BIOREMEDIATION IN NORTHERN CLIMATES: HOW CAN PETROLEUM HYDROCARBON BIODEGRADATION BE ASSESSED IN SOILS UNDER SEASONAL FREEZE-THAW CONDITIONS?

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ABSTRACT

Accurate assessment for bioremediation feasibility plays a critical role in developing cost-effective and efficient remediation strategies adapted to northern climates, where warm seasons are short and accessibility is limited. Using a pilot-scale bioremediation system, this study quantitatively assessed bioremediation feasibility under dynamic site temperatures, including summers and non-summers, at a northern contaminated site. A total of 15 first-order rate constants were derived from previous pilot-scale experiments carried out under dynamic or fixed average temperatures of a cold site. The biodegradation rate obtained from pilot-scale testing was two times slower than that of the microcosm-scale experiments, which have been widely used for laboratory-based assessments. Site temperature variability and seasonality at cold sites greatly influences biodegradation kinetics. Using pilot-scale experiments under site-representative freeze-thaw temperatures, the unfrozen water content, which is a requirement for microbial activity in freezing soils, was predicted based on a TEMP/W model. The simulated unfrozen water content was in excellent agreement with the measured unfrozen water content during soil freeze-thaw. The correlation between unfrozen water content and microbial CO_2 production was statistically significant. This study suggests that on-site bioremediation for cold site soils is feasible in summers and can be extended to seasonal freeze-thaw months.

Key Words: Bioremediation, Petroleum Hydrocarbons, Seasonal Soil Freeze-Thaw, Unfrozen Water Content, Pilot-Scale Experiment, Biodegradation Rate, Cold Climates.

INTRODUCTION

The remediation of contaminated sites in northern Canada is a pressing environmental concern. An estimated 2,600 contaminated sites exist in the three northern territories (Northwest Territories, Nunavut, and Yukon) and 182 of these are classified as high priority sites in terms of environmental and health risks associated with the contaminants (Federal Contaminated Sites Inventory 2013). Federal government liability has been estimated at $1.5 billion for northern contaminated sites, which typically include mine sites, drilling sites, abandoned military stations, oil spill sites, and non-managed disposal sites (Office of the Auditor General of Canada 2012). At these sites, petroleum hydrocarbons are some of the most frequently identified contaminants. Petroleum hydrocarbons, which are hydrophobic organic compounds, including toxic and carcinogenic hydrocarbon fractions, are persistent in northern cold soils and serve as long-term contaminant sources for inhabitants and unique northern ecosystems.

Bioremediation is the use of microorganisms to degrade or detoxify contaminants in the environment and has been considered a cost-effective and non-destructive remediation technology for petroleum hydrocarbon-contaminated soils in northern cold regions. Cold-adapted, indigenous, hydrocarbon-degrading bacteria are able to survive and remain metabolically active in petroleum hydrocarbon-contaminated soils and groundwater at low temperatures, and thus have been the focus of bioremediation efforts at contaminated sites in cold regions, including the Arctic and Antarctica (Snape et al. 2008).

However, since many of the contaminated sites in northern climates are located in remote regions and site accessibility is limited during seasonal freezing and thawing months, assessing the bioremediation feasibility for hydrocarbon-contaminated soils still largely relies on controlled, laboratory-based biodegradation experiments. The costs of risk management, remediation and monitoring are prohibitively increased by cold climate seasonality, associated logistics and the environmental sensitivity of frozen ground. An accurate assessment of contaminated soils is required for enhancing hydrocarbon biodegradation and for implementing cost-effective and successful bioremediation at remote northern sites. Understanding site-specific environmental conditions and rate-limiting factors such as site temperature dynamics and the scale of the bioremediation system should be considered during the design of laboratory experiments.

The majority of previous laboratory-based assessments about optimal soil and nutrient conditions for enhancing indigenous hydrocarbon-degrading bacteria have been generally performed at microcosm scales (3 to 1200 g). However, larger field-scale or on-site pilot-scale bioremediation systems (e.g., 3600 m^3) at cold sites are likely to be influenced by greater soil heterogeneity. Although microcosm-scale experiments are useful for indicating the bioremediation potential of contaminated soils, the system scale may significantly influence the rate and extent of hydrocarbon biodegradation (Davis et al. 2003).

Furthermore, previous bioremediation feasibility studies have been predominantly performed at fixed, low temperature incubations, with temperatures ranging from near 0 to +25°C, which is not representative of realistic, periodic and seasonal temperature regimes at northern sites. Therefore, this study will investigate the influence of system-scale and site-representative temperature dynamics on the rate and extent of hydrocarbon biodegradation in a cold site soils.

On the other hand, current remedial strategies and on-site activities, regardless of the type of remediation technology, have often been designed to be effective only during short summers (2 to 4 months per year) due to the colder climate, slower degradation rates and limited site accessibility (Chang et al. 2011a; Paudyn et al. 2008). On-site remediation systems are left dormant after short treatment seasons when seasonal freezing begins. However, Chang et al. (2011a) showed that hydrocarbon biodegradation did not cease at sub-zero temperatures during soil freezing and thawing at seasonal soil temperature changes, which potentially provides important insight for understanding biodegradation activity in semi-frozen and frozen contaminated soils and for developing bioremediation strategies that are feasible during seasonal freeze-thaw months. Changes in unfrozen water availability become a critical rate-limiting factor for maintaining hydrocarbon biodegradation in semi-frozen and frozen hydrocarbon-contaminated soils.

In this study, unfrozen water availability was predicted using a numerical model (TEMP/W) and compared to measured unfrozen water content in the soil of a pilot-scale tank subjected to seasonal freeze-thaw temperatures. The correlation between unfrozen water content and microbial CO_2 production was investigated.

In summary, the specific objectives of the present study are to (1) compare the rates and extents of hydrocarbon biodegradation for microcosm and pilot-scale experiments, (2) determine favorable nutrient dosages for enhancing microbial activity in contaminated soils, (3) compare the rates of hydrocarbon biodegradation under fixed and dynamic temperature regimes, including seasonal freeze-thaw conditions, and (4) simulate unfrozen water availability during seasonal freeze-thaw and assess its correlation with microbial activity. Field-aged petroleum hydrocarbon-contaminated soils were shipped from a Resolution Island site in Nunavut. Microcosm- and pilot-scale bioremediation experiments were conducted in a large-scale cold room in order to program site-representative temperature dynamics, including seasonal freeze-thaw temperature regimes.

MATERIALS AND METHODS

Contaminated Soils

Unsaturated petroleum hydrocarbon-contaminated soils were shipped from a Resolution Island (RI) site (61°30'N 65°00'W) in Nunavut. The soils were classified as sand (gravel: 27%; sand: 71%; silt and clays: 1%) and the initial pH of site soils was 4.3. In accordance with four carbon-number-based fractions (F1-F4) from the CWS PHC (Canada-Wide Standard for Petroleum Hydrocarbons in Soils), the majority of hydrocarbon contaminants in the soils were semi-volatile (F2: >C10-C16) and non-volatile (F3: >C16-C34). Volatile (F1: C6-C10) and heavier hydrocarbon fractions (F4: >C34) were negligible. An abundance of viable, aerobic hydrocarbon-degrading bacteria (2×10^2 to 1×10^5 CFU/g) were enumerated in the soils at incubation temperatures ranging from 4 to 25°C. Catabolic genes (*AlkB*, *ndoB*, *phnAc*, and *xylE*) encoding for petroleum hydrocarbon biodegradation were detected in the contaminated soils. The feasibility of aerobic biostimulation in field-aged hydrocarbon contaminated soils from the site was therefore indicated. Many of the results from physical, chemical and microbiological characterizations of the RI soils were reported in Chang et al. (2010).

Microcosm- and Pilot-Scale Bioremediation Experiments

Nutrient-amended and unamended (control) soils were prepared at both the microcosm and pilot scales. The weights of wet unsaturated contaminated soils used in the microcosm- and pilot-scale experiments were 0.5 and 300 Kg, respectively. The microcosm experiments were conducted using 1-L glass jars. For the scaled-up studies, the pilot-scale tanks (1 m long × 0.65 m wide × 0.3 m high) equipped with activated carbon and moisture traps to capture volatile hydrocarbons were placed in a large-scale cold room. The temperature programs for site temperature scenarios were constructed based on the 20-year climatic data available from Environment Canada.

The soil treatment included nutrient amendment and soil buffering. Water-based, 20:20:20 commercial fertilizer (20% total N: 20% P_2O_5: 20% K_2O) was prepared to make samples with 50, 100 and 250 milligrams of nitrogen per kilogram of wet soil in order to determine the favorable loading of nutrients for the site soils. The site soils were acidic and buffered by adding 2000 mg $CaCO_3$/Kg. Soil pore gas concentrations of carbon dioxide (CO_2) and oxygen (O_2) were monitored using a gas monitor

(ATX 620, Industrial Scientific Co.). The soil samples were collected at intervals of 20 days and CWS PHC analyses were adopted for petroleum hydrocarbon analyses (CCME 2001). The detailed analytical protocols are described elsewhere in Chang et al. (2011a). The 15 rate constants for hydrocarbon biodegradation were determined based on a first-order kinetic model. Of these, nine rate constants were derived from previous series of pilot-scale experiments (Chang et al. 2010, 2011a,b).

The change in unfrozen water availability was simulated using TEMP/W, which is a commercial numerical modeling software (GEO-SLOPE 2008). Unfrozen water content curves in response to soil freeze-thaw were generated for the input data. The measured data for unfrozen water content and CO_2 production during soil freeze-thaw were derived from Chang et al. (2011a). The simulated and measured unfrozen water contents were compared and the correlation between CO_2 production (i.e., microbial activity) and unfrozen water content was evaluated.

RESULTS AND DISCUSSION

Effects of the Scale of the System on Biodegradation Rates for Petroleum Hydrocarbons

The present study indicated a significant difference in the bioremediation performance between the microcosm- and pilot-scale experiments. It is notable that TPH biodegradation was enhanced more easily at the microcosm scale (500 g) than in the pilot-scale system (300 Kg) at 5.5°C. Using a first-order kinetic reaction model, the rate constants, *K*, were determined from TPH data for both systems. As shown in Figure 1, the $K_{microcosm}$ of 0.01 day^{-1} was two times higher than the $K_{pilot\text{-}scale}$ of 0.005 day^{-1}. The calculated half-life for TPH biodegradation was 44 to 68 days (95% confidence interval) at the microcosm scale and 99 to 202 days (95% confidence interval) at the pilot scale.

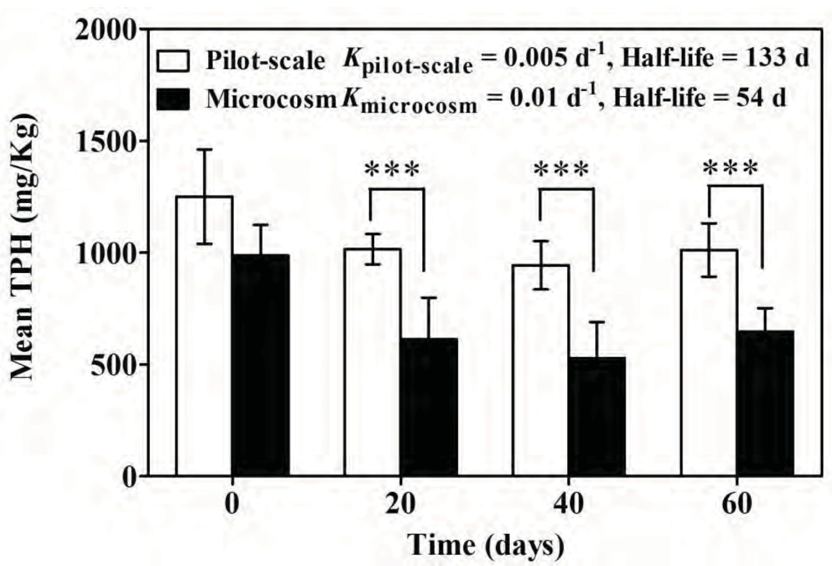

*Figure 1. The effects of system scale on the rate and extent of petroleum hydrocarbon biodegradation at 5.5°C. The standard deviation is presented as bars. ***: p-value < 0.0001 (obtained by two-way ANOVA). TPH: Total Petroleum Hydrocarbons.*

Approximately 40% of the initial TPH were removed at the microcosm scale. In the pilot-scale system, the percent removal of TPH was 21%. The two-way analysis of variance (ANOVA) confirmed that the effects of scale and time on TPH-biodegradation were statistically significant (the effects of both scale and time resulted in a p-value < 0.0001). In unamended soils (control), no significant TPH removal occurred in both the microcosm- and pilot-scale experiments.

The TPH chromatograms obtained from the GC/FID analyses (Gas Chromatography equipped with a Flame Ionization Detector) showed the degradation of a variety of petroleum hydrocarbon fractions, including the peak and hump areas of the two different systems. The hump area, which represents Unresolved Complex Mixtures (UCMs), potentially includes structurally complex hydrocarbons with branched and unsubstituted alkyl chains. At the microcosm scale, the UCM humps after the biotreatment were significantly smaller than those of the pilot scale. After the 60-day biotreatment, the final TPH concentrations in the microcosm- and pilot-scale experiments were 648±104 mg/Kg and 1012±119 mg/Kg, respectively. Based on the TPH-data analyses, it is necessary to consider the effects of scale when microcosm systems are used for predicting *in situ* rates and extents of petroleum hydrocarbon biodegradation in the RI landfarm soils. Although the RI site soils are relatively homogenous sandy soils in terms of particle distributions and soil compositions, the scale effect is most likely due to nutrient mass transfer limitations, buffering reagent amendments and the slower diffusive flux of oxygen through soil phases in larger systems. Considering the scale effect is therefore also important for scaling up from pilot to field scale, especially due to a potentially significant increase in the heterogeneity of site soils.

Soil Treatments for Enhancing Indigenous Microbial Activity in Contaminated Soils

A high dosage of nutrient supply to contaminated soils inhibits microbial activity and a low nutrient dosage delays the onset of microbial activity. The selection of nutrient concentrations and types is thus important for stimulating indigenous hydrocarbon-degrading bacteria in contaminated soils and for maximizing the effectiveness of soil bioremediation.

At the pilot-scale, all the nutrient amendments (50, 100, and 250 milligrams of nitrogen per kilogram of wet soil) stimulated aerobic microbial activity, which was estimated based on soil pore gas concentrations of CO_2 and O_2 (Figure 2). The nutrient amendments effectively increased CO_2 production and O_2 consumption, indicating enhanced microbial activity in petroleum hydrocarbon-contaminated soils at 5.5°C. Conversely, in untreated contaminated soils (control), no significant changes in microbial respiration activity over 60 days were indicated. The degree of microbial enhancement varied with nitrogen (N) concentration. Within the range of concentrations used (50 to 250 mg-N/Kg), a nitrogen dosage of 100 mg-N/Kg provided the most favorable conditions for microbial enhancement in the contaminated soils. Similarly, previous studies consistently indicated that moderate concentration of nitrogen (i.e., 100 mg-N/Kg) successfully stimulated hydrocarbon biodegradation in cold region soils (Braddock et al. 1997). In this study, high nitrogen concentrations (> 250 mg-N/Kg) inhibited biodegradation activity, which was likely due to toxic effects and osmotic stress on hydrocarbon-degrading bacteria caused by high nitrogen concentrations.

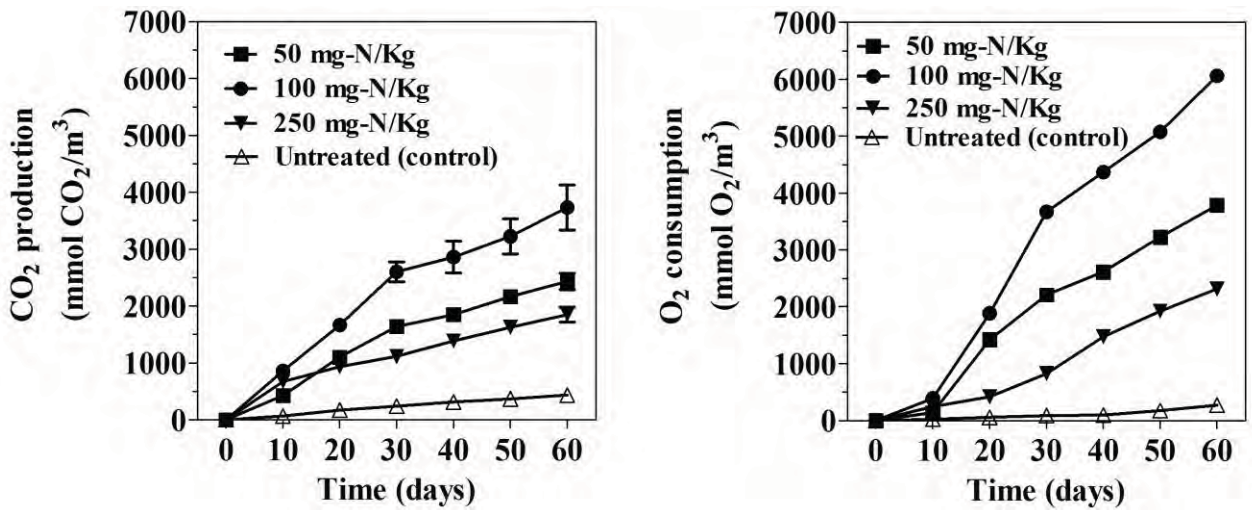

Figure 2. CO_2 production and O_2 consumption in the contaminated soils that received 0, 50, 100 and 250 mg-N/Kg in pilot-scale bioremediation systems.

<u>Impact of Site Temperature Dynamics on the Rate of Hydrocarbon Biodegradation</u>

A total of 15 first-order rate constants for petroleum hydrocarbon biodegradation in site soils were derived from the pilot-scale bioremediation assessments that were supplied with favorable nutrient dosages of 50 and 100 mg-N/Kg. The assessments were carried out under dynamic summer temperatures varying from +1 to 10°C (dynamic temperature regime), under fixed average temperatures of +5.5°C (fixed average temperature) and under extended seasonal freeze-thaw temperatures from -5 to +4°C (seasonal freeze-thaw).

Figure 3 demonstrates the distribution of rate constants for the three different temperature regimes. One-way ANOVA tests showed that the difference in the rate constant datasets was statistically significant (p-value < 0.0001). In addition, Bonferroni's test for the comparison of datasets indicated that the effects of summer temperature dynamics, when compared to a fixed average temperature, are also statistically significant. The mean rate constant for the realistic summer temperature dynamics was 0.017±0.004 day^{-1}, which is over three times higher than the mean rate constant of 0.0048±0.002 day^{-1} for the fixed average summer temperature. Under the seasonal freeze-thaw temperature regime, the mean rate constant was 0.0046±0.002 day^{-1}, which is comparable to that of the fixed temperature experiments. In particular, the rate constant for the biodegradation of semi-volatile hydrocarbons (C10-C16) was 0.006 day^{-1} in the seasonal freeze-thaw temperature regimes including subzero temperatures (half-life: 116 days; R^2 = 0.94, first-order kinetic model). Although the rate of hydrocarbon biodegradation under the seasonal freeze-thaw conditions was slower, hydrocarbon biodegradation activity did not cease during soil freeze-thaw at sub-zero temperatures ranging from -5 to 0 °C (Chang et al. 2011a).

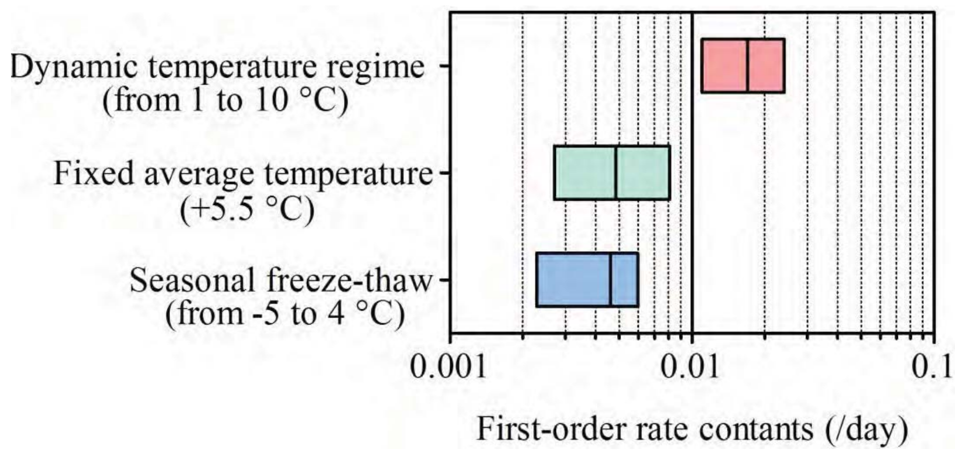

Figure 3. A total of 15 first-order rate constants were derived from a series of previous pilot-scale experiments under the three different temperature scenarios. The nutrient concentrations of 50 and 100 mg-N/Kg were supplied to the contaminated soils.

Extended Microbial Activity as a Function of Unfrozen Water Content in Freezing and Thawing Soils

The change in unfrozen water content in petroleum hydrocarbon-contaminated soils is a key factor for enhancing hydrocarbon biodegradation in semi-frozen and frozen soils (Siciliano et al. 2008). The onset and extent of microbial activity in relation to soil freezing and thawing were positively correlated to changes in unfrozen water content in contaminated soils (Chang et al. 2011a) and, thus, the prediction of unfrozen water content is important for developing bioremediation strategies extended to seasonal freeze-thaw conditions in cold-climate environments.

As shown in Figure 4, the time-dependent model based on a finite element method (TEMP/W model) for the prediction of unfrozen water provided a simulated quantity and distribution of unfrozen water in field-aged hydrocarbon contaminated soils in a pilot-scale soil tank subjected to site-representative seasonal freeze-thaw temperatures. Figure 4(A) shows a snapshot of the distribution of unfrozen water in semi-frozen soils during a soil phase change on Day 30. The simulated unfrozen water content was in excellent agreement with the measured unfrozen water content (Figure 4).

Characteristic curves for unfrozen water and other parameters such as the freezing-point depression (T_f) and the effective endpoint of unfrozen water (T_{ef}) are specific to different soils and their properties, which includes hydrocarbon contamination. The determination of the characteristic curve for unfrozen water for contaminated sandy soils played a critical role in accurately predicting unfrozen water content in unsaturated, hydrocarbon-contaminated soils. The simulated results based on conventional unfrozen water characteristics for uncontaminated sandy soils did not match the measured unfrozen water content in contaminated soils.

The unfrozen water in semi-frozen and frozen soils provides a nutrient reservoir for maintaining microbial metabolism and therefore hydrocarbon biodegradation can continue under the seasonal freeze-thaw conditions. For the hydrocarbon biodegradation assessment in the pilot-scale experiment, Chang et al.

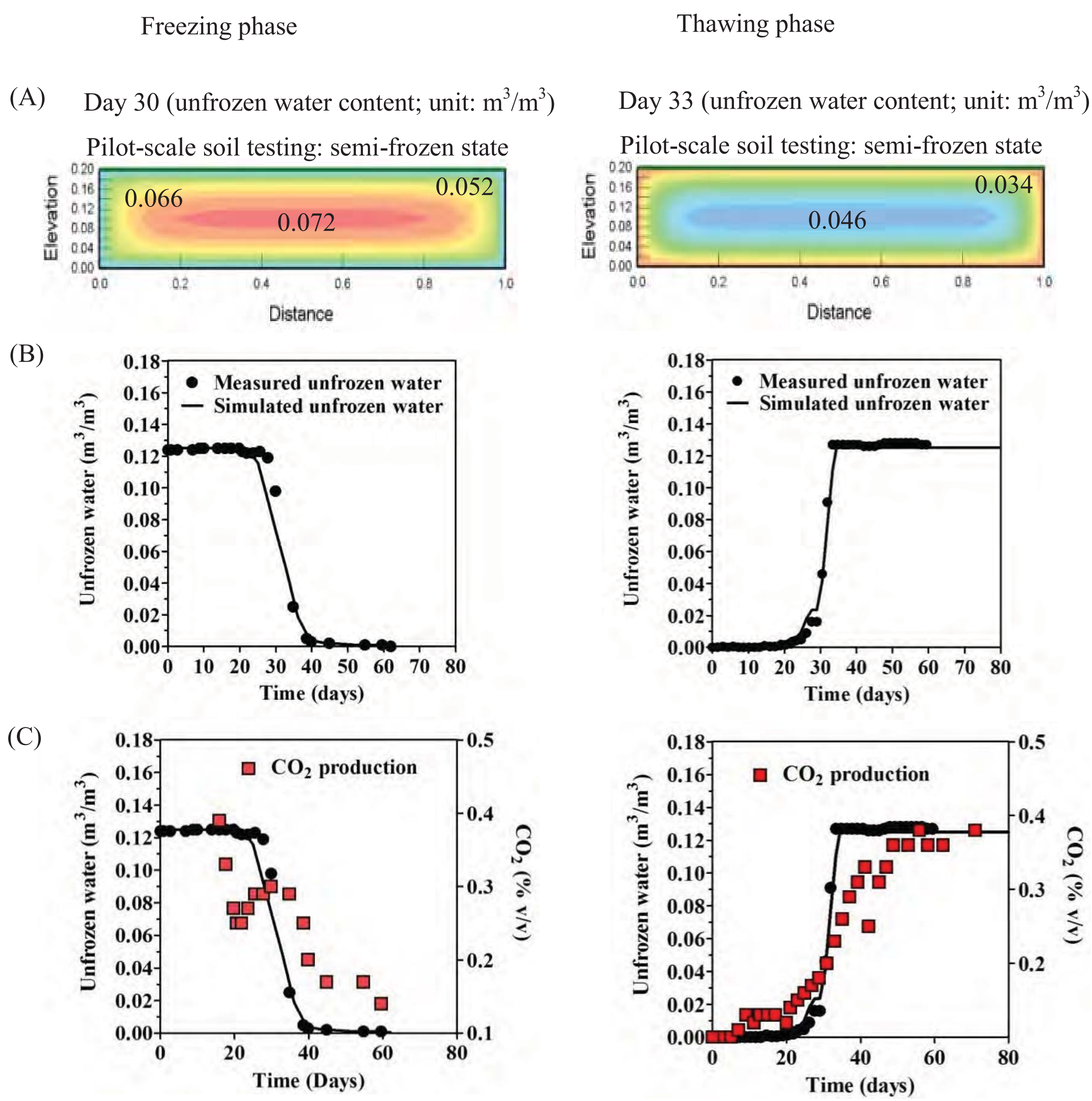

Figure 4. Results of unfrozen water content simulation using TEMP/W: (A) unfrozen water distribution in semi-frozen soils (B) comparison of simulated and measured unfrozen water content (C) correlation between unfrozen water content and microbial CO_2 production.

(2011a) reported that in the nutrient-amended hydrocarbon-contaminated soils, the total removals of semi-volatile (F2: C10-C16) and non-volatile (F3: C16-C34) hydrocarbons were 52% and 16%, respectively, during the soil freeze-thaw phase. Robust biomarker analyses (i.e., bicyclic sesquiterpenes) and ^{14}C-hexadecane mineralization results explicitly showed the progress of hydrocarbon biodegradation (not abiotic loss) in seasonally freezing and thawing contaminated soils. In the previous study (Chang et al. 2011a), increases in bacterial *alkB* genes (hydrocarbon degradation genes) and 16S rRNA gene copy numbers (total bacterial populations) were observed in the semi-frozen state of the soil in which ice and unfrozen water co-exist. Molecular-based microbial community analyses showed that hydrocarbon-degrading bacteria (*Corynebacterineae*- and *Alkanindiges*-related strains) that are adapted to the frozen and unfrozen states of the soils sequentially emerged in response to soil freezing and thawing and, thus, hydrocarbon biodegradation continued to progress in seasonal freeze-thaw temperature regimes. Therefore, time-dependent changes in unfrozen water content should be accurately assessed. This study represents an extension of the study by Chang et al. (2011a) to investigate the feasibility of predicting unfrozen water content in the RI contaminated soils.

There was a very strong correlation between unfrozen water availability and microbial respiration activity. In Table 1, the correlation analyses indicated a significant positive correlation between unfrozen water content and microbial activity (i.e., CO_2 production) in freezing and thawing hydrocarbon-contaminated soils. Correlation coefficients (Pearson r) during the freezing and thawing phases were 0.73 and 0.94, respectively. Therefore, the change in unfrozen water content in freezing and thawing biotreated contaminated soils can be an effective indicator of microbial activity under seasonal freeze-thaw conditions. This study suggests that the prediction of unfrozen water quantity and distribution in field-aged hydrocarbon-contaminated soils during the seasonal freeze-thaw period can be used for developing bioremediation strategies extended beyond conventional active treatment seasons in cold-climate environments, which are limited to short summers.

Table 1. Results of statistical correlation analyses for unfrozen water content and CO_2 production.

	Freezing phase	**Thawing phase**
Pearson r (correlation coefficient)	0.73	0.94
P value (two-tailed)	0.002	< 0.0001
Is the correlation significant? (α = 0.05)	Yes	Yes
R^2	0.53	0.88

CONCLUSIONS

The majority of laboratory assessments for bioremediation feasibility for hydrocarbon-contaminated soils in cold climates are based on microcosm-scale soil experiments at fixed average temperatures for cold sites. In this study, the rates and extents of hydrocarbon biodegradation in the scale-up bioremediation systems were significantly different from the microcosm-scale systems. The first-order rate constants obtained from the pilot-scale experiments were two times smaller than those obtained from the microcosm experiments. Compared to the pilot-scale experiments, biodegradation was also extended to higher molecular weight hydrocarbon fractions at the microcosm-scale. The microcosm experiments may overestimate the bioremediation performance that is achievable in the field. Inorganic, N-based nutrient supplies of 50 and 100 mg-N/Kg resulted in significant microbial enhancement in hydrocarbon-contaminated soils from the RI site. When comparing to conventional laboratory fixed temperature scenarios (averaged for summers), it becomes clear that the impacts of dynamic site temperature scenarios are important for estimating the achievable rate constants for petroleum hydrocarbon biodegradation in the field at the RI site.

In the pilot-scale experiment under the dynamic site temperature conditions, changes in simulated and measured unfrozen water content in freezing and thawing contaminated soils correlated significantly to microbial activity estimated based on CO_2 production. The quantity and distribution of unfrozen water during soil freeze-thaw can be predicted in hydrocarbon-contaminated soils using the TEMP/W model when characteristic curves for unfrozen water in hydrocarbon-contaminated soils are available. The predictive model for unfrozen water content can be an effective indicator for microbial activity and can be seen as a framework for developing a seasonality-based bioremediation strategy that is extended to freeze-thaw seasons, beyond the short, active summer treatment seasons at northern contaminated sites.

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Overcoming Northern Challenges

Proceedings of the 2013 Northern Latitudes Mining Reclamation Workshop and

38th Annual Meeting of the Canadian Land Reclamation Association

Whitehorse, Yukon September 9 – 12, 2013

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Northern Latitudes Mining Reclamation Workshop

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Polster	Natural Processes: An Effective Model For Mine Reclamation
Dustin	Implementation of contaminated water management system upgrades to allow for dewatering of two open pits at the Vangorda Plateau, Faro Mine Complex, Yukon
Kempenaar, Marques and McClure	Tools for Arctic Revegetation: What's in Your Toolbox?
Smreciu, Gould, and Wood	Establishment of Native Boearl Plant Species On Reclaimed Oil Sands Mining Disturbances
Keefer	Twin Sisters Native Plant Nursery
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Stewart and Siciliano	Biological Soil Crusts and Native Species for Northern Mine Site Restoration
Nadeau and Huggard	Restoration Planning and Application of Ecological Succession Principals
Simpson	Defining Disturbance and Recovery - the influence of landscape specific ecological responses to oil and gas linear disturbances in Yukon

NORTHERN LATITUDES MINING RECLAMATION WORKSHOP

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The first Workshop was held in Whitehorse, Yukon Territory, Canada in 2001 and it has been held every two years since, alternating between Canada and Alaska. The primary sponsors of the Workshop include the Yukon Geological Survey, Indian and Northern Affairs Canada, Natural Resources Canada, US Department of the Interior Bureau of Land Management, and the State of Alaska Department of Natural Resources.

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- To collect information relating to land reclamation and publish periodicals, books and leaflets which the Association may think desirable.
- To encourage education in the field of land reclamation.
- To provide awards for noteworthy achievements in the field of land reclamation.

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