumed creating an anaerobic environment.

At ambient temperatures mean biodegradation rates ranged from 0.003 to 0.017 day$^{-1}$. The ambient temperature for this study ranged from 4–28°C. The slowest rate was reported for total BTEX followed by total xylenes, benzene, ethylbenzene, m/p-xylenes, o-xylene and toluene with the fastest rate. Data normalized to $+5^\circ C$ using the $Q_{10}$ rule ranged from 0.001 day$^{-1}$ for total BTEX to 0.010 day$^{-1}$ for toluene. The general order of biodegradation rates with respect to compounds remained the same, however, the scatter in the rates was dramatically reduced by 33 to 66%. By normalizing the data to $+5^\circ C$ a more accurate and concise representation of anaerobic biodegradation rates has been compiled for cold climates.

The most recent and comprehensive overview of biodegradation rates for fuel hydrocarbons was completed by Suarez and Rifai (1999). One component of the study involved reviewing 75 BTEX studies under anaerobic conditions for first order biodegradation rates. Although Suarez and Rifai (1999) do not provide temperatures for much of their reported collection of rates the ambient temperature ranged from 6-36°C. Figure 1 provides a comparison of the rates contained in the database from this study with the rates collected by Suarez and Rifai (1999).
Table 1: Summary of first order biodegradation rate data under anaerobic conditions from this study.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Number of Field Rates</th>
<th>Number of Lab Rates</th>
<th>Total Number of Rates</th>
<th>Mean (day(^{-1}))</th>
<th>Minimum (95% confidence)</th>
<th>Maximum (95% confidence)</th>
<th>Average ambient temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ambient</td>
<td>5°C</td>
<td>10°C</td>
<td>ambient</td>
<td>5°C</td>
<td>10°C</td>
<td></td>
</tr>
<tr>
<td>Benzene</td>
<td>15</td>
<td>18</td>
<td>12</td>
<td>8</td>
<td>0.004</td>
<td>0.002</td>
<td>0.011</td>
</tr>
<tr>
<td>Toluene</td>
<td>18</td>
<td>26</td>
<td>14</td>
<td>9</td>
<td>0.017</td>
<td>0.011</td>
<td>0.014</td>
</tr>
<tr>
<td>Ethylbenzene</td>
<td>12</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td>0.010</td>
<td>0.005</td>
<td>0.008</td>
</tr>
<tr>
<td>m/p-Xylenes</td>
<td>8</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>0.003</td>
<td>0.005</td>
<td>0.003</td>
</tr>
<tr>
<td>o-Xylene</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.000</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Total Xylenes</td>
<td>6</td>
<td>10</td>
<td>32</td>
<td>3</td>
<td>0.019</td>
<td>0.008</td>
<td>0.014</td>
</tr>
<tr>
<td>Total BTEX</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.002</td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
</tr>
</tbody>
</table>

**Note:** Other category includes crude oil and diesel fuel.

Figure 1. Range of BTEX biodegradation rates under anaerobic conditions from field and laboratory data.

The data used to generate the bar graph are shown in Tables 1 and 2. Table 1 provides the data from this study and Table 2 provides the data obtained from Suarez and Rifai (1999). The bar lengths represent the 95% confidence interval on the mean. Note that Suarez and Rifai do not report rates for total xylenes, total BTEX or other categories. The bar for Suarez and Rifai’s ethylbenzene data was set at the lower limit of 0.0001 to reflect a data set where the lower 95% confidence limit was negative due to high variance.

A comparison of biodegradation rates from our study to Suarez and Rifai’s data shows their rates are higher for all compounds except for o-xylene. This may result from the higher ambient temperature range reported for the studies and/or the large number of laboratory data used in their analysis (47 to 61% of total rates as compared to 11 to 31% for data in this study). Biodegradation rates obtained...
from laboratory studies are generally higher than field rates because of the favorable conditions for biodegradation. Laboratory biodegradation rate data can be useful in better understanding individual field conditions; however, it is important to closely mimic field conditions. Suarez and Rifai reported a temperature range for their laboratory studies of 10 to 36°C, which is higher than the range of groundwater temperatures reported in the field studies (6 to 22°C). The temperature range for our lab (4-28°C) and field (4-27°C) data sets were identical.

Table 2. Summary of first order biodegradation rate data under anaerobic conditions from Suarez and Rifai (1999).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Number of Field Rates</th>
<th>Number of Lab Rates</th>
<th>Total Number of Rates</th>
<th>Mean</th>
<th>Minimum (95% confidence)</th>
<th>Maximum (95% confidence)</th>
<th>Average ambient temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzene</td>
<td>45</td>
<td>59</td>
<td>104</td>
<td>0.008</td>
<td>0.005</td>
<td>0.011</td>
<td>NR</td>
</tr>
<tr>
<td>Toluene</td>
<td>43</td>
<td>63</td>
<td>106</td>
<td>0.232</td>
<td>0.110</td>
<td>0.354</td>
<td>NR</td>
</tr>
<tr>
<td>Ethylbenzene</td>
<td>33</td>
<td>36</td>
<td>69</td>
<td>0.148</td>
<td>0.025</td>
<td>0.321</td>
<td>NR</td>
</tr>
<tr>
<td>m-Xylene</td>
<td>30</td>
<td>43</td>
<td>73</td>
<td>0.062</td>
<td>0.037</td>
<td>0.087</td>
<td>NR</td>
</tr>
<tr>
<td>p-Xylene</td>
<td>25</td>
<td>22</td>
<td>47</td>
<td>0.037</td>
<td>0.011</td>
<td>0.063</td>
<td>NR</td>
</tr>
<tr>
<td>o-Xylene</td>
<td>27</td>
<td>43</td>
<td>70</td>
<td>0.015</td>
<td>0.008</td>
<td>0.022</td>
<td>NR</td>
</tr>
<tr>
<td>m-/p-Xylenes</td>
<td></td>
<td></td>
<td></td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>o-Xylene</td>
<td></td>
<td></td>
<td></td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Total Xylenes</td>
<td></td>
<td></td>
<td></td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Total BTEX</td>
<td></td>
<td></td>
<td></td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
</tbody>
</table>

NR = Not reported

As shown by the data, biodegradation rates for field applications can be easily over estimated if the information within the data set is not completely understood. Our data set is compiled from TEAPs that dominate during natural attenuation (methanogenesis, sulphate reduction and iron reduction) and has been normalized to +5°C and +10°C. By normalizing the data to cold climate temperatures, the scatter in the data is minimized creating a data set that appears to be more reliable, that can be used by regulators and practitioners for estimating natural attenuation rates in field settings.
3.2 Soil Remediation Study

The data set discussed above focused on groundwater data, while this second study focused on soil remediation data. In total, 47 field case histories at 36 sites were investigated for their application of bioremediation to petroleum hydrocarbon impacted soils in the Arctic and other cold climates. A summary of projects investigated classified primarily by the type of treatment used, and further divided based on parameter analyzed and rate of degradation achieved can be obtained from Environment Canada as referenced in the method’s section. The survey revealed that biopiles and landfarming were the primary methods used at 66% (31 of 47) and 28% (13 of 47) of the sites, respectively. Singular applications of in-situ soil washing, bio-sparging, and bio-venting were also documented and found to be successful in the reduction of PHC in soils. Treatment volumes for full scale biopiles typically ranged from 450 to 6500 m$^3$, although volumes as high and low as 15,700 and 50 m$^3$, respectively, were reported. Treatment volumes for full scale landfarms typically ranged from 350 to 3200 m$^3$, with volumes as high as approximately 8500 m$^3$ reported.

Contaminants including diesel fuel, jet fuel, kerosene, heating oil, gasoline, motor oil, transformer oil, hydraulic oil, glycol, and transmission fluid oil were all found to have been successfully biodegraded in a variety of soil types including sand, silty clay, clay, shoreline sediments, silts, crushed granitic rock, and granular fill. Listed in Table 3 are ranges of contaminant concentrations and biodegradation rates reported.

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Concentrations (mg/kg)</th>
<th>Biodegradation Rates (mg/kg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTEX</td>
<td>58-300</td>
<td>0.39 – 0.83</td>
</tr>
<tr>
<td>MOG (mineral oil and grease)</td>
<td>1,400 – 7,200</td>
<td>9.1 - 74</td>
</tr>
<tr>
<td>PAH (polycyclic aromatic hydrocarbons)</td>
<td>730</td>
<td>51</td>
</tr>
<tr>
<td>PHC (petroleum hydrocarbons, C$<em>{10}$-C$</em>{30}$)</td>
<td>2,100 – 9,800</td>
<td>2.1 - 100</td>
</tr>
<tr>
<td>TPH (total petroleum hydrocarbons, C$<em>{6}$-C$</em>{25}$)</td>
<td>200 – 200,000</td>
<td>0.26 - 760</td>
</tr>
<tr>
<td>Total Oil</td>
<td>7,000 – 12,000</td>
<td>12 - 26</td>
</tr>
</tbody>
</table>

For those case histories for which regulatory criteria was provided (7 cases), 71% of those managed to successfully meet their respective criteria (4 biopiles and 1 landfarm). Both cases where regulatory criteria was not met occurred in landfarm applications; in one case, the active layer was remediated to below criteria, while the permafrost layer was not, and in the other case, less frequent tilling (in one trial of four, the three other trials met criteria) appears to be the cause of its failure to meet criteria. Duration of treatment for biopiles was found to range from 9 to 103 weeks, with a mean duration of approximately 28 weeks. Duration of treatment for landfarm applications was found to range from 5 to 52 weeks, with a mean duration of approximately 31 weeks.

Aeration was provided to the impacted soils in a variety of methods: passive aeration, mechanical aeration, and tilling (ranging in frequency from daily throughout the treatment season, to initially with application of amendment). Generally, soils were watered as necessary in order to provide a suitably moist environment for hydrocarbon reducing bacteria (HRB), although there were some applications that did not receive any additional water.

A variety of amendments were used, including different combinations of fertilizer (in the form of agricultural grade fertilizers, organics, and sewage sludge), microorganisms (sewage sludge and mixed culture cells), biosurfactants (Biosolve), and bulking agents (wood shavings, peat moss, and wood chips). For those sites where fertilizer was applied (81% of those investigated), the following are some nutrient ratios observed:

- C:N = 60:1 to 100:15;
- C:P = 50:1 to 800:1;
- C:N:P = 100:7.5:0.5; and

Nineteen sites (40% of those investigated) were found to involve the application of biosurfactants, the details of which were not available. Microbe addition was practiced at 40% of the sites surveyed, while the addition of bulking agents was practiced at 6% of the sites surveyed.

Although temperatures were not provided for most case histories, soil temperatures when compared to air temperatures were on average approximately 8-28°C warmer, and were observed to not fall below 2°C.

Costs provided per tonne of soil treated include design, equipment, material, mobilization/demobilization, manpower, monitoring, water management and reporting. Urban sites were found to range in costs from $15-90/tonne, while more remote northern sites were found to range from $30-150/tonne. Generally, the cost per tonne to treat impacted soils was observed to decrease with increasing volumes encountered.

The following generalizations have been noted regarding conditions to augment biodegradation of PHC impacted soils:

1. All soil types, contaminant types, and concentrations encountered in the surveyed sites appear to respond positively to bioremediation efforts. There were no cases encountered where PHC reduction was not observed.
2. The frequency of aeration, whether mechanical, passive, or as tilling, appears to be directly related to the rate of PHC reduction;
3. The addition of nutrients and biosurfactants appear to also increase the rate of PHC reduction in soils. Based on the applications investigated in this study, there is insufficient evidence to confirm the benefits of microorganism addition.
4. It appears that with appropriate configuration (determined through pilot studies), temperature constraints can be mitigated. In two of the case studies, biological activity was found to maintain
soil temperatures in the range of 20-30°C during winter months (Pouliot et al. 1999 and 2001). In addition, the use of plastic covers at one site was found to increase soil temperatures by an additional 30-49% (Mohn et al. 2001).

4. CONCLUSIONS

The groundwater data provided herein may be used for preliminary calculations of intrinsic bioremediation at sites in cold climates. The groundwater data from numerous sites have been normalized to temperatures of +5°C and +10°C using the Q_{10} rule to attempt to account for the impact of temperatures in cold climate groundwater. The complete groundwater database can be obtained from Environment Canada guidance document for the implementation of bioremediation in the Canadian Arctic. The results compiled from this study will contribute to an Environment Canada guidance document for the implementation of bioremediation in the Canadian Arctic.

5. REFERENCES

5.1 Groundwater Biodegradation Study


5.2 Soil Remediation Study


