Bioremediation of Petroleum Hydrocarbons in Cold Regions

This practical guide to bioremediation in cold regions is designed to aid environmental practitioners, industry, and regulators in the remediation of petroleum spills and contaminated sites in cold regions. Remediation design and technology used in temperate climates does not necessarily work in cold climates, and cleanup takes longer due to shorter treatment seasons, sub-freezing temperatures, ground freezing and thawing, and limited bioactivity. Environmental engineers and scientists from eight countries working in the polar regions combine their experiences and expertise with petroleum contamination in this book. It contains in-depth discussions on regulations, freezing and frozen ground, identification and adaptations of cold-tolerant bacteria, contaminant transport in cold soils and permafrost, temperature effects on biodegradation, analytical methods, treatability studies, and nutritional requirements for bioremediation. Emphasis is given to practical and effective bioremediation methods for application in cold regions using case studies and cost-benefit analyses. Emerging technologies are also discussed. This volume will be an important reference for students, researchers, and practitioners of environmental science and engineering.

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> Bioremediation of Petroleum Hydrocarbons in Cold Regions

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> Dedicated to Peter J. Williams, whose inspiration and leadership pioneered the study of contaminants in freezing ground

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Preface

Bioremediation of Petroleum Hydrocarbons in Cold Regions is written by a multi-disciplinary group of scientists and engineering professionals working in polar regions. The monograph is designed as a state-of-the-art guidance book to assist industry, environmental practitioners, and regulators with environmental cleanup in cold climates. The book can also be used for environmental science and remediation engineering seniors and graduate students who are preparing for a career in professional environmental practice or applied scientific research. The intent of this book is to articulate conditions unique to our cold regions, and present practical and cost-effective remediation methods for removing petroleum contamination from tundra, taiga, alpine, and polar terrain.

Oil and its refined products represent a significant proportion of the pollution found in the Arctic and Antarctic. This pollution is encountered at former military and industrial sites, scientific research stations, rural communities, and remote airstrips, while recent spills and releases tend to be associated with resource development and transportation mishaps. Bioremediation is recognized as potentially the most cost-effective technology for removing petroleum contaminants from ecosystems in cold regions.

Permafrost, suprapermafrost water, tundra, cold-tolerant microorganisms, short summers and long, dark winters, cold air and ground temperatures, and annual freezing and thawing of the active layer are but a few environmental characteristics of cold regions. Their prevalence limits practical remediation methods and has led to the development of innovative and pragmatic biore-mediation schemes for use at contaminated sites in cold climates. Case studies and costs (where known) are integrated in appropriate chapters to aid decision making.

xiv Preface

Scope

For the purposes of defining the scope of this volume, we define *bioremediation* as any process that involves biological processes, through the action of biota such as bacteria, fungi, and plants, in the transformation and breakdown of petroleum hydrocarbons. Since abiotic breakdown, such as evaporation, is often an important or even dominant mechanism in some biologically active treatment systems, this process is not excluded from the broad definition of bioremediation used here. However, purely abiotic treatments, such as thermal incineration and *in situ* chemical oxidation, are not considered in detail here.

Petroleum Hydrocarbons are hydrogen and carbon based compounds derived from rock, mineral or natural oils, and any of their derivatives. From a remediation perspective, this book mainly considers crude oil and its refined products such as diesel, kerosene, aviation fuels, and gasoline.

Cold Regions are Arctic and sub-Arctic, Antarctic and sub-Antarctic, and alpine geographic regions that exhibit permafrost or experience seasonally frozen ground. Additionally, maritime subpolar regions that do not freeze but share many other environmental characteristics are also included.

Future initiatives

We recognize that this book is incomplete in many regards. For example, there is little integrated information from the many disseminated mid-latitude and high-altitude fuel spills. Similarly, reliable information from Russia and China is sadly lacking. Furthermore, the focus of this volume is soil treatment; much still needs to be done with respect to treating petroleum-contaminated water in cold regions.

We welcome further information and criticism. Please send comments to Dennis Filler at ffdmf@uaf.edu.

Glossary

Absorption is retention of a solute within the mass of a solid rather than on its surface.

Action value is a contaminant concentration beyond which remediation is considered necessary.

Active layer (or seasonally thawed layer) is the upper realm of soil or permafrost that experiences annual freezing and thawing as a function of temperature.

Adsorption is retention of a solute by the surface of a solid rather than within its mass.

Air freezing index (AFI) is the index representing the number of negative (T < 0 °C) degree–days between the highest and lowest points on a curve of cumulative daily average air temperatures versus time (degree–days) for a given location. AFI is calculated as the area below the freezing isotherm drawn through such a curve.

Air sparging is an *in situ* technology where air is injected into contaminated water-saturated soils, or aquifers, at high flow rates $(10-20 \text{ m}^3/\text{h})$ to promote contaminant volatilization.

Air thawing index is the index representing the annual total thawing degree–days (i.e. area above the freezing isotherm) of the average daily temperature curve for a given location.

Allochthonous microorganisms are nonindigenous bacteria.

Amino acids are organic acids that contain a carboxyl group (COOH) and amino group (NH₂), linked together into polypeptide chains to form proteins that are essential to all life.

xvi Glossary

Ammonia volatilization is loss of ammonia gas (NH₃) from the soil into the atmosphere.

Ammonification is the production of ammonia resulting from the bacterial conversion of organic nitrogenous compounds.

Anthropogenic refers to man-made in origin.

Autochthonous refers to native soil bacteria whose populations do not change rapidly in response to the addition of specific nutrients.

Autotrophs are organisms that produce complex organic compounds from simple inorganic molecules, e.g. soil bacteria that mineralize ammonium into nitrate (i.e., autotrophic nitrification).

Bioaugmentation is soil amendment with non-indigenous microorganisms (inoculation), bioproducts, or engineered microbes to enhance the biodegradation of contaminants.

Bioavailability is the fraction of the total of a chemical in the surrounding environment (i.e. water, sediment, soil, suspended particles) that can be taken up by organisms.

Biodegradation is the breaking of intramolecular bonds (or *breakdown*) of organic substances by microorganisms to derive energy.

Biogenic interference is the fraction of natural organic matter that cannot be distinguished from petroleum in a standard petroleum analysis.

Biopile is a remediation technique involving heaping contaminated soils into piles and stimulating aerobic microbial activity within the soils through aeration and/or addition of minerals, nutrients, and moisture.

Bioreactor is a closed-vessel system in which biological, chemical, and physical processes occur, and that permits control of process parameters (e.g. temperature, moisture, oxygen, pH, and nutrients).

Bioremediation is any biological process, through the action of bacteria, fungi, plants, or managed biodegradation, that transforms or breaks down environmental contaminants.

Biosparging is an *in situ* technology where air is injected into contaminated water-saturated soils, or aquifers, at low flow rates ($<5 \text{ m}^3/\text{h}$ or 3 cfm per point) to promote aerobic biodegradation. Volatilization is typically less than that of the standard air sparging system.

Glossary xvii

Biostimulation is the modification of the environment (e.g., by the addition of nutrients, oxygen, etc.) to stimulate the rate of biological degradation of contaminants by indigenous microorganisims.

Bioventing is the process of injecting air (i.e., aeration) into subsurface soils to stimulate *in situ* bioremediation using soil vapor extraction systems.

Capillary forces are mechanical forces exerted on the soil-water-gas system at the pore scale. The combined effect of *surface tension* and effective *contact angle* occurs at the interface between water, air and soil in the interconnected soil pores. Surface tension is the tangential force acting at the interface between the water and air vapor, caused by the difference in attraction between liquid and gaseous molecules. The effective contact angle is the equilibrium angle of contact of water on the soil particle surface, measured within the water at the contact line where the three phases (liquid, solid, and gas) meet.

Capillary fringe is the zone of soil immediately above the water table that *wicks* water up from the underlying water table (i.e., soil suction) and retains this water by capillary forces. As most soil pores are completely filled with water at the base of the capillary fringe, but only the smallest soil pores are water filled at the top, moisture content of the capillary fringe decreases with increasing distance above the water table. Furthermore, because the size of the soil pores define the capillary rise of the water column, and since soil pore size is highly heterogenous, the height of capillary rise has a ragged upper surface.

Chromatography is an analytical technique for separating substances by adsorption on media for which they have different affinities. A *chromatogram* is the graphical output of the chromatograph, in which different peaks or patterns correspond to different components of the separated mixture.

Cold regions are Arctic and sub-Arctic, Antarctic and sub-Antarctic, and alpine lands that exhibit permafrost or experience seasonally frozen ground.

Cometabolism is the simultaneous metabolism of two compounds, in which the degradation of the second compound requires the presence of the first compound.

Denitrification is the process of reducing nitrates and nitrites, most commonly by soil bacteria (denitrifying bacteria), into nitrogen-containing gases (e.g., N_2 , N_2O , NO, NO₂). Denitrification is primarily an anerobic process.

End Point is a contaminant concentration that once reached, remediation is considered to be complete.

xviii Glossary

Engineered remediation is the design, analysis, and/or construction of works for the removal of environmental contaminants from media such as soil, groundwater, sediment, or surface water.

Engineered bioremediation is the design, analysis, and/or construction of works for the removal of environmental contaminants from media such as soil, ground-water, sediment, or surface water utilizing microorganisms to enhance bioactivity.

Enzyme is a compound, usually a protein, that acts as a catalyst to a specific chemical reaction.

Evolutionary operation is the process of optimizing a system(s) to maximize its performance. In bioremediation, this process is used to fine-tune systems in order to maximize treatment effectiveness and minimize operation and maintenance costs.

Extraction (*solvent*) is a method to separate compounds based on their solubility in various solvents. Petroleum compounds are extracted out of an aqueous phase into an organic phase for chromatography and quantitation.

Field trial refers to a treatability study or pilot study that is implemented at a contaminated site generally to test the performance of a particular remediation technique under the conditions in which it will be used.

Freeze exclusion is the displacement of petroleum from soil pores as soil water freezes.

Funnel and gate is an *in situ* system of cut-off walls (e.g., sheet pile, slurry, or composite walls) and a central gate (e.g., permeable reactive barrier) equipped with permeable reactive or non-reactive media, used to control and treat contaminated water.

Genotypic adaptations refer to changes in an organism's genome over evolutionary time scales.

Heterotrophs are organisms that are only able to utilize organic sources for biosynthesis.

Homologues are identical compounds except for their frequency of repetition (homologous).

Humic substances comprise the refractory, mainly hydrophilic, high molecular weight, chemically complex and polyelectrolyte-like fraction of natural organic matter (NOM).

Glossary xix

Hydrolysis is chemical reaction involving cleavage of a molecular bond by reaction with water.

Hydrophilic means water attracting.

Hydrophobic means water repelling.

Hydrophobic bonds (or *interactions*) occur where compounds possess non-polar properties and are brought into close association in aqueous environments due to their repulsion to water.

Hyperthermophiles (or extreme thermophiles) are microorganisms with a thermotolerance range above 70 $^{\circ}$ C, up to about 115 $^{\circ}$ C.

Immiscible fluids are liquids or gases that cannot mix to form a homogenous mixture.

Indigenous microorganisms are microorganisms that naturally exist in a particular environment, and that usually have a selective, competitive advantage in that environment.

Insolation is incoming solar radiation, or solar heating.

Intrinsic bioremediation (or natural attenuation) is bioremediation by natural processes (e.g., biodegradation, abiotic transformation, mechanical dispersion, dilution, and sorption) that reduces contaminants in the environment.

Landfarming is land treatment of soil for degradation or transformation of contaminants by a combination of volatilization and biodegradation by resident microorganisms. A common practice is to place the soil as a shallow layer within a bermed and lined treatment cell (or biocell), occasionally amend the soil with nutrients and water to stimulate biodegradation, and regularly till (aerate) the soil to mix and aid contaminant volatilization.

Magic number refers to an arbitrary cleanup value arrived at when one considers a trigger value as the trigger, action and remediation target.

Matric potential, one of two major components of soil water potential, is the result of the attraction between soil particles (primarily clay size – less than 0.002 mm in diameter) and water.

Mesocosms are small-scale bioassays designed to simulate natural soil conditions for the purpose of evaluating conditions supportive of bioremediation. A mesocosm treatability study can be conducted in the field, and results can be used to assess biodegradation rates and for estimating treatment duration and remediation costs.

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Mesophiles are soil microorganisms with a thermotolerance range of 15 °C to 45 °C, that grow optimally between 20 °C to 40 °C, and that can survive at low temperatures (less than 4 °C) without growing.

Methanogenisis is conversion of short-chain organic compounds by anerobic microorganisms to methane, carbon dioxide, and inorganic byproducts.

Microbial consortia are mixed populations of interacting microorganisms.

Microcosms are small-scale experimental bioassays used to evaluate conditions supportive of bioremediation. This type of treatability study does not attempt to simulate a soil ecosystem, but results can be used to infer bioremediation potential and treatment effectiveness.

Microfauna are microscopic and/or very small animals (e.g., protozoans and nematodes) that live in a particular environment.

Mineralization is the breakdown of organic compounds (e.g., organic residues in soil) into inorganic materials (e.g., mineral nutrients that can be utilized by plants).

Natural attenuation is the unassisted biodegradation, evaporation, adsorption, metabolism, or transformation of contaminants in soil and water by microorganisms.

Nitrification is the process of oxidizing ammonia and organic nitrogen to nitrite (mainly by bacteria of the genus *Nitrosomonas*) followed by the oxidation of these nitrites into nitrates (mainly by bacteria of the genus *Nitrobacter*). Nitrification is an aerobic process.

Nitrogen demand refers to the amount of nitrogen required for degradation of a specific amount of contaminant.

Non-aqueous phase liquid (*NAPL*) is an organic liquid that is relatively insoluble in water.

Oligotrophs are organisms able to survive on very low nutrient concentrations.

Osmotic potential, a major component of soil water potential together with matric potential, that results from the interaction of water molecules and dissolved salts.

Permafrost is soil that remains frozen for two or more consecutive years.

Permeable reactive barrier is an open *in situ* reactor system designed to achieve contaminant mass transfer reactions, as with bioreactors, but with somewhat less control.

Glossary xxi

Permeability is a measure of the ability of a material to transmit fluids.

Permeases are membrane transport proteins that facilitate the diffusion of a specific molecule in or out of the cell.

Petroleum Hydrocarbons are hydrogen and carbon based compounds derived from rock, mineral or natural oils, and any of their derivatives.

Phenotypic adaptations refer to changes in the structural and physiological properties of organisms in response to a genetic mutation or a change in the environment.

Photooxidation is ultraviolet light-induced oxidation for destruction of organic contaminants.

Phytoremediation is the use of plants to remediate contaminated soils or water.

Pilot study generally refers to a scaled down test of a remediation system or component. The study may take place at the contaminated site to simulate operation under field conditions, or in the laboratory as a *bench-scale study*.

Polar regions are geographic regions above the Arctic Circle (N66°33'39") and south of the Antarctic Circle (N66°33'38"), characterized by long, dark and cold winters followed by a few light summer months. Land in these regions includes the high-Arctic, coastal plains and archipelagos of North America, northern portions of Greenland, Scandinavia and Russia, and Antarctica.

Pore ice is ice that occurs in the pores of soils and rocks.

Proteins are long polypeptide chains often bonded with nucleic acids, lipids, etc., essential to the diet of microorganisms in the composition of cellular structures and in enzymatic processes.

Psychrophiles are soil microorganisms with a thermotolerance range of -5 °C to 25 °C, that grow optimally around 10 °C, and usually do not grow above 20 °C.

Psychrotolerant microbes (psychrotrophes) have the ability to grow at low temperatures (less than 4 °C), but optimally between 20 °C to 30 °C.

Psychrotrophic is the ability to grow best at temperatures below 20 °C.

Recalcitrant means resistant to microbial degredation.

Rhizosphere is the soil region in the immediate vicinity of growing plant roots including the region of the soil modified as a result of the presence of a root.

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Rhizodegradation is the transformation of contaminants in soil proximal to plant roots by organisms associated with vegetative species.

Segregated ice is ice that occurs as discrete layers or ice lenses in soil.

Sequestering of a contaminant compound occurs when the compound becomes less available or is wholly unavailable to microorganisms for biodegradation.

Soil warming is passive (solar or water conducted) or active (mechanized) heating of soil to enhance biodegradation of contaminants.

Soil water potential is a measure of physical and chemical potential (or energy) of the soil water. Its two major components are matric and osmotic potentials.

Sorption refers to both absorption and adsorption, or the retention of solutes in solution in the solid mass and on surface of the solid.

Sulfate reduction is the conversion of reducible forms of sulfur (e.g., sulfate, thiosulfate) to reduced forms (e.g., sulfides, elemental sulfur) by bacterial anaerobic respiration.

Suprapermafrost water is water that occurs in the active layer above permafrost.

Surface freezing index is the cumulative number of degree–days below 0° C for the surface temperature (of the soil, ground, etc.) during a given time period.

Surrogates are organic compounds that are similar to analytes of interest in extraction and chromatography, but which are not normally found in environmental samples. These compounds are spiked into method blanks, calibration and check standard, samples (including duplicates and quality control reference samples) and spiked samples prior to analysis. Analysis typically includes calculations of percent recoveries for each surrogate used.

Taliks are layers or bodies of unfrozen soil in permafrost. An open talik is completely surrounded by permafrost, a closed talik is a thawed zone bordered by permafrost and the ground surface and a through talik is bordered by unfrozen layers beneath the permafrost and the ground surface.

Thermokarst is soil deformation (subsidence) that results from the thawing of ground ice and consolidation of the thawed soil.

Thermophiles are organisms that live under conditions of high heat and acid. In the soil these organisms usually have a thermotolerance range of 40 °C to 70 °C, grow optimally between 50 °C to 60 °C, and usually do not grow above 65 °C.

Glossary xxiii

Treatability studies are *in vitro* microcosms with individual bacterial species or soil consortia incubated in liquid or slurry media, mesocosm studies with soils and natural microfauna, or field trials, used to evaluate conditions supportive of bioremediation.

Trigger value is a conservative soil contaminant value (or concentration) below which environmental impacts are considered unlikely. Soil contamination in excess of a trigger value should instigate further assessment, although such values are often used as action values and remediation endpoints.

Vadose zone is unsaturated soil above saturated soil or the water table, and includes the zone of capillary rise.

Volatilization is the transformation of a solid or liquid contaminant into vapor. In bioremediation, volatilization occurs naturally by wind and as consequence of the relative partial pressures of contaminant and air, or is artificially induced by mechanized systems (e.g., tilling, bioventing).

Water holding capacity is the field capacity of soil to store water after saturation, and once gravitational water is drained.

Weathering of a contaminant refers to the degradation of more biodegradable compounds via biotic or abiotic reactions and the formation of an aged residue.

Zeolites are predominantly alumino-silicate hydrated minerals (e.g., chabaite, clinoptilolite, heulandite, natrolite, stilbite) that have a micro-porous structure that can readily accommodate a wide variety of cations (e.g. Na⁺, K⁺, Ca²⁺, Mg^{2+}). There are 48 naturally occurring and more than 1500 synthesized zeolite types known.

ACRONYMS

ABS	acrylonitrile butadiene styrene
AFI	air freezing index
ATI	air thawing index
BTEX	benzene, toluene, ethylbenzene, xylenes
CAF	coarse adjustment factor
CALM	Circumpolar Active Layer Monitoring program
CAM	cold-adapted microorganism
C:N	carbon to nitrogen ratio
C:P	carbon to phosphorus ratio
cPCR	competitive polymerase chain reaction
CRREL	Cold Regions Research & Engineering Laboratory
CRN	controlled release nutrients

xxiv Glossary

CSP	cold-shock proteins
CSR	cold-shock response
DEA	diethylamine extraction solvent
DEG	diesel-electric generator
DNA	deoxyribonucleic acid
DRBO	diesel range biogenic organics
DRO	diesel-range organics
FID	flame ionization detection
GC	gas chromatography
GRO	gasoline-range organics
GRPH	gasoline-range petroleum hydrocarbons
HDPE	high-density polyethylene
LNAPL	light non-aqueous phase liquid
LOI	loss of ignition
m.a.a.t.	mean annual air temperature
MAE	microwave assisted extraction
MS	mass spectrometry
MPN	most probable number
NAPL	non-aqueous phase liquid
NOM	natural organic matter
N:P:K	ratio of nitrogen to phosphorus to potassium
PAH	polycyclic aromatic hydrocarbon
PCB	polychlorinated biphenyls
PCR	polymerase chain reaction
PHC	petroleum hydrocarbons
PRB	permeable reactive barrier
RRO	residual range organics
RRBO	residual range biogenic organics
SAB	Special Antarctic Blend
SFI	surface freezing index
TEB	thermally enhanced bioremediation
TPH	total petroleum hydrocarbons
USEPA	United States Environmental Protection Agency