



Understanding Temperature Effects on Bioremediation and Cleanup Timeline: Case Studies

June 1, 2023

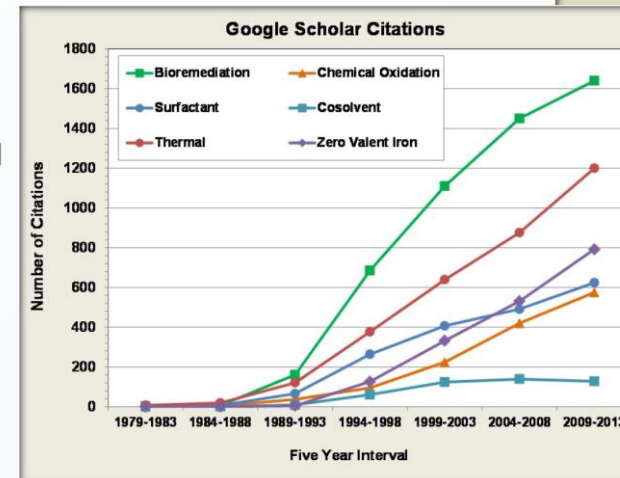
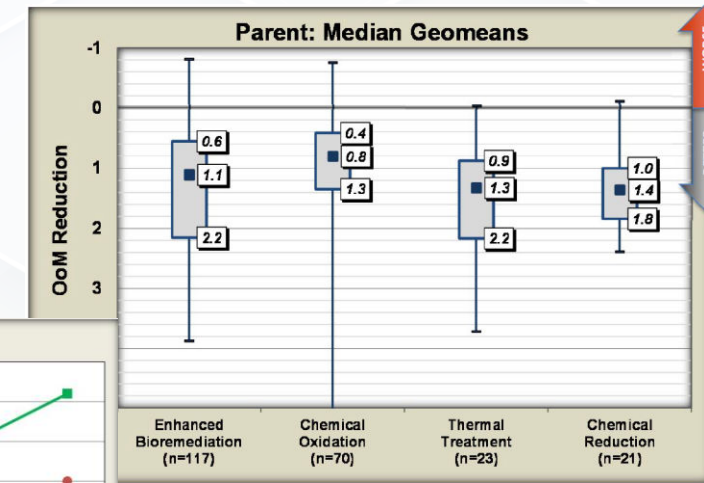
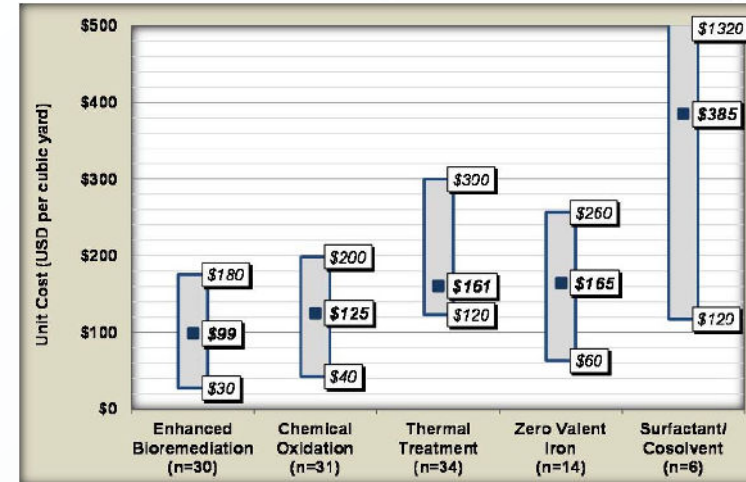
B.J. Min, M.Eng., P.Eng.
Jongho Won, Ph.D., P.Eng.

Outline

- Background – Basics of Bioremediation
- Bioremediation Myth & Misperception
- Temperature Effect on Bioremediation
 - Microbial Growth, Biodegradation Rate, and Cleanup Timeline
- Bioremediation Enhancement Options
- Pilot/Field Implementation Cases under Cold Temperature

Why Bioremediation?

- Cost-effective & sustainable approach
- Less energy required
- Less destructive cleanup process
- Low operations and maintenance
- No negative impact on receptors on site during/after remediation
- long lasting effectiveness and responsive cleanup for residual contaminants from back-diffusion
- Many proven studies & field cases

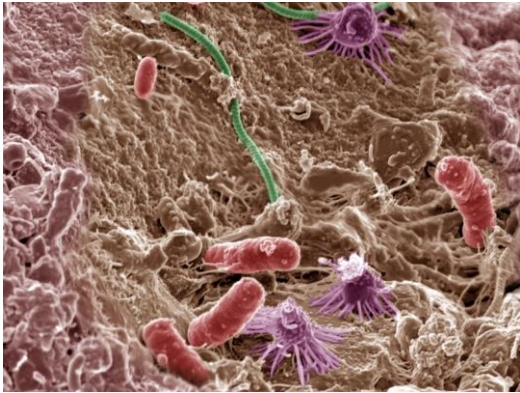


(McGuire et al. 2016)

What Bioremediation Options Available?

Microbial Bioremediation

Microbes



(Courtesy of Pacific Northwest National Laboratory)

Mycoremediation

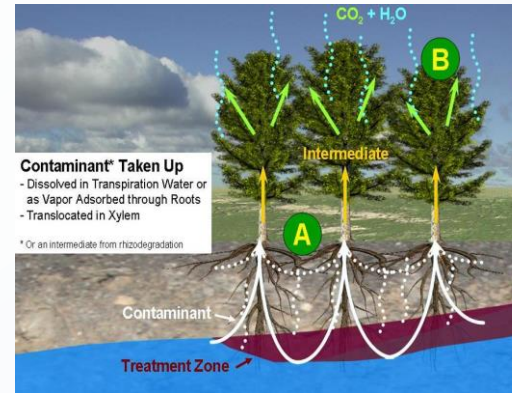
Fungi (e.g. mushroom)



(TRIUM test site)

Phytoremediation

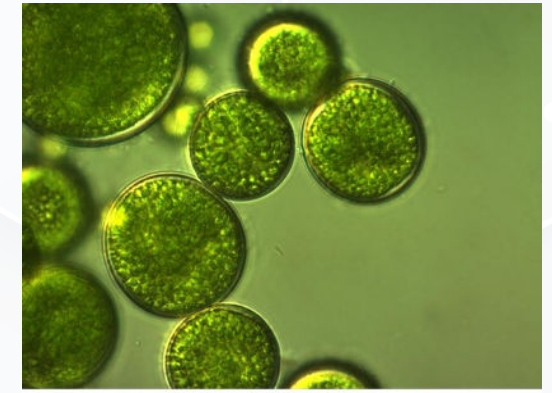
Plants & Trees



(ITRC Guideline)

Phycoremediation

(Micro)Algae

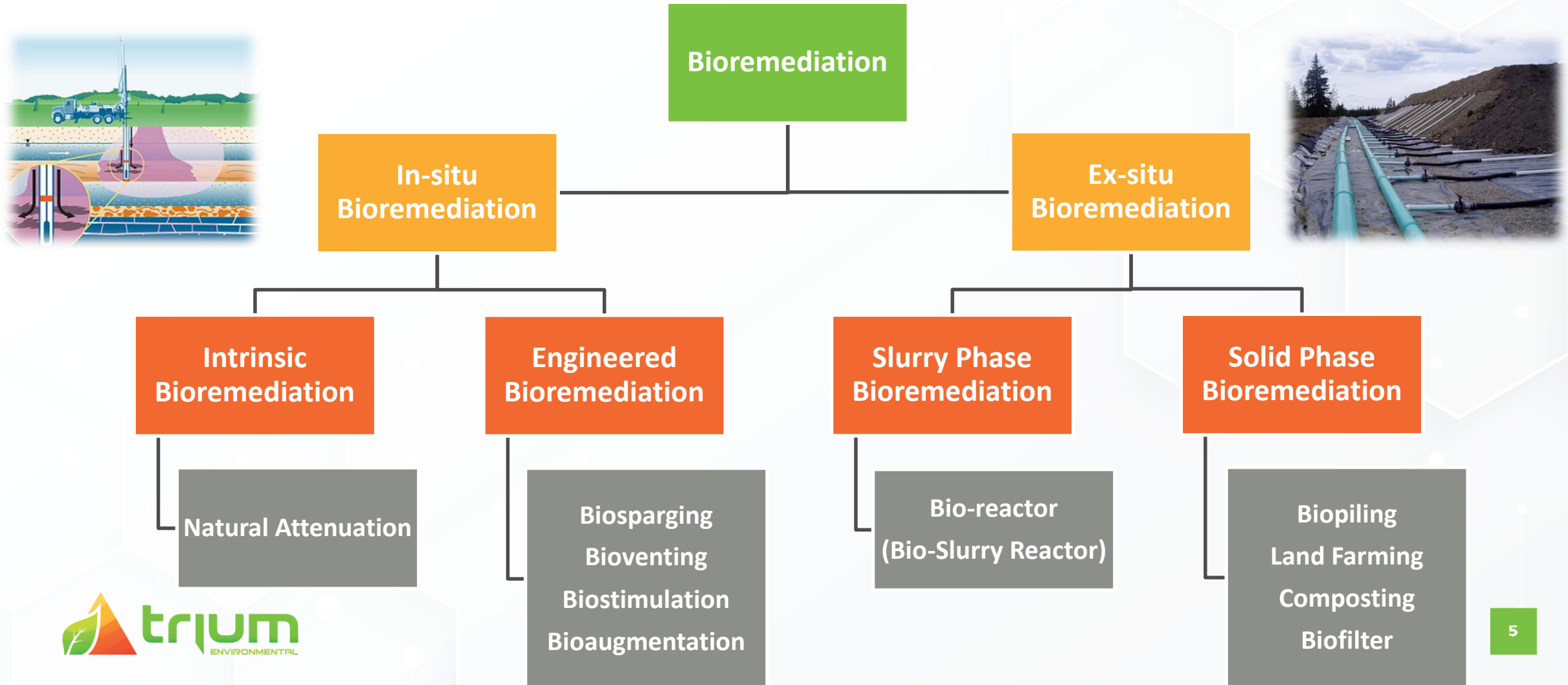


(Shutterstock)

Other Bioremediation

- Zooremediation (e.g. fish)
- Mix cell culture system (bacteria + algae + fungi)
- Vermiremediation (earthworms + microbes)

What Bioremediation Methods Available?



How Bioremediation Works?

- Reduction-Oxidation (Redox) Reaction based
- Microbial growth (anabolism) using energy released during biological redox reactions
- Contaminants oxidized as electron donors (e.g. PHC) or reduced as electron acceptors (e.g. PCE)

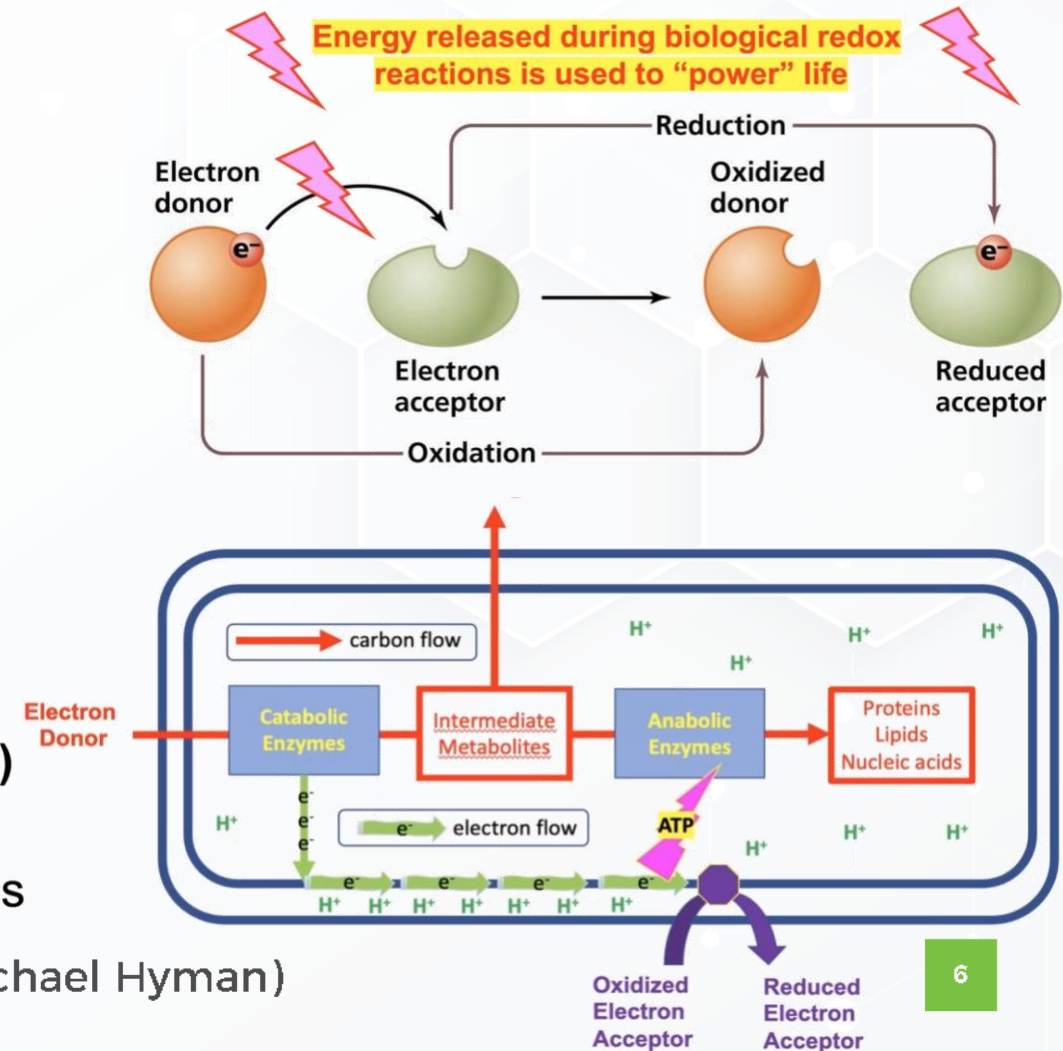
ATP = Adenosine triphosphate (cash energy currency of cell)

Metabolism = Catabolism + Anabolism

ABC of Metabolism: Anabolism **B**uilds **C**atabolism **D**egrades

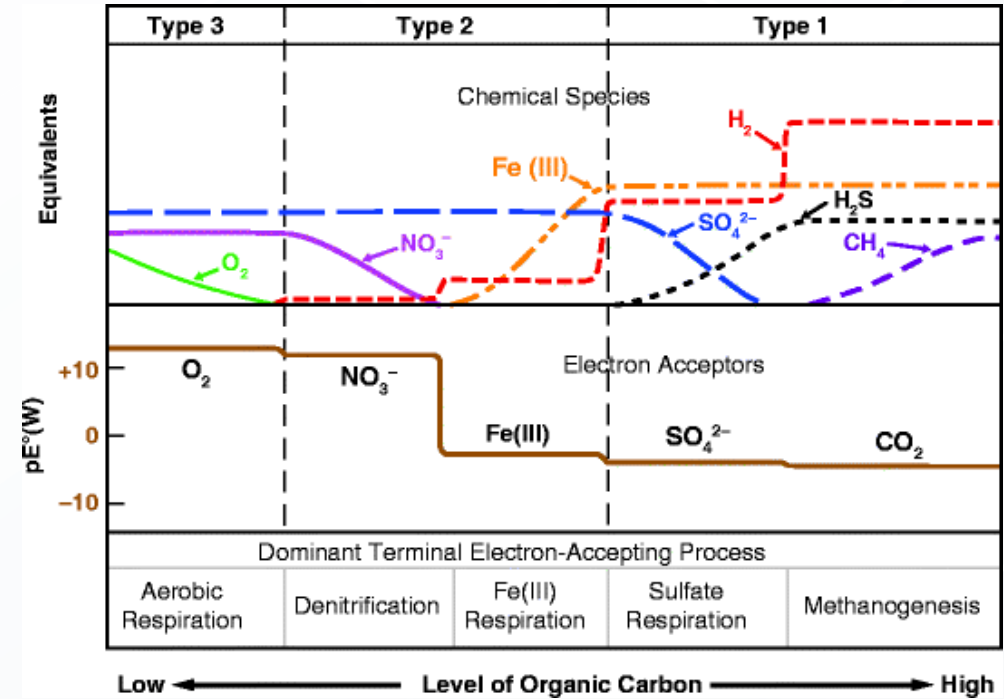


(Courtesy of Dr. Michael Hyman)

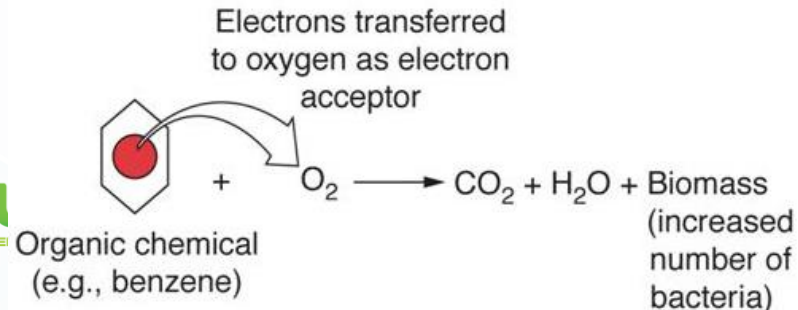


Aerobic vs Anaerobic Bioremediation

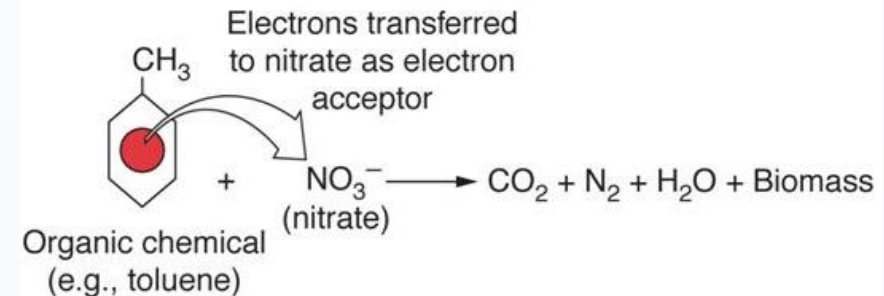
- **Aerobic Biodegradation**
 - Organics (e.g. hydrocarbons) are oxidized
 - Oxygen is reduced to water by gaining electrons (electron acceptor)
- **Anaerobic Biodegradation**
 - Organics (e.g. toluene) are oxidized
 - Organics (e.g. PCE) & inorganics (e.g. nitrate, sulphate) are reduced by gaining electrons (electron acceptor)



Aerobic biodegradation



Anaerobic biodegradation



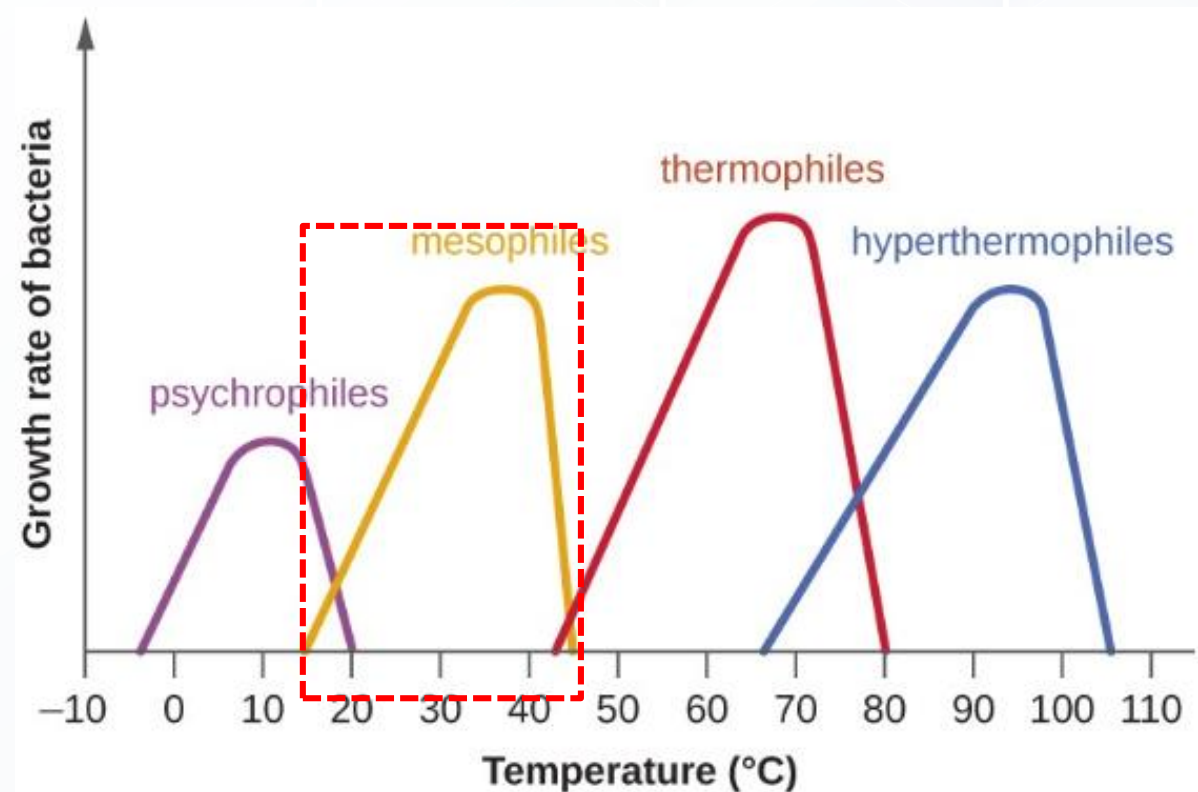
Bioremediation Myth & Misperception

- **Myth 1 - No Effectiveness of Bioremediation in Cold Region**
 - Fact - Slow microbial growth (or activity) in cold conditions but still works and enhanced biodegradation comes back under warm temperature
- **Myth 2 - Too Long Treatment Time**
 - Fact - Typically months to years of treatment time but can be reduced by engineered (enhanced) bioremediation approaches
- **Myth 3 - Effective for Not Too Bad Soils Only**
 - Fact - Bioremediation is versatile to treat wide range of concentrations and contaminant types, being supported by many proven cases
- **Myth 4 - Potential Stall of Toxic Daughter Products**
 - Fact - Well designed and managed system can prevent potential stall of undesired byproducts (e.g. abiotic/biotic reductive dechlorination for VC)
- **Myth 5 - Microbes & nutrients are all we need**
 - Fact - Other inhibitory factors including contaminant/metal toxicity, bioavailability, geochemical factors (pH, electron donors/acceptors, etc.) should be considered

Effect of Temperature in Microbial Growth

- Low Temperate Influences on:
 - Bacterial growth
 - Enzymes enrichment
 - Extracellular polysaccharides
 - Biosurfactant production (bioavailability of contaminants)
 - Fermentation of electron doner to produce hydrogen (anaerobic bioremediation)
 - Consequently, biodegradation rate

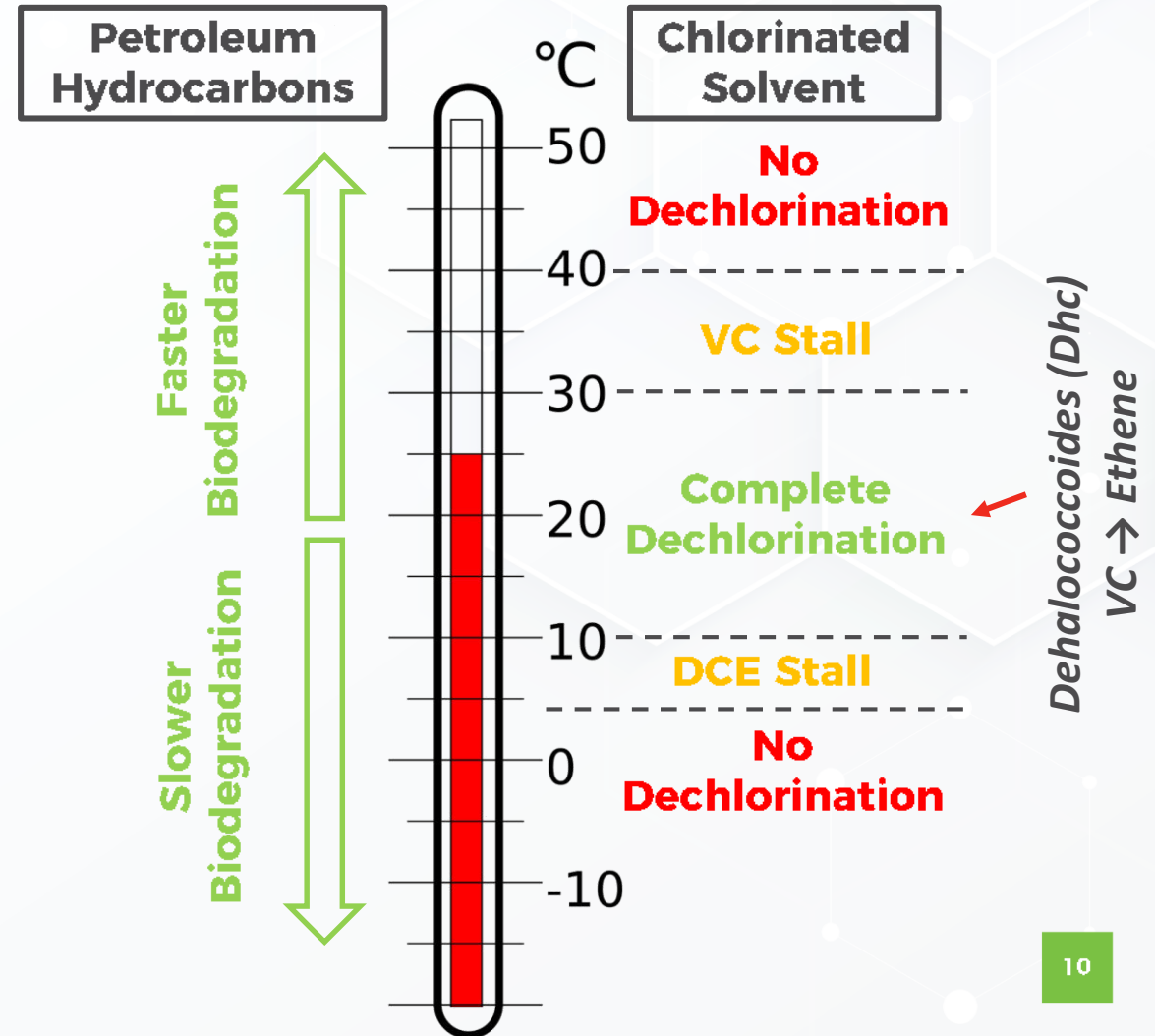
Many contaminant degraders are mesophiles (15-45 °C)



(LumenLearning)

Effect of Temperature in Bioremediation

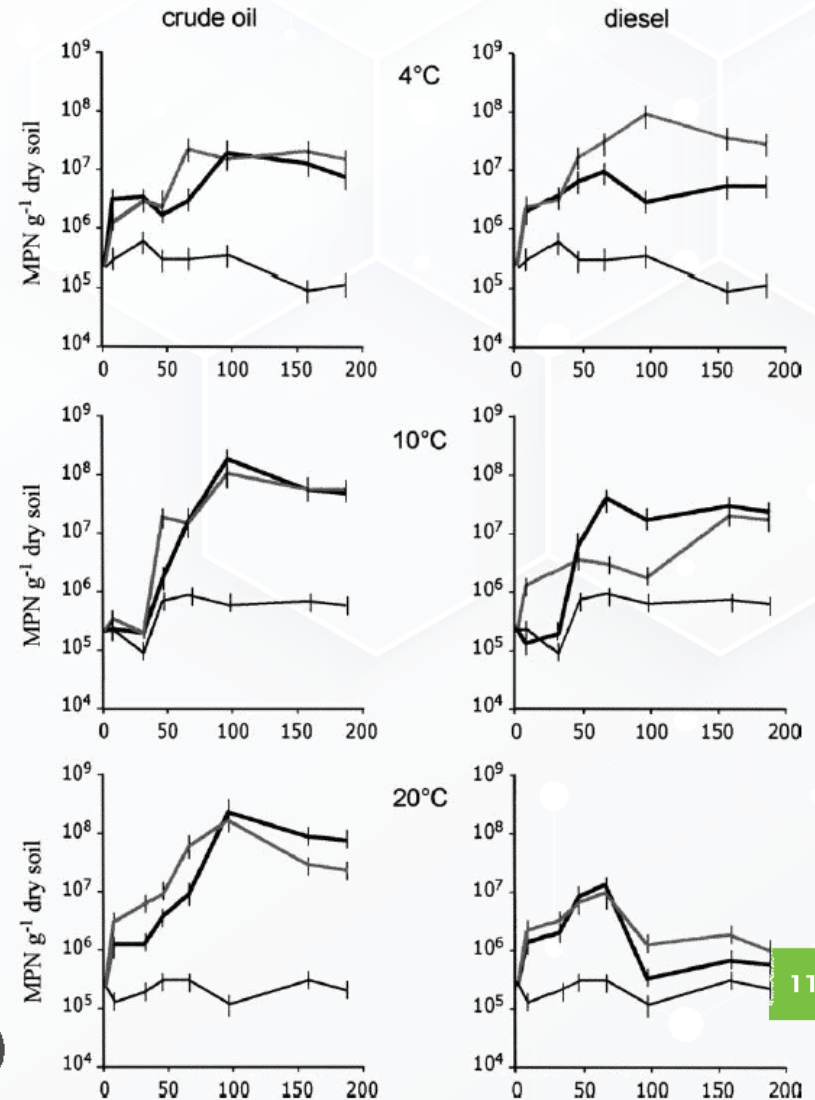
- PHC biodegradation available under wide temperature conditions (from freezing to warm)
- Slower PHC biodegradation under cold temperature
- Reported dechlorination of TCE to ethene: 4 - 40 °C
- Complete dechlorination typically observed at 10 - 30 °C
- Incomplete dechlorination at <10 °C or > 30 °C



(Heiman et al. 2007)

Effect of Temperature in Bioremediation

- GW Temperature and Hydrocarbon Degrading Bacteria Growth
 - Optimal growth in crude oil contaminated soil at 20 °C
 - Optimal growth in diesel contaminated soil at 10 °C
 - Relatively lower but still high bacterial population at 4 °C
 - $> 10^7$ MPN / g soil

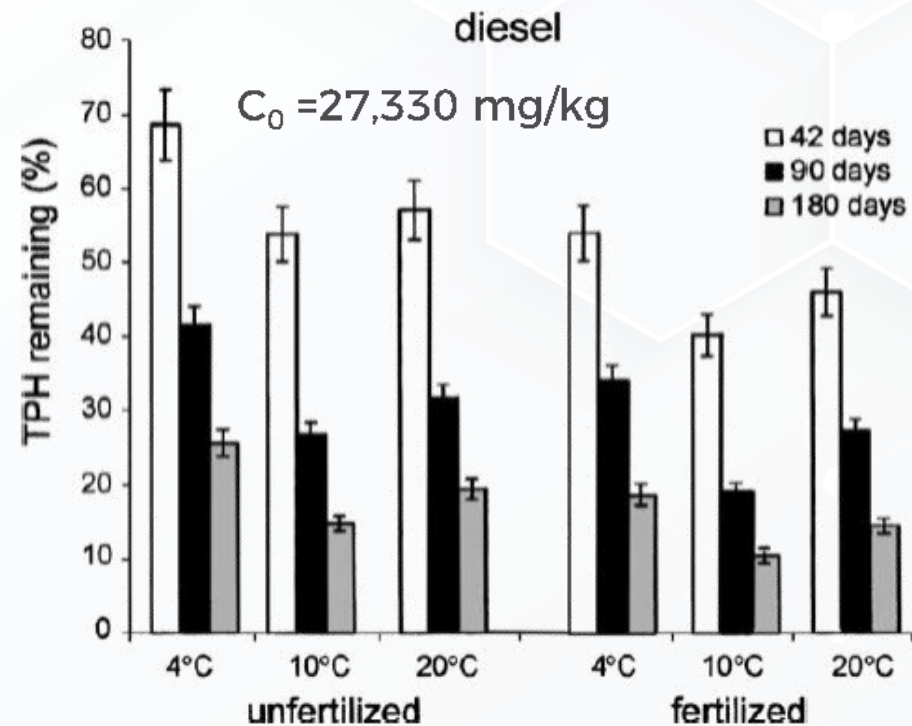
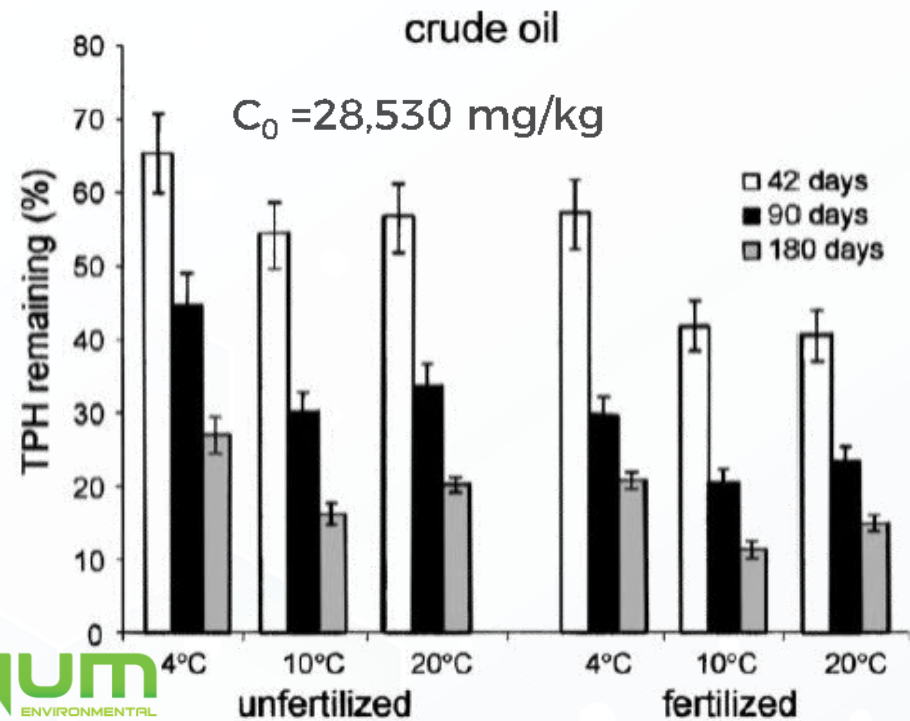


Thin: control
Thick: Fertilized
Gray: Unfertilized

(Coulon et al. 2005)

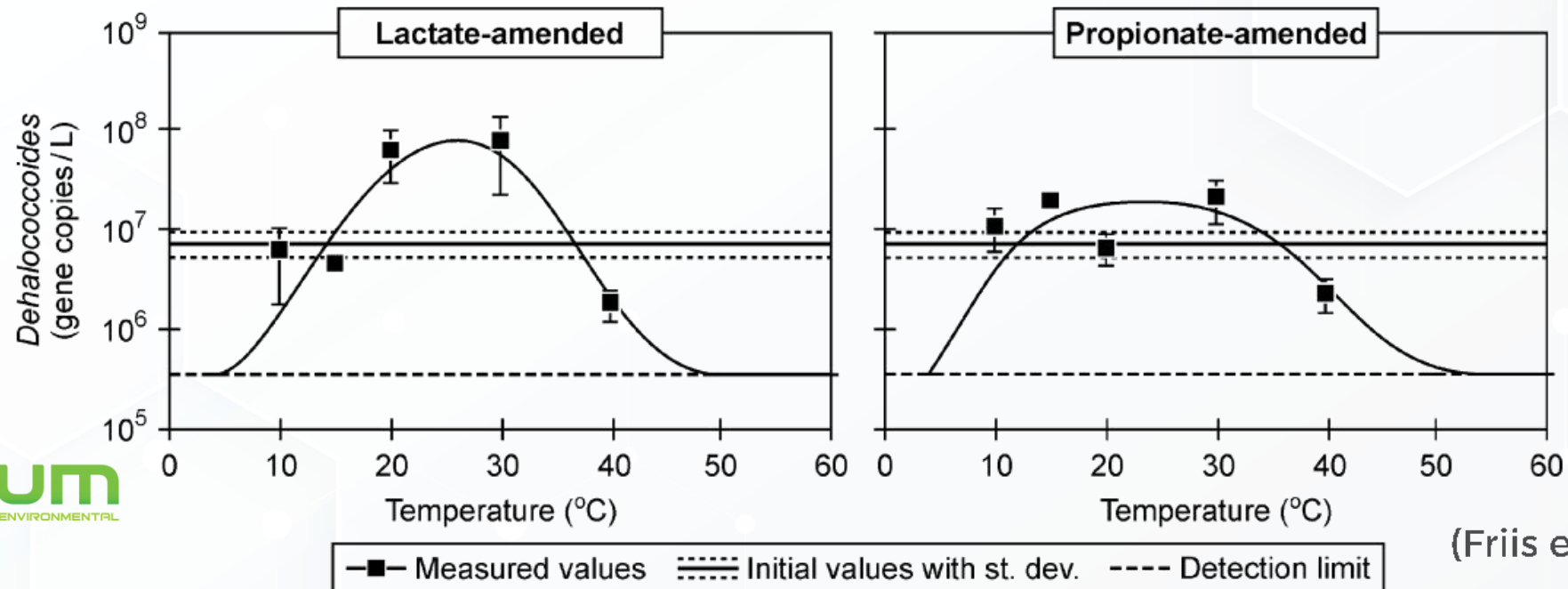
Effect of Temperature in Bioremediation

- GW Temperature and Hydrocarbon Biodegradation
 - ~ 80 % TPH degradation with nutrient addition at 4 °C (in 180 days)
 - ~ 90 % TPH degradation with nutrient addition at 10 °C (in 180 days)



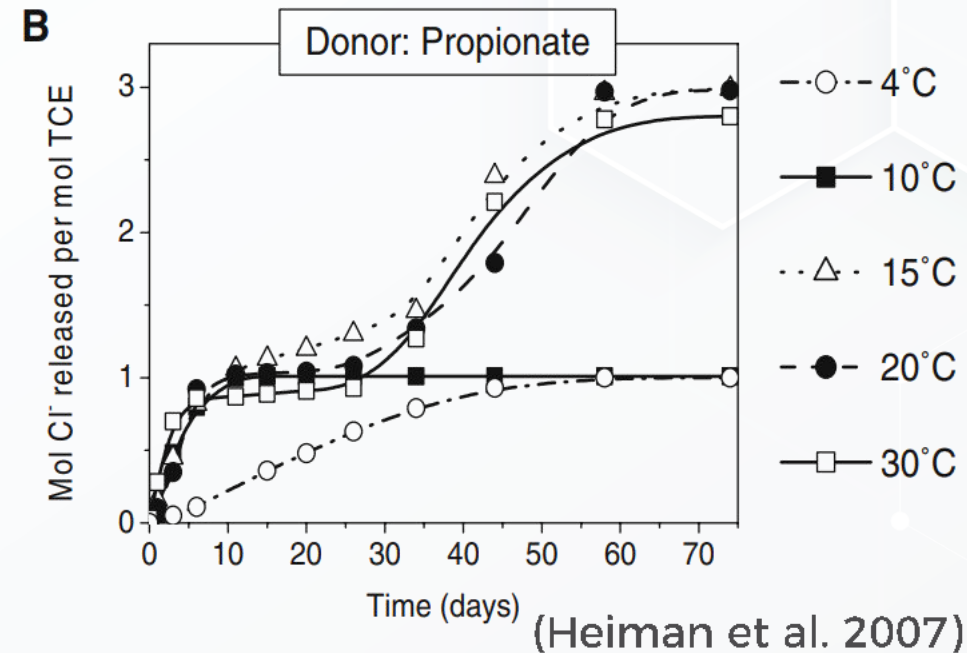
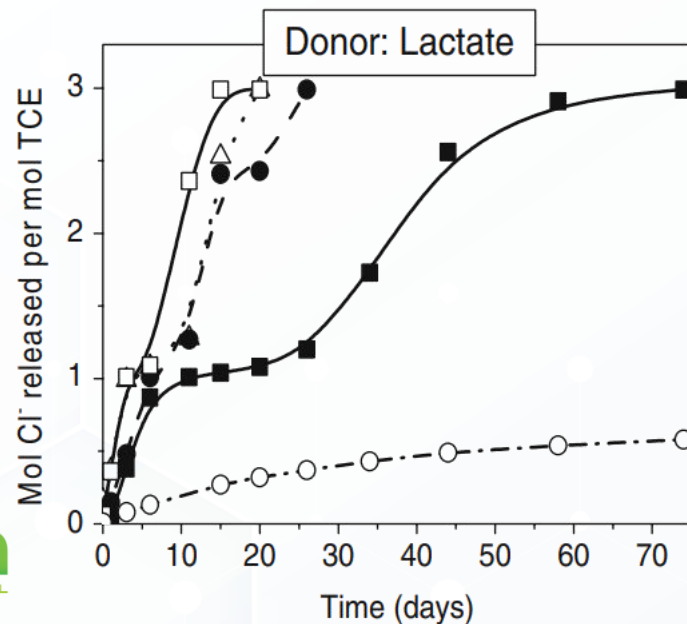
Effect of Temperature in Bioremediation

- GW Temperature and *Dehalococcoides (Dhc)* Growth
 - *Dehalococcoides (Dhc)* - Key bacterial species for complete dechlorination
 - Optimal *Dhc* growth at 20 - 30 °C
 - An order of magnitude lower growth at 10 °C but still high population (~ 10⁷/L)
 - **Type of electron donor** is also important factor in bacterial growth



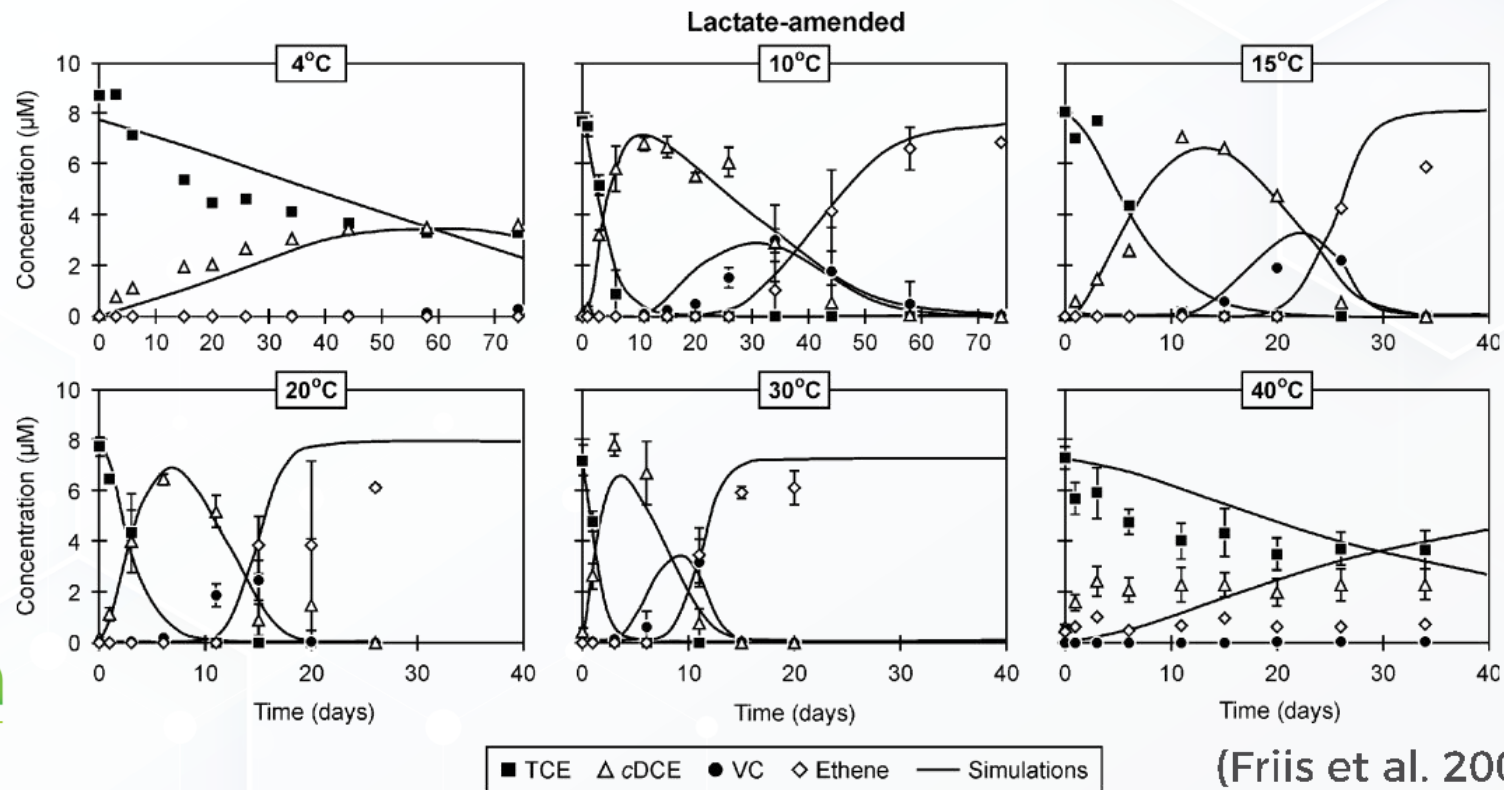
Effect of Temperature in Bioremediation

- GW Temperature and Electron Donor Fermentation
 - Optimal temperature for electron donor fermentation: 20 to 30 °C
 - Relatively faster dechlorination with lactate addition as an electron donor
 - Another evidence for importance of electron donor type
 - Relatively slower dechlorination but continually occurs at 4 °C



Effect of Temperature in Bioremediation

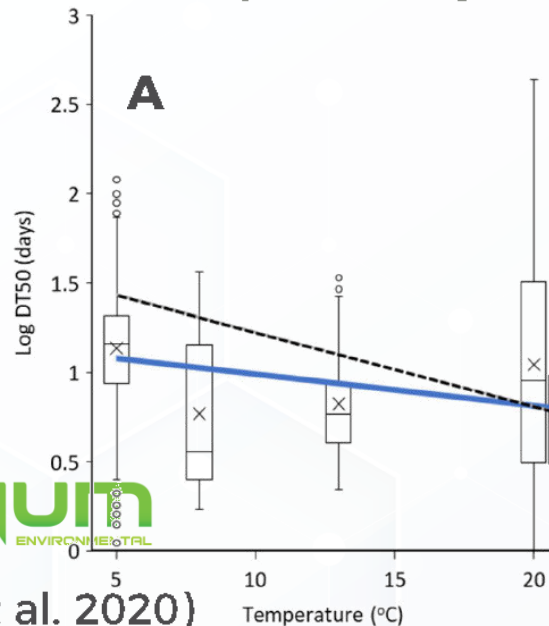
- Temperature and Dechlorination
 - No ethene production at 4 °C (incomplete dechlorination)
 - Initial ethene detection at Day 11 at 30 °C



(Friis et al. 2007)

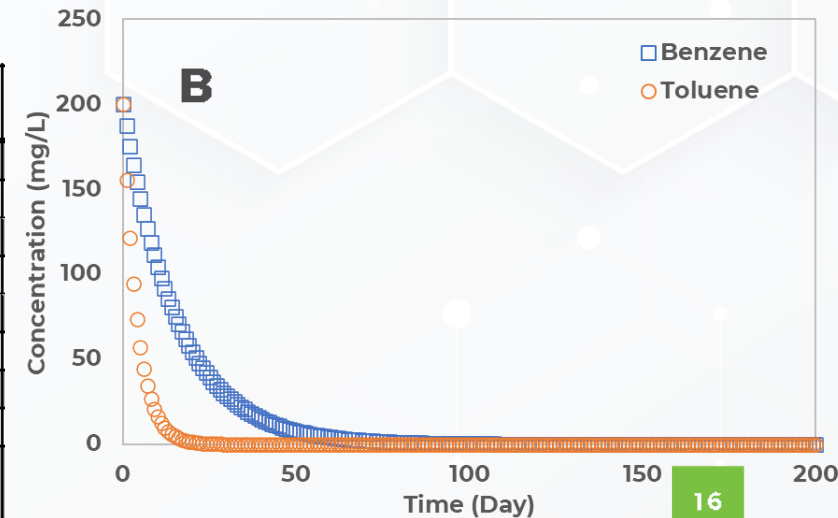
Bioremediation & Cleanup Time

- Estimation with the First order Biodegradation Rates
 - A. Degradation half time (DT50)
 - Time required to reduce by 50% of initial concentration
 - No lag phase $\frac{\ln(2)}{k}$ with lag phase $X_0 + \frac{\ln(2)}{k}$
 - B. Complete degradation time: $C_t = C_0 e^{-kt}$



	Benzene Day ⁻¹	Toluene Day ⁻¹
Number of Field Rates	68	59
Number of Lab Rates	82	76
Total Number of Rates	150	135
Mean	0.065	0.250
Std. Dev.	0.275	0.705
95% Confidence Interval	0.044	0.119
Minimum (95% confidence)	0.021	0.131
Maximum (95% confidence)	0.109	0.369
Average ambient temperature	NR	NR

NR = Not reported



Bioremediation & Cleanup Time

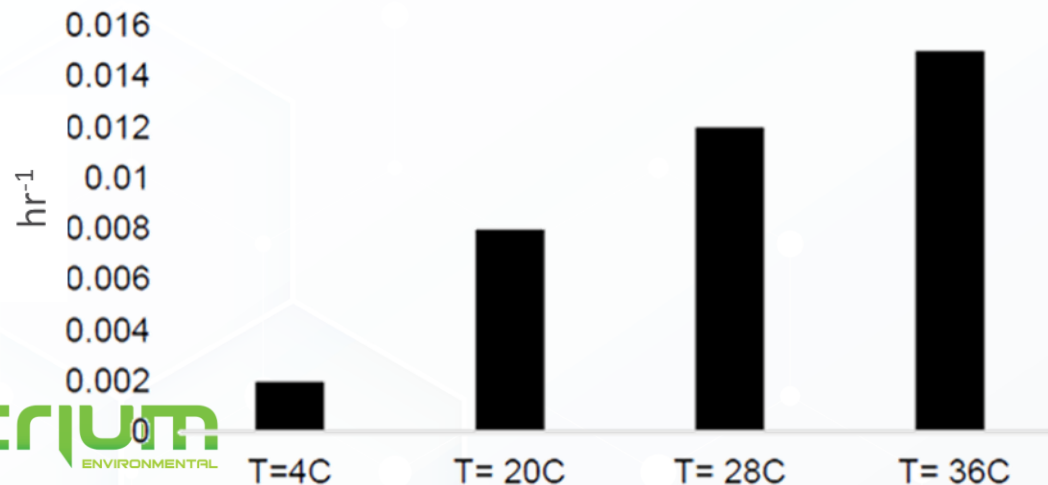
- Subsurface Temperature and Biodegradation Rate

- Rule of Thumb: $Q_{10} = 2$

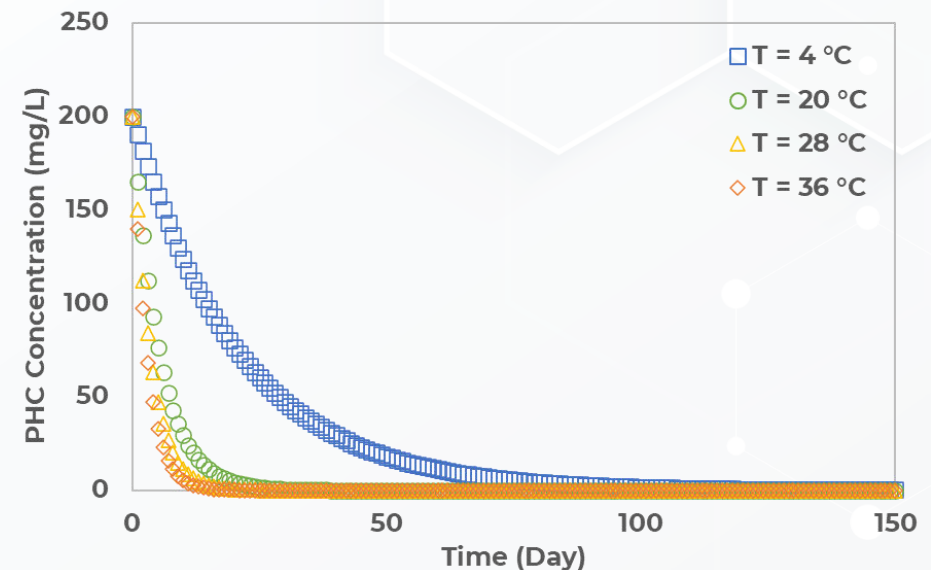
- Q_{10} : ratio of a first-order rate constant at a specific temperature to the rate constant at a temperature 10 °C lower
- Degradation rates double for every 10 °C increase within temperature range for bacterial growth

$$Q_{10} = \left[\frac{k_2}{k_1} \right]^{10/\Delta T}$$

Biodegradation rates

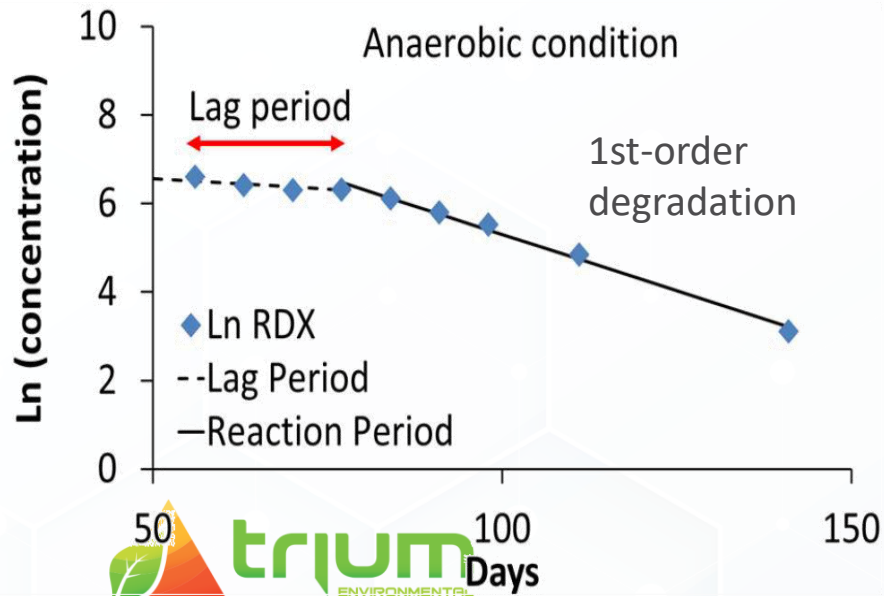


(Yadav and Gupta 2022)

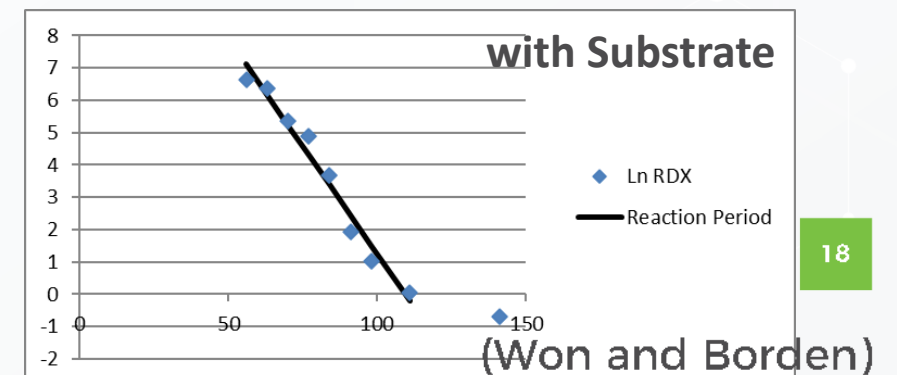
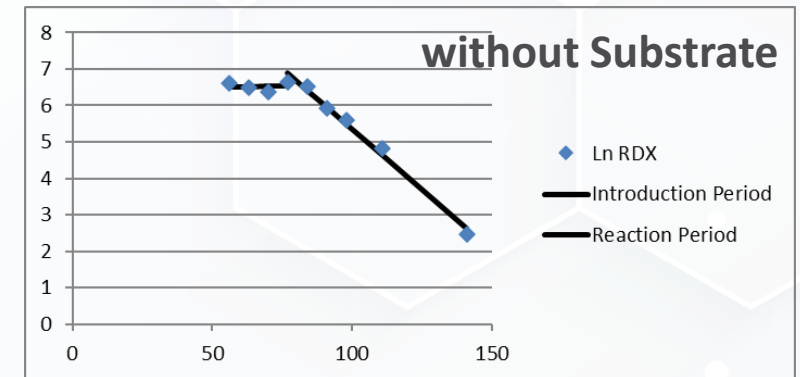
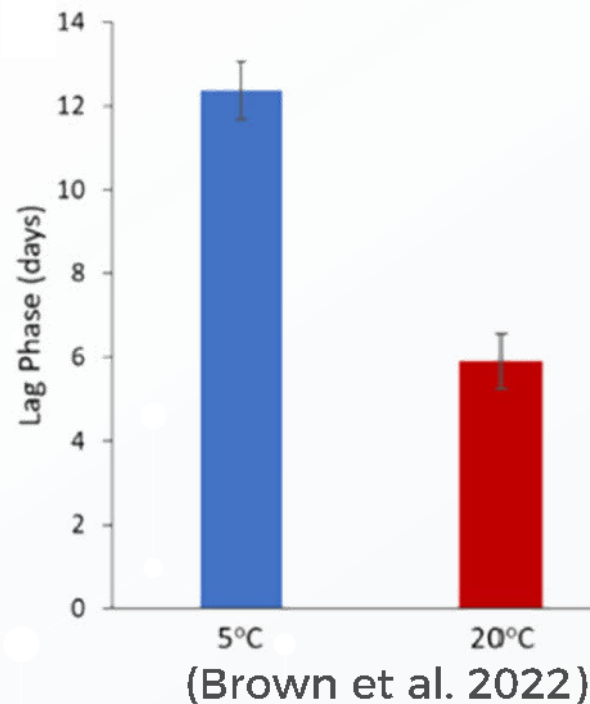


Bioremediation & Cleanup Time

- Consideration of Lag Period
 - No or limited degradation of target contaminants during bacterial growth
 - Longer lag period at lower temperature
 - Lag phase could be overcome through biostimulation or bioaugmentation



(Won and Borden 2016)

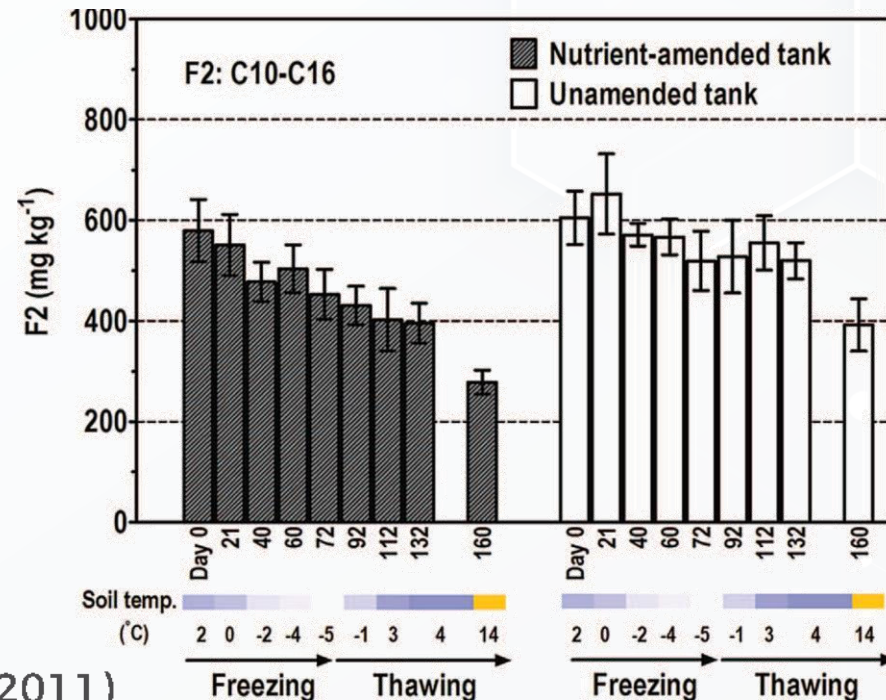
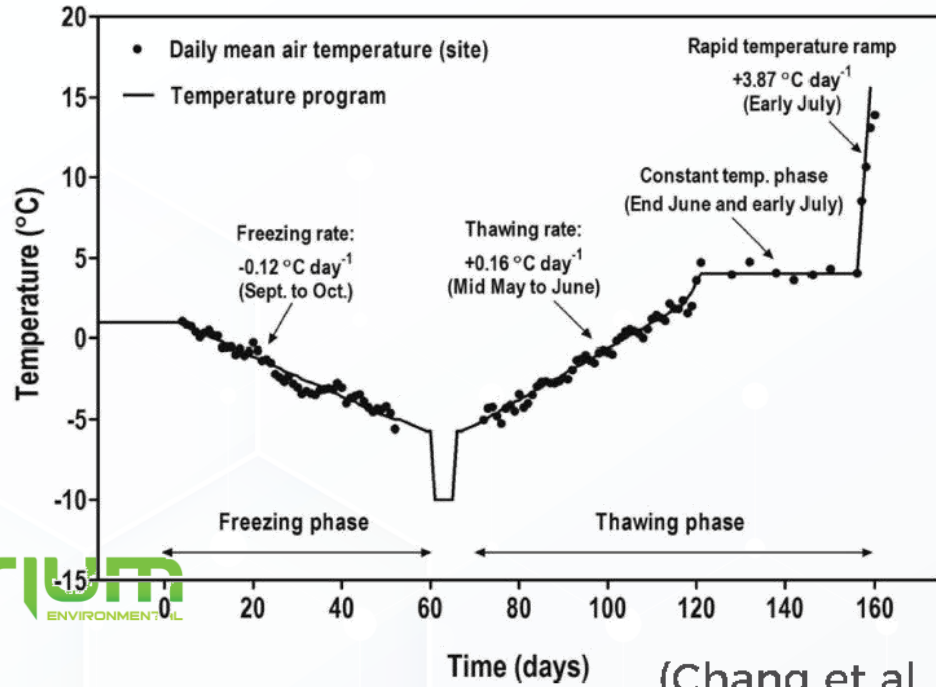


Bioremediation Enhancement Options

- Combination with Other Remediation Technologies
 - ChemOx + Aerobic bioremediation
 - Residual oxygen enhances bioremediation
 - Abiotic/biotic reductive dechlorination
 - ZVI/Iron sulfide + Electron donor/nutrient injection
- Thermal Enhancement
 - Temperature increase for enhanced bacterial growth and other reactions such as production of fatty acids
 - Increased water temperature dissolves fewer gases (e.g. oxygen), resulting in faster anaerobic conditions but more solids such as electron donor/nutrient

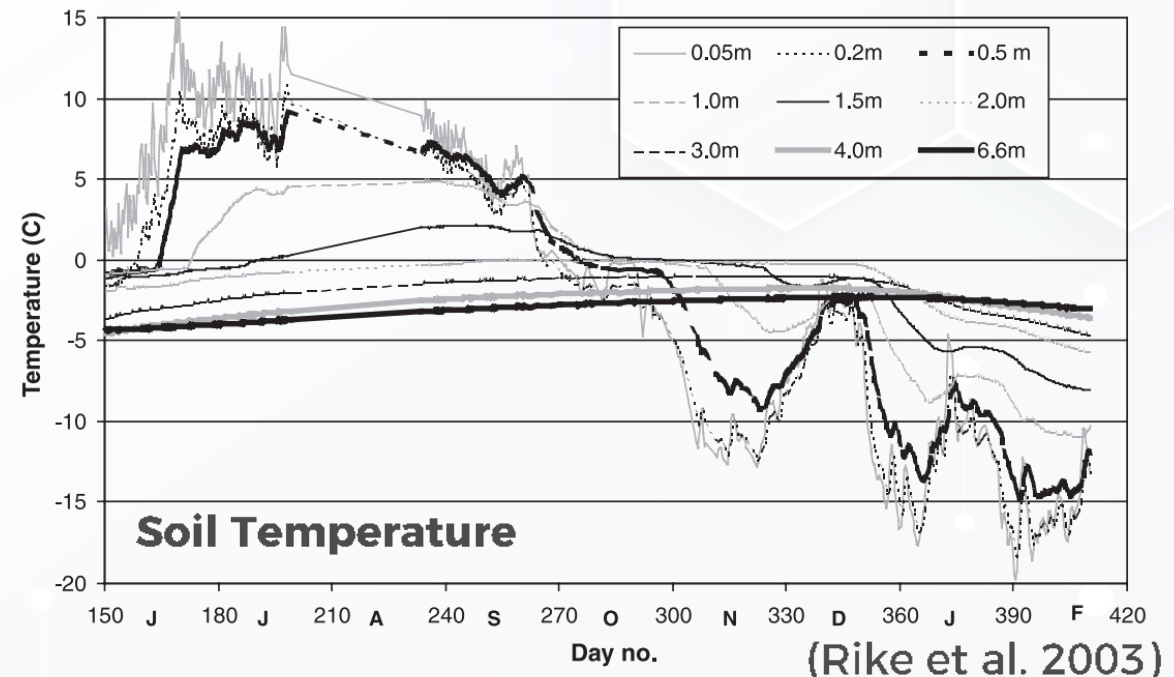
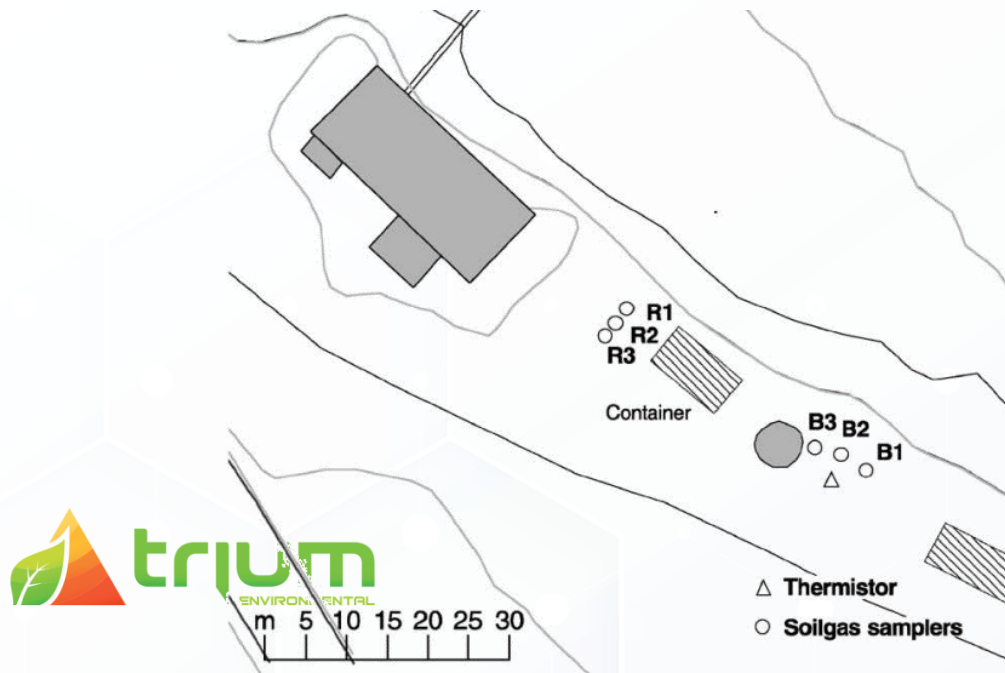
Pilot Scale Study under Cold Temperature

- Example: Aerobic Bioremediation (Military Site, USA)
 - PHC contaminated site with ~1,300 mg/kg TPH
 - Monitored room temperature during study: - 5 ~ 15°C
 - Slow but continual bacterial growth and PHC degradation with **Nutrient Amendment** even under freezing condition



Field Implementation under Cold Temperature

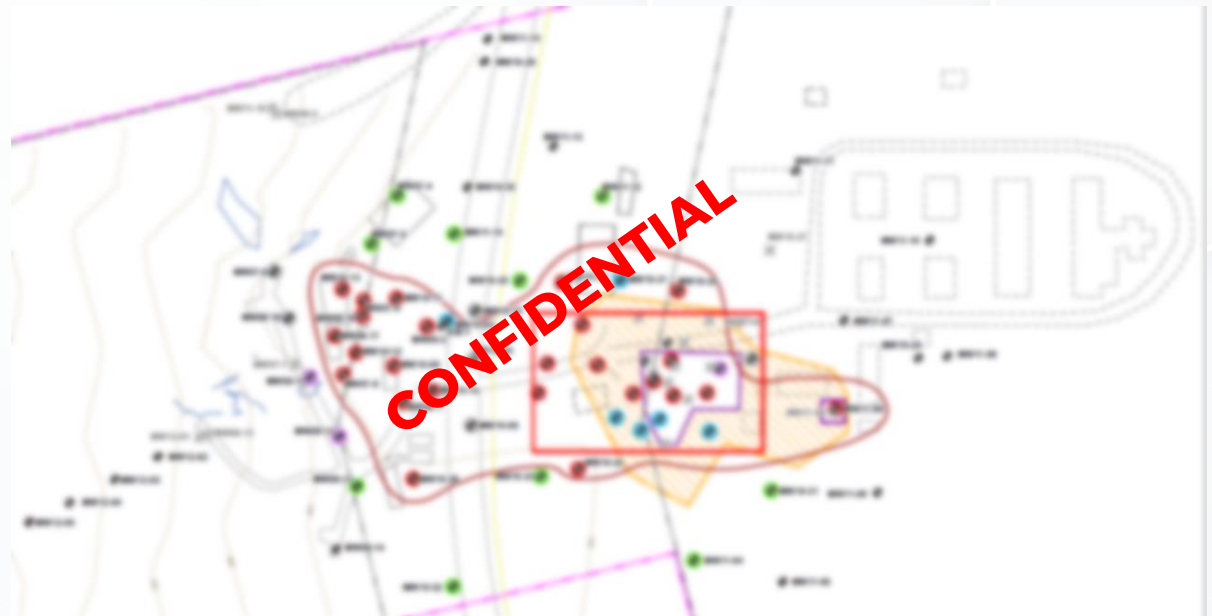
- Example: Aerobic Bioremediation (Longyearbyen, Norway - Permafrost)
 - Oil contaminated site with 205 to 21,500 mg/kg TPH
 - Continuous permafrost zone with annual air temperature of - 6 °C
 - Diesel degraders: 10^3 to 10^5 MPN / g soil (**Bacteria Growth under cold temperature**)
 - TPH degradation rates: 3.01 to 7.09 mg / kg soil / day



Field Implementation under Cold Temperature

- Anaerobic Bioremediation (Northern BC in Canada)
 - BTEX and PHC contaminated site
 - DO: < 1 mg/L, ORP: - 60 mV → **Good conditions for anaerobic bioremediation**

Parameter	Max Conc. (µg/L)
Benzene	111
Toluene	9.4
Ethylbenzene	319
Xylene, Total	2,060
EPH C10-C19	8,300
LEPH	8,230



Field Implementation under Cold Temperature

- Anaerobic Bioremediation (Northern BC in Canada)
 - Injected specialized anaerobic microbial culture along with anaerobic water in Winter
 - GW temperature in a range of 7 to 8 °C during/after injection
 - Remedial monitoring results indicate continuous reduction of the contaminants



SUMMARY

- Temperature is one of key factors in successful bioremediation
- Bioremediation is still viable option in cold region
 - Relatively slower bacterial growth and biodegradation rates under cold temperature but rebounded under temperature $> 10\text{ }^{\circ}\text{C}$
 - Previous cases have proven
- Adverse temperature effect can be overcome through engineered bioremediation approaches such as **Stimulation and/or Augmentation**

SUMMARY

Chemical Remediation

Bioremediation

Oxidation	Oxidants	Peroxide, Ozone, Permanganate, Persulphate	Aerobic approach	Additives: Oxygen, Substrates (Carbon Source - optional)
	Chemicals of Concern	Petroleum Hydrocarbons, Dry Cleaning Solvents, Other Organics	Chemicals of Concern	Petroleum Hydrocarbons, Other Organics
Reduction	Reductants	Zero-valent Iron (ZVI), Calcium Polysulfide, Ferrous Iron, Sodium Dithionite	Anaerobic approach	Additives: Sulphate, Substrates (Electron Donor / Acceptor)
	Chemicals of Concern	Dry Cleaning Solvents, Heavy Metals	Chemicals of Concern	BTEX, Dry Cleaning Solvents, Heavy Metals

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Questions?

B.J. Min, M.Eng., P.Eng.

bjmin@triuminc.com

Headquarter:

#130, 239 Mayland Place NE, Calgary, AB

+1-403-932-5014



Operation Hub:

#10, 240057 Frontier Cres. SE, Rocky View County, AB

Satellite:

Burnaby, BC, Toronto, ON
Seoul, Beijing, Hong Kong



info@triuminc.com
www.triuminc.com

