

# Bioremediation in extremes: feasibility of scale-up biostimulation of petroleum hydrocarbon contaminated sub-Arctic soils during soil freezing

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## Summary

Petroleum hydrocarbon contamination has occurred at many sites in the Arctic/sub-Arctic due to leaks from on-ground fuel storage tanks and accidental spills. Penetration and transport of petroleum liquids to groundwater and through fissured/ fractured permafrost have been reported and its environmental impacts have been documented in several studies.

It is generally accepted that bioremediation is a feasible, less disruptive and low-cost remedial option for contaminated sites in cold and remote regions. On-site experiments and field operations of bioremediation using landfarming and biopile technologies have been successfully tested during the warmer summer months where soil surface temperatures are generally above 0 °C. A number of laboratory biodegradation experiments have been conducted to evaluate petroleum biodegradation feasibility and associated microbial activity, and these have been performed typically at temperatures of 5 to 10°C that approximate the average summer site temperatures. However, little is known about the biodegradation of petroleum hydrocarbons in cold soils impacted by freezing stress that is common to the active layers at the Arctic/sub-Arctic sites. Cold-adapted bacteria isolated from cold regions are metabolically active even at extreme subzero temperatures and resistant to cold-shock stresses.

We conducted an experiment to assess the feasibility of petroleum hydrocarbon biodegradation and to elucidate the microbial community response and activity in field-aged soils from a sub-Arctic site in Resolution Island site (NU) under a freezing temperature regime. Controlled, pilot-scale biodegradation experiments were carried out in 1 m long × 0.65 m wide × 0.3 m deep tanks which were placed in a large-scale cold room where freezing temperatures representative of the site were maintained. Weathered petroleum-contaminated soils shipped from the site were amended with inorganic nutrients and pH buffering agents that were predetermined at the optimal level. A control soil tank was maintained and was not amended with nutrients and buffering agents but was maintained at the same temperature regime.

Soil freezing drastically changed the water availability below the freezing-point depression temperature, presumably imposing osmotic stresses to the microbial population present in the unfrozen liquid water. However, significant microbial respiration by soil gas O<sub>2</sub>-CO<sub>2</sub> was measured during soil freezing. Quantifiable biodegradation activity (over 10% reduction) was detected in semi-volatile hydrocarbon fractions (>C10-C16). Under the multiple environmental stress, significant numbers of viable heterotrophs and hydrocarbon-degraders (>10<sup>5</sup> CFU/g) were detected. An increase in 16s rRNA gene copy numbers over time provided additional evidence of survival and growth of bacterial populations under freezing conditions. PCR-DGGE analyses indicated emergence of freeze-tolerant populations. Overall, these results suggest that microbial populations are active under freeze-thaw conditions and may be able to provide modest reductions in petroleum hydrocarbon levels beyond the summer season.