



# Fostering Sustainable Remediation: Debunking Cold Climate Myths and Harnessing Bioremediation in Canada

October 12, 2023

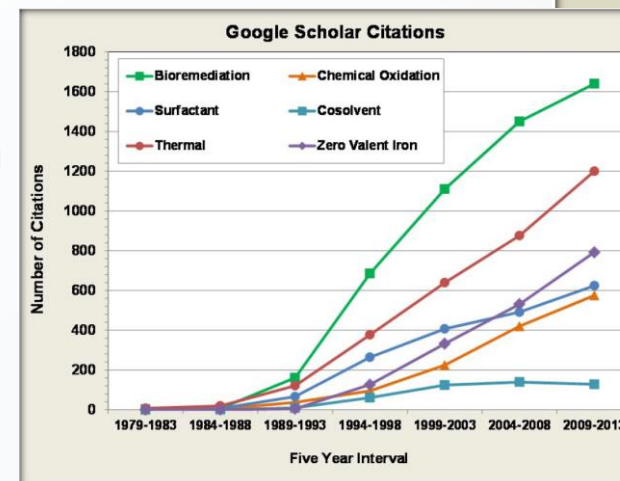
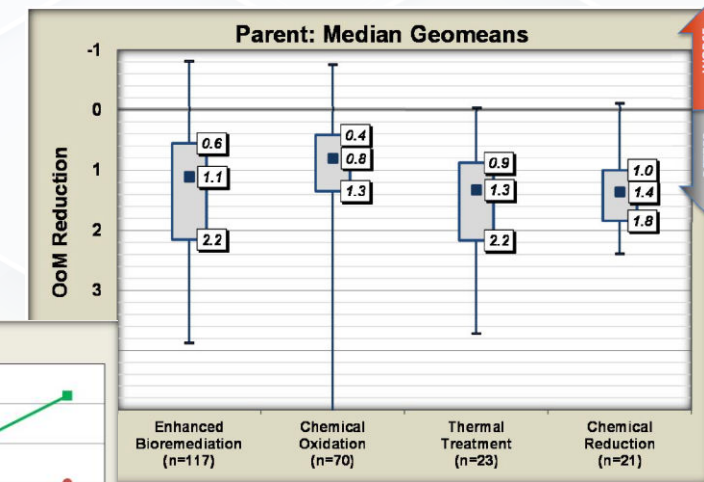
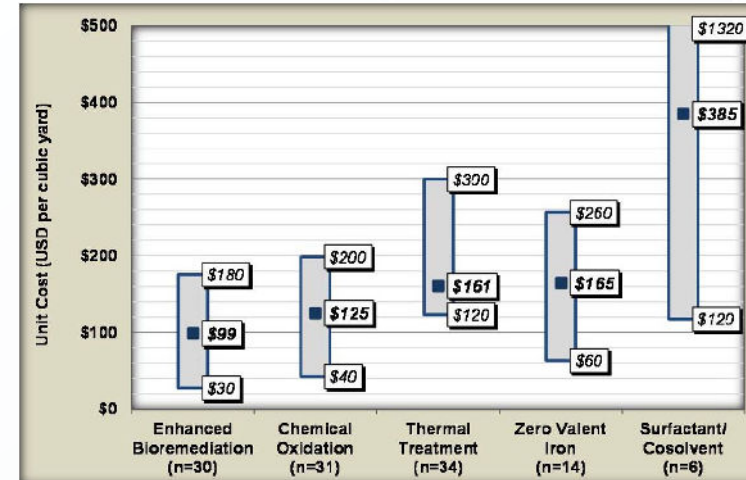
Jongho Won, Ph.D., P.Eng.

# Outline

- Background – Basics of Bioremediation
- Bioremediation Myths & Misperceptions
- 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

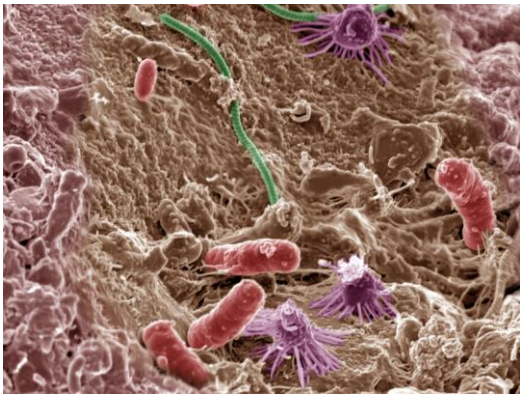


(McGuire et al. 2016)

# What Bioremediation Options Available?

## Microbial Bioremediation

Microbes



(Courtesy of Pacific Northwest National Laboratory)

## Mycoremediation

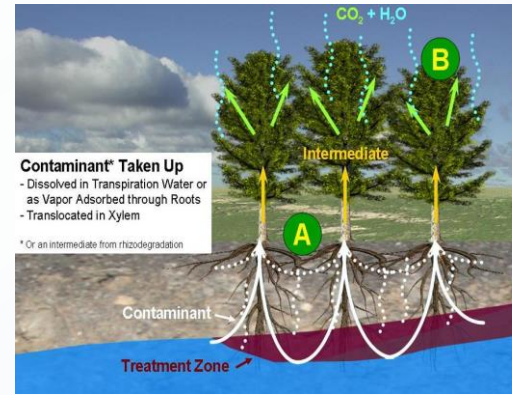
Fungi (e.g. mushroom)



(TRIUM test site)

## Phytoremediation

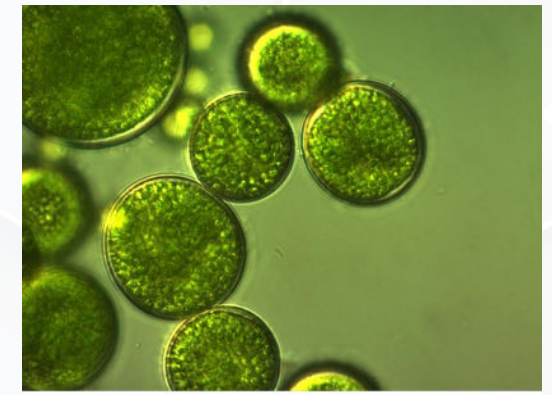
Plants & Trees



(ITRC Guideline)

## Phycoremediation

(Micro)Algae

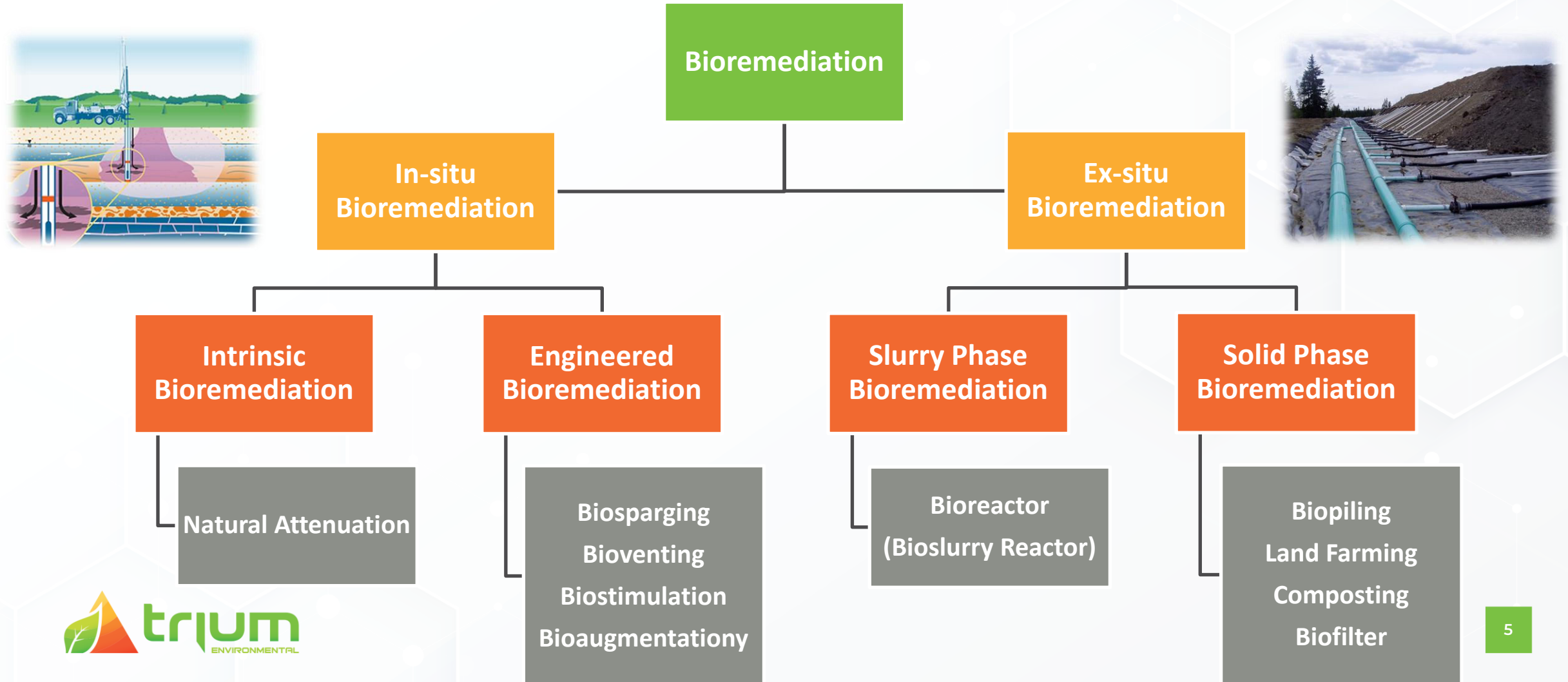


(Shutterstock)

## 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)

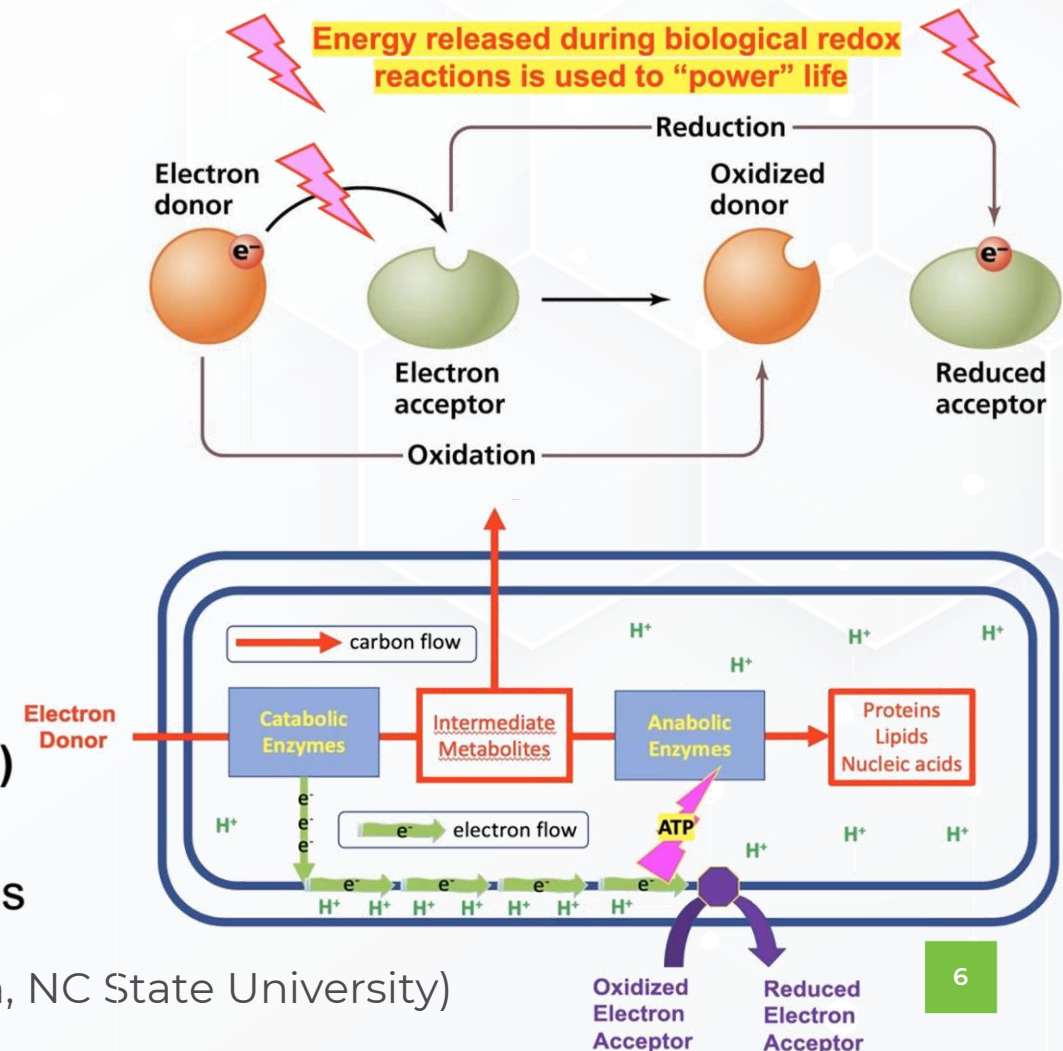
**ATP** = Adenosine triphosphate (cash energy currency of cell)

**Metabolism** = Catabolism + Anabolism

**ABC of Metabolism: A**nabolism **B**uilds **C**atabolism **D**egrades



(Courtesy of Dr. Michael Hyman, NC State University)



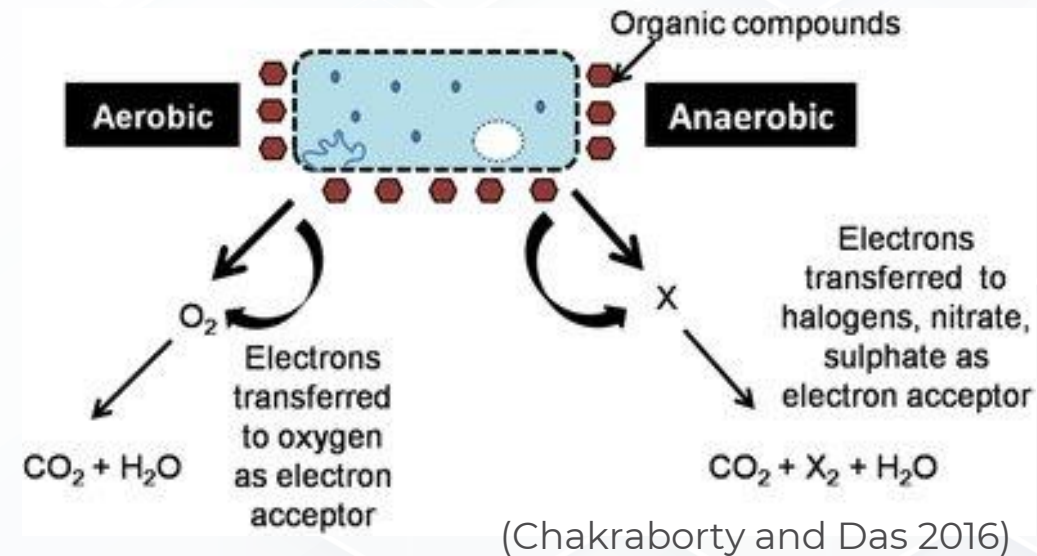
# Aerobic vs Anaerobic Bioremediation

- **Aerobic Biodegradation**

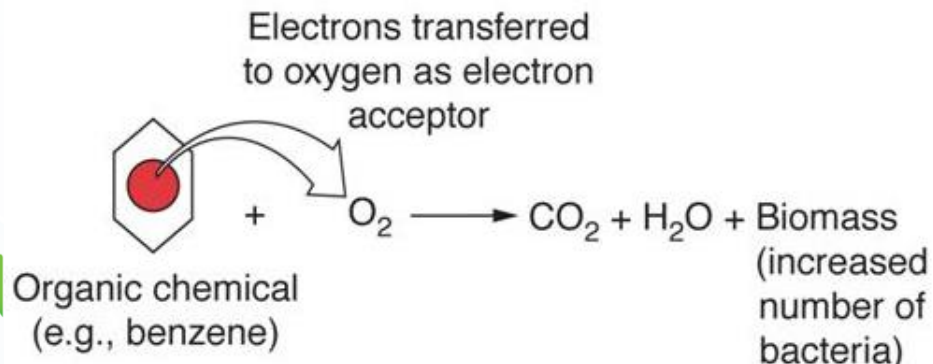
- Organics (e.g. hydrocarbons) are oxidized
- Oxygen is reduced to water by gaining electrons

- **Anaerobic Biodegradation**

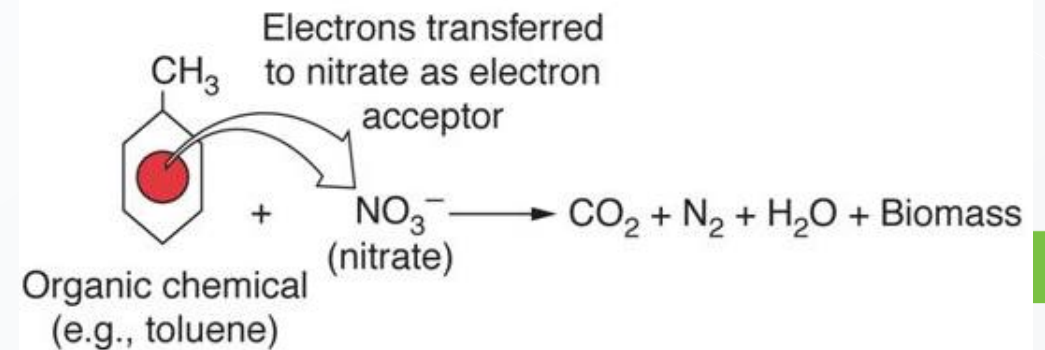
- Organics (e.g. toluene) are oxidized
- Organics (e.g. PCE) & inorganics (e.g. nitrate, sulphate) are reduced by gaining electrons



*Aerobic biodegradation*



*Anaerobic biodegradation*



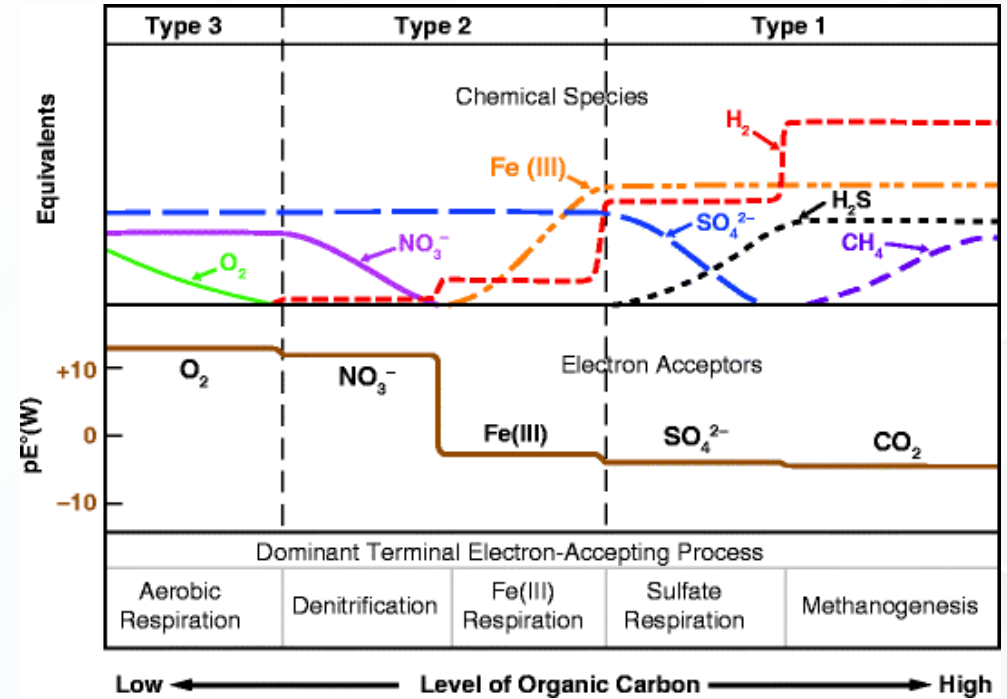
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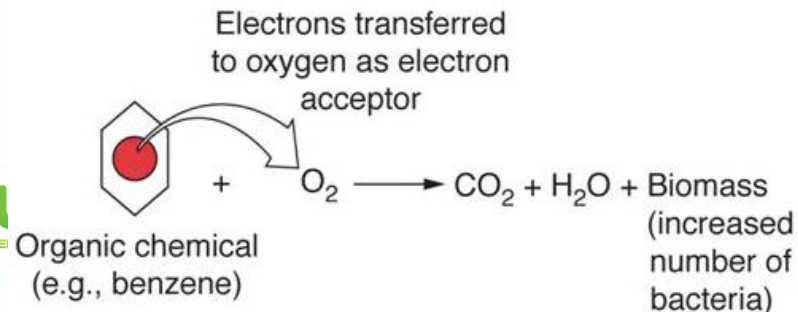
- **Anaerobic Biodegradation**

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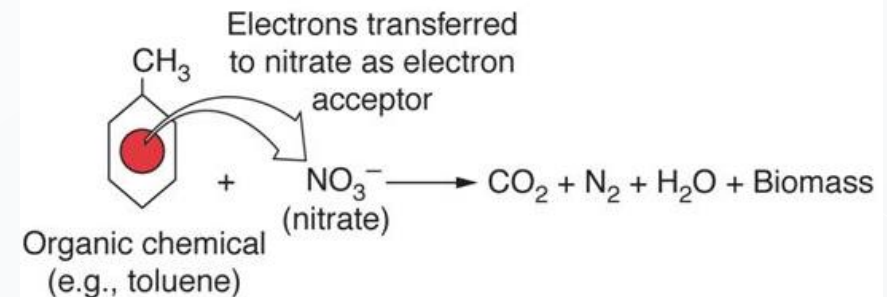


(Henry 2010)

*Aerobic biodegradation*



*Anaerobic biodegradation*





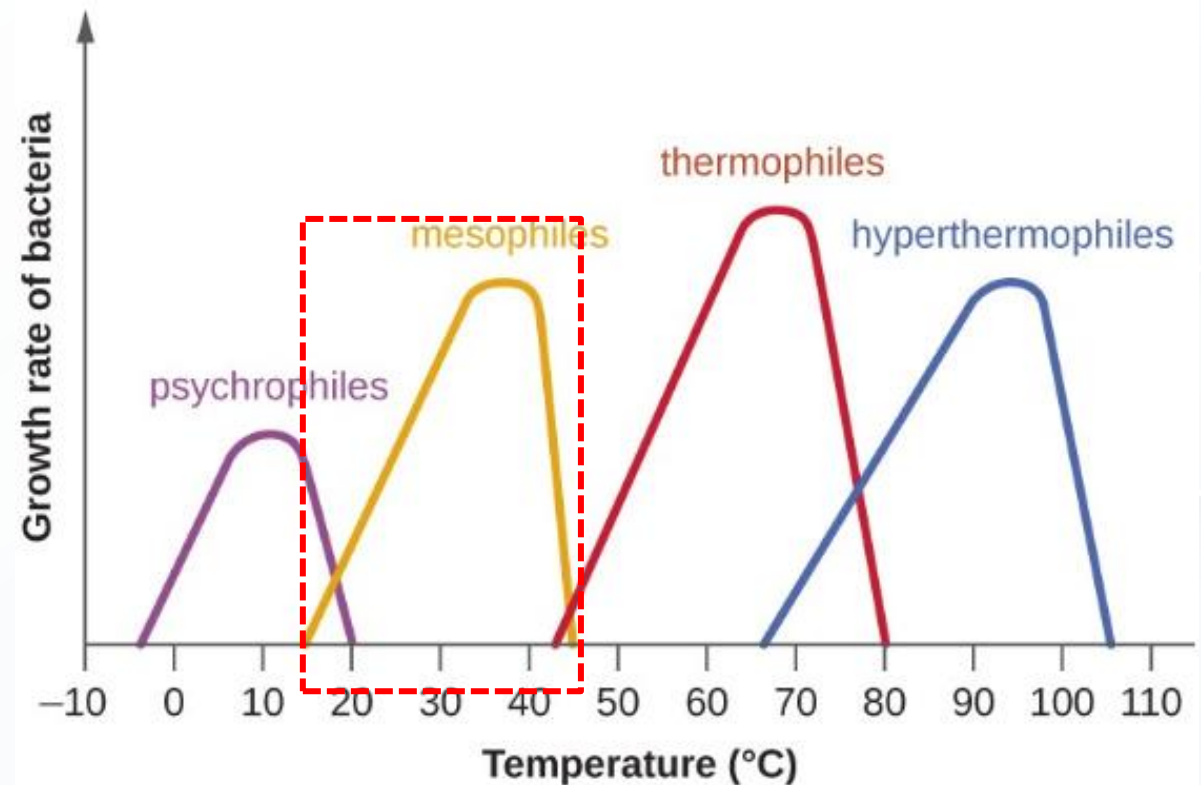
# Bioremediation Myths & Misperceptions

- **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

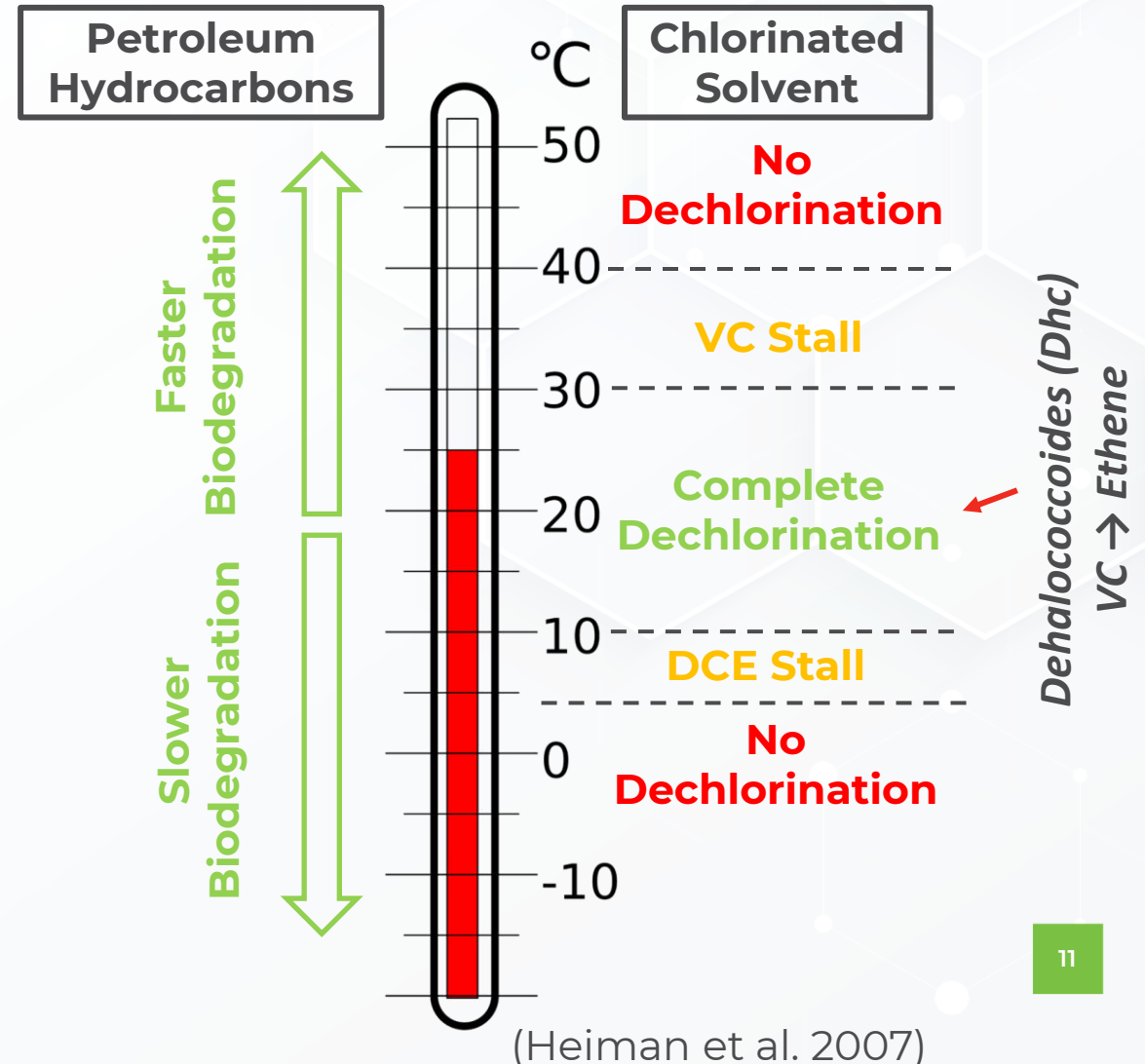
**Many contaminant degraders are mesophiles (15-45 °C)**



(Source: LumenLearning)

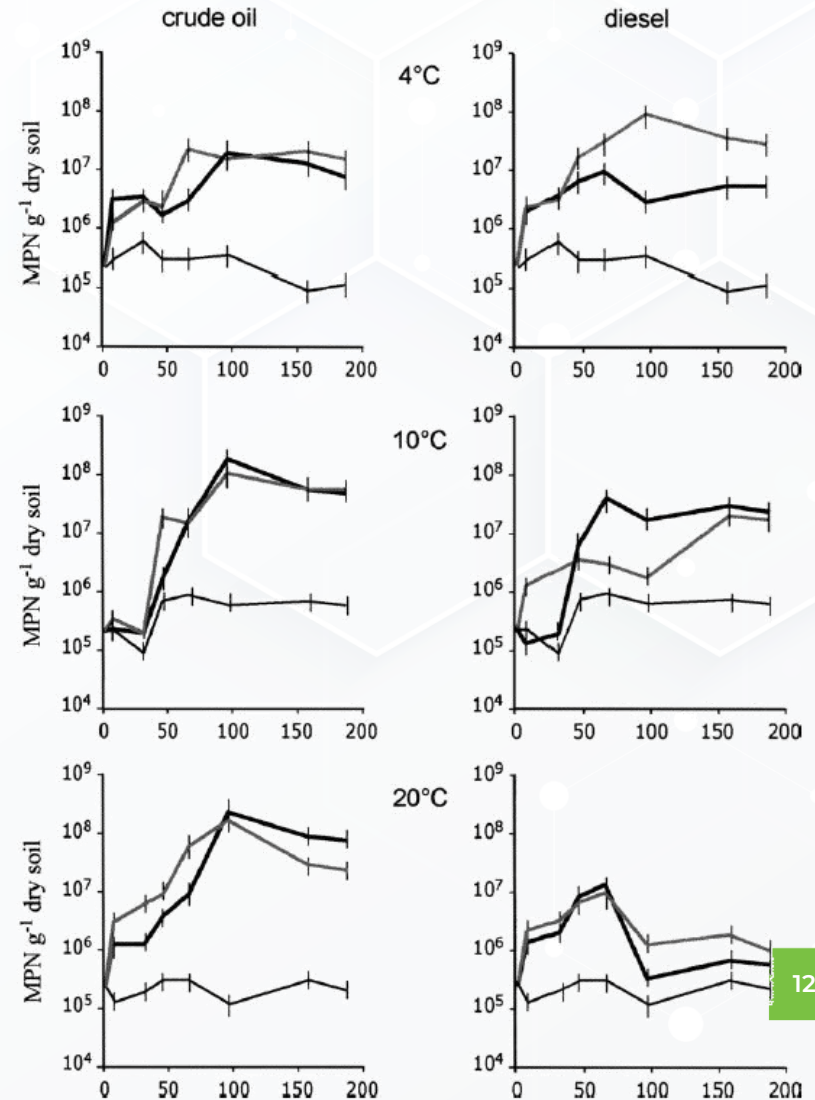
# Effect of Temperature in Bioremediation

- 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



# 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

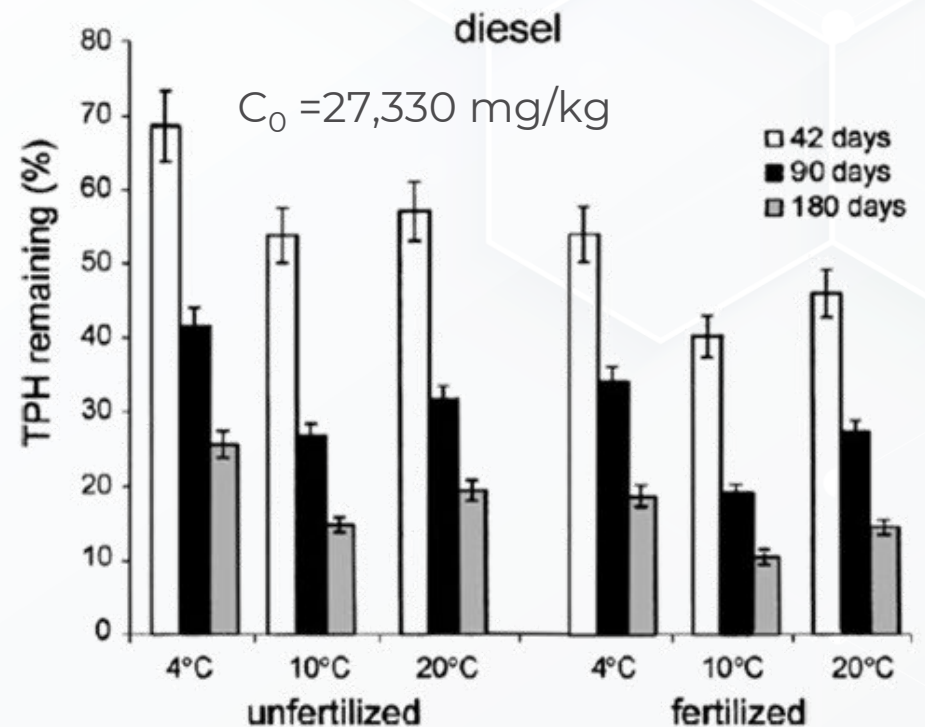
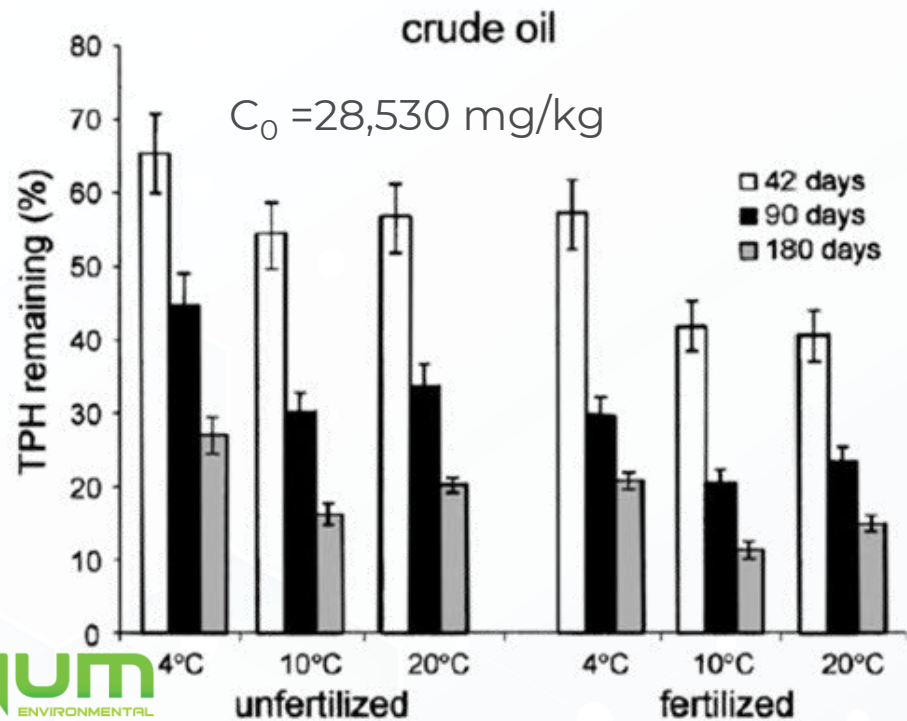


Thin: control  
Thick: Fertilized  
Gray: Unfertilized

(Coulon et al. 2005)

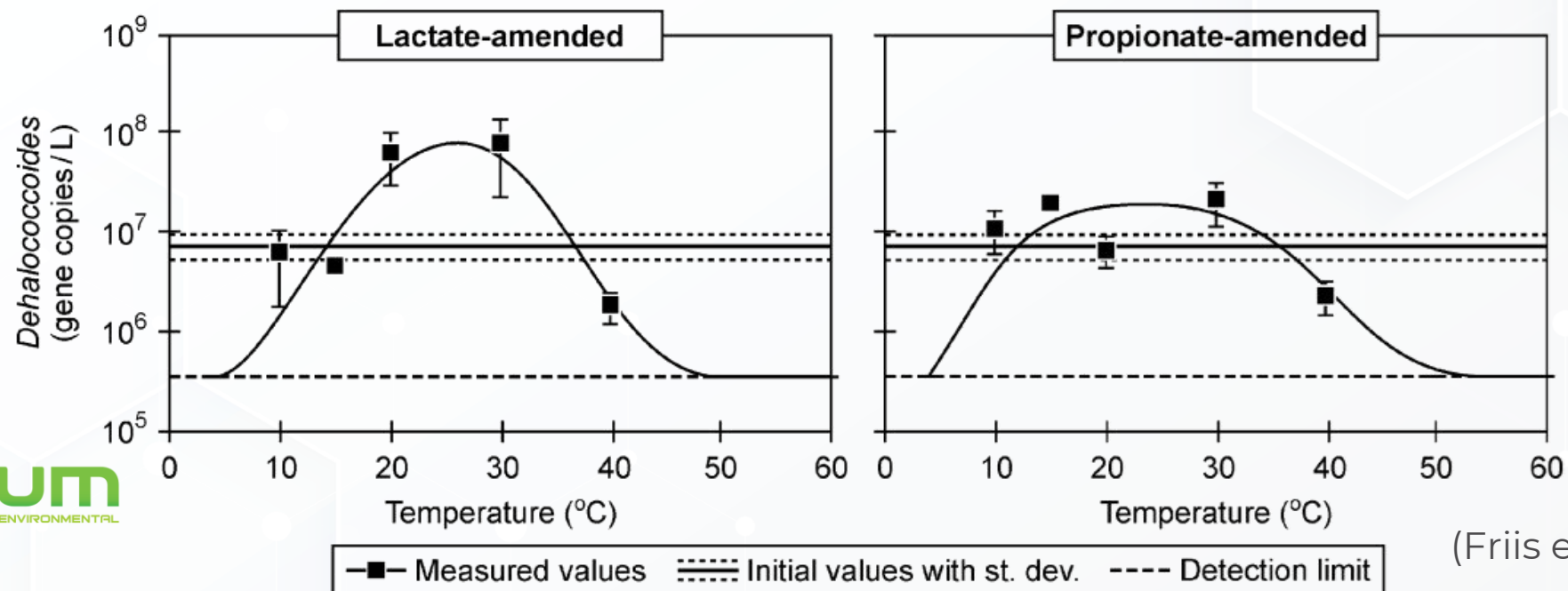
# Effect of Temperature in Bioremediation

- 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)



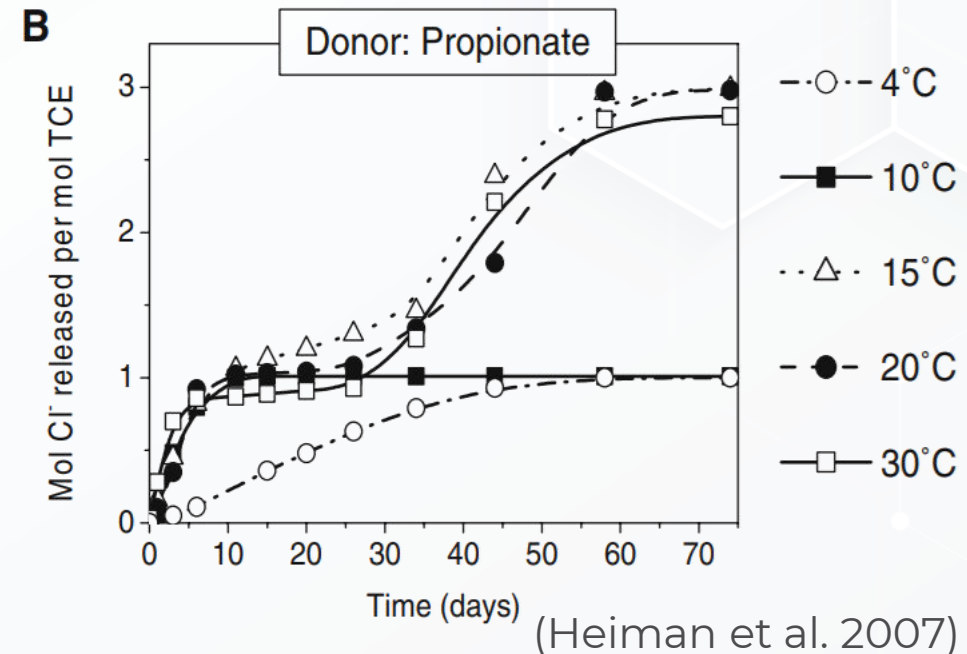
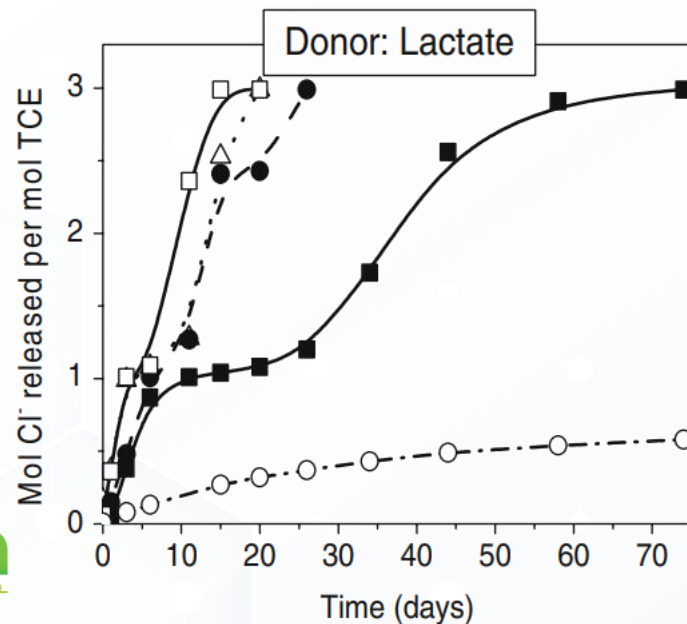
# Effect of Temperature in Bioremediation

- 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 ( $\sim 10^7$ /L)
  - **Type of electron donor** is also important factor in bacterial growth



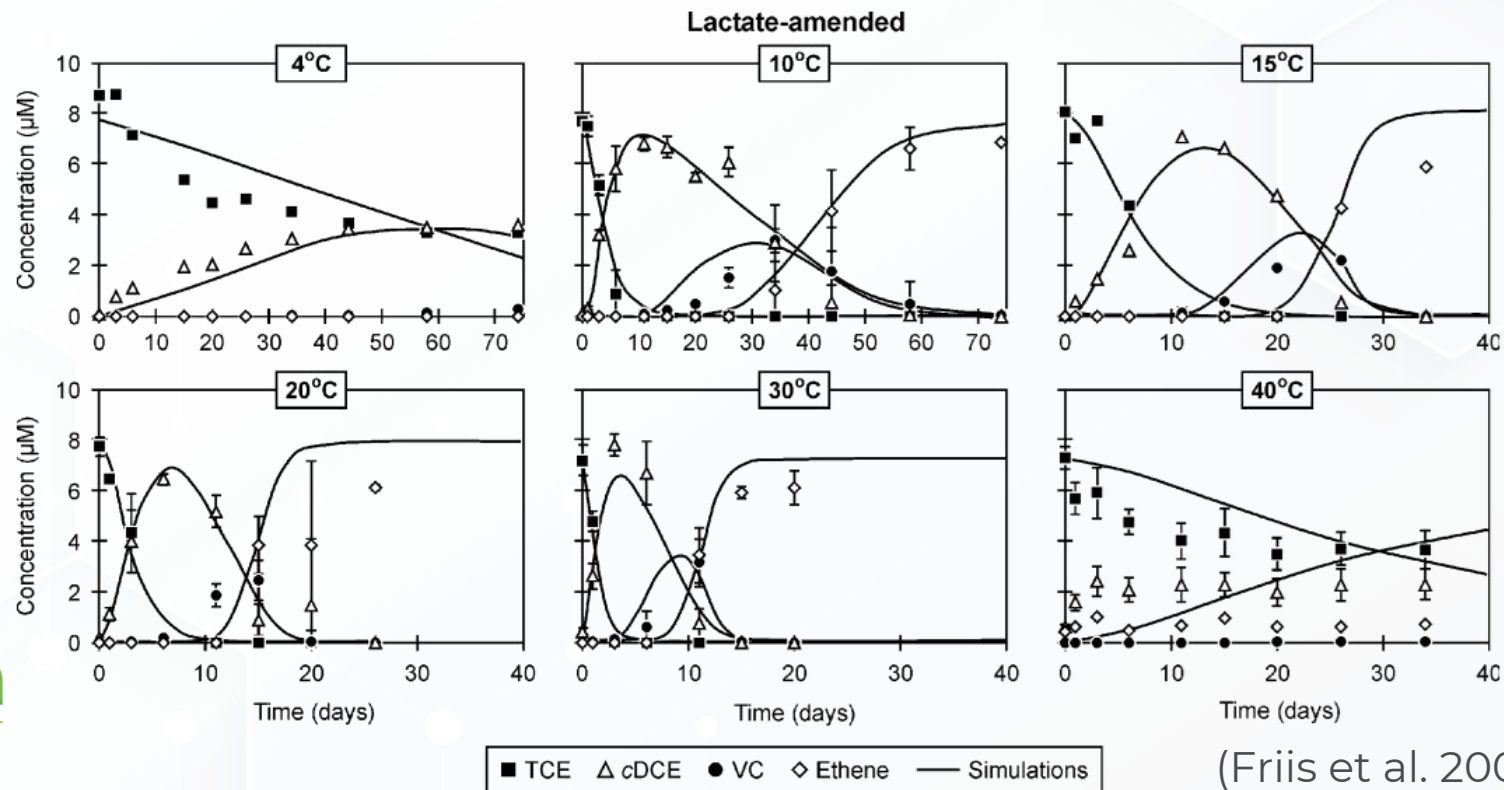
# Effect of Temperature in Bioremediation

- GW Temperature and Electron Donor Fermentation
  - Optimal temperature for electron donor fermentation: 20 - 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

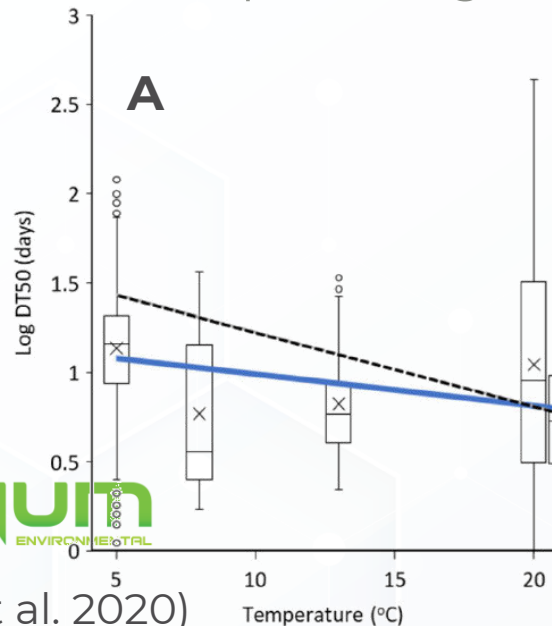


(Friis et al. 2007)



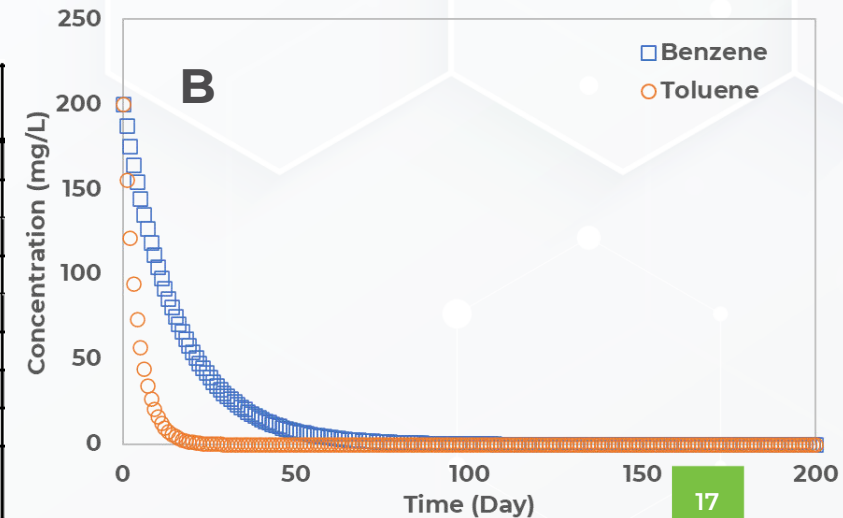
# Bioremediation & Cleanup Time

- 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 <sup>-1</sup>	Toluene Day <sup>-1</sup>
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

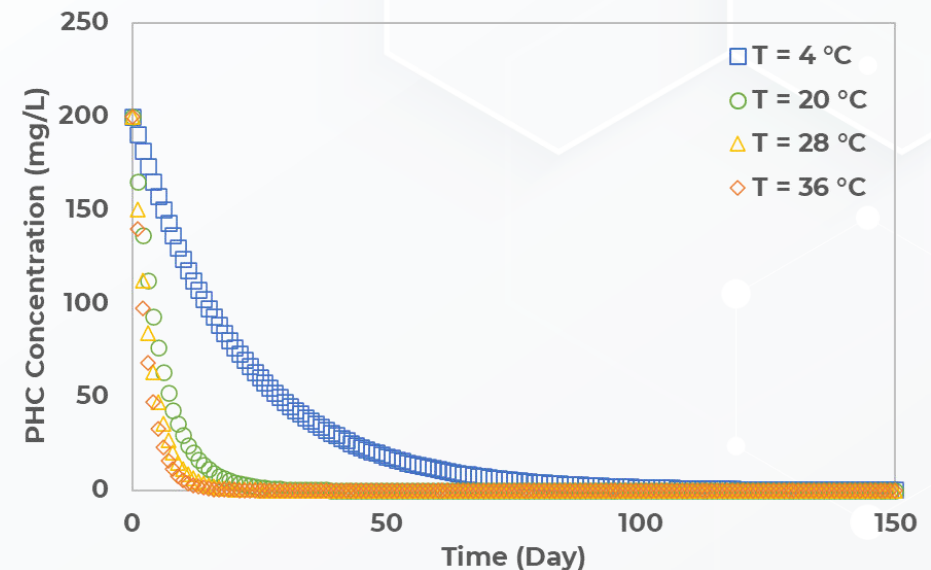
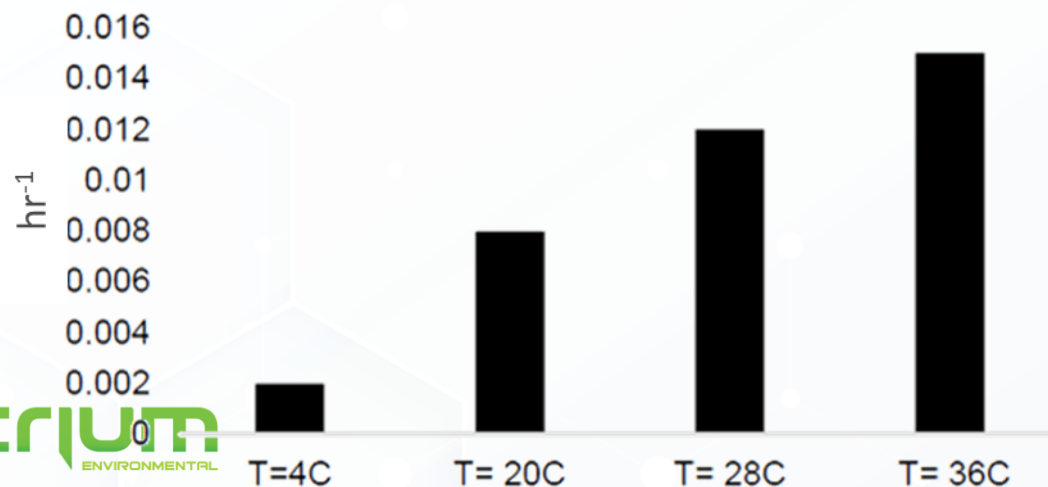
- Subsurface Temperature and Biodegradation Rate

- 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

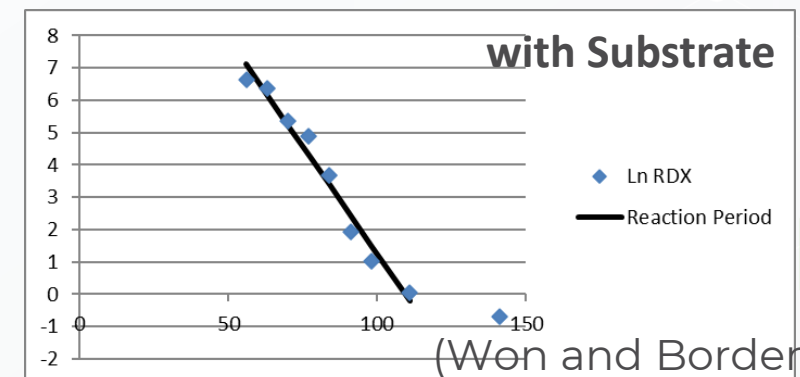
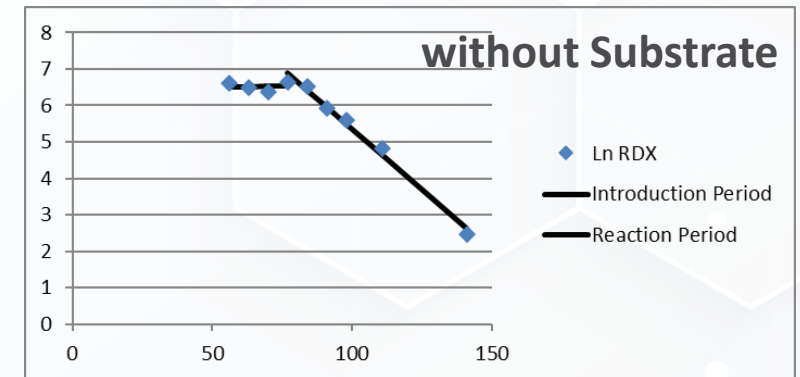
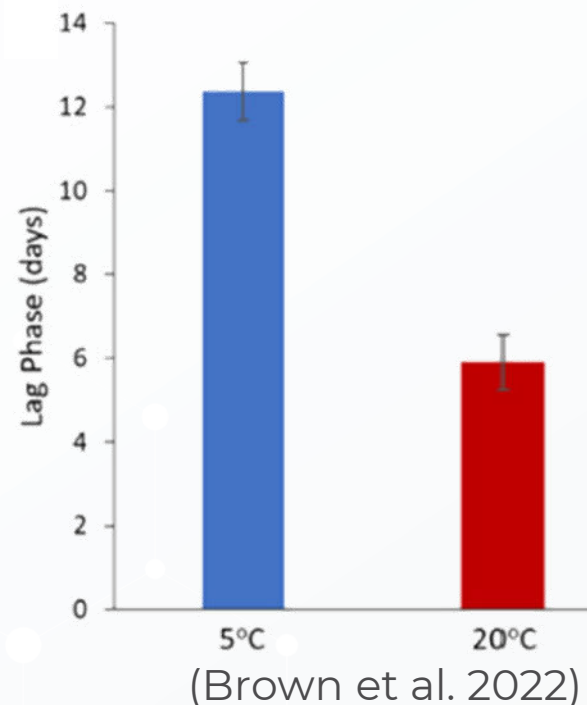
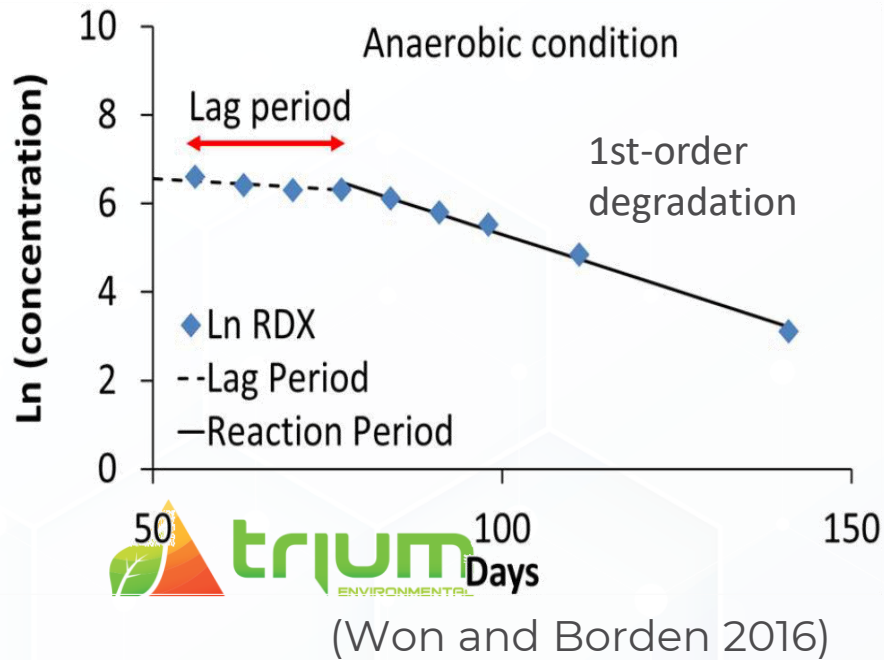
$$Q_{10} = \left[ \frac{k_2}{k_1} \right]^{10/\Delta T}$$

Biodegradation rates



# 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



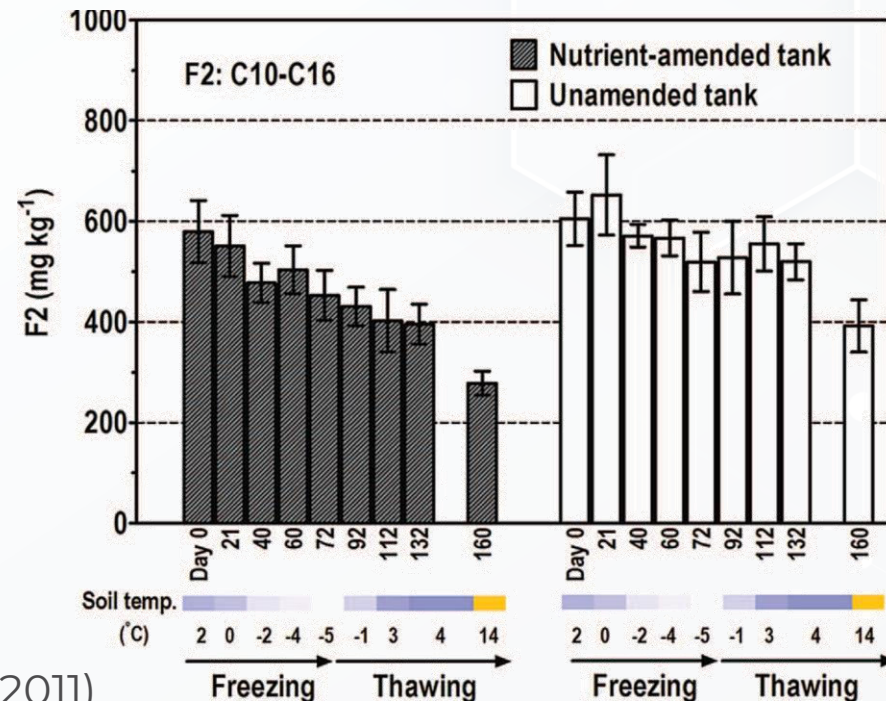
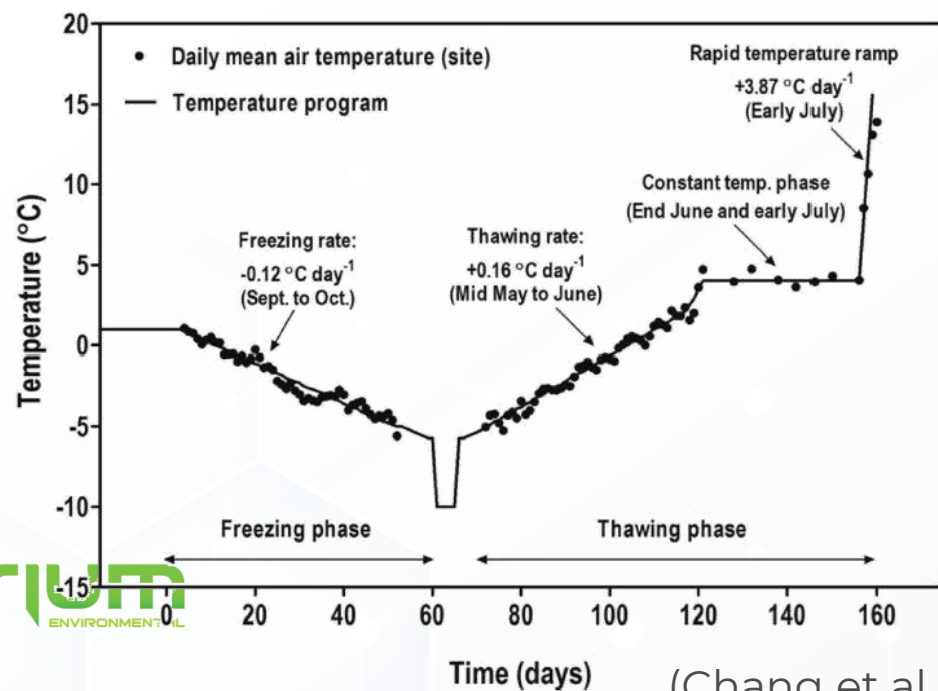
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# Bioremediation Enhancement Options

- 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

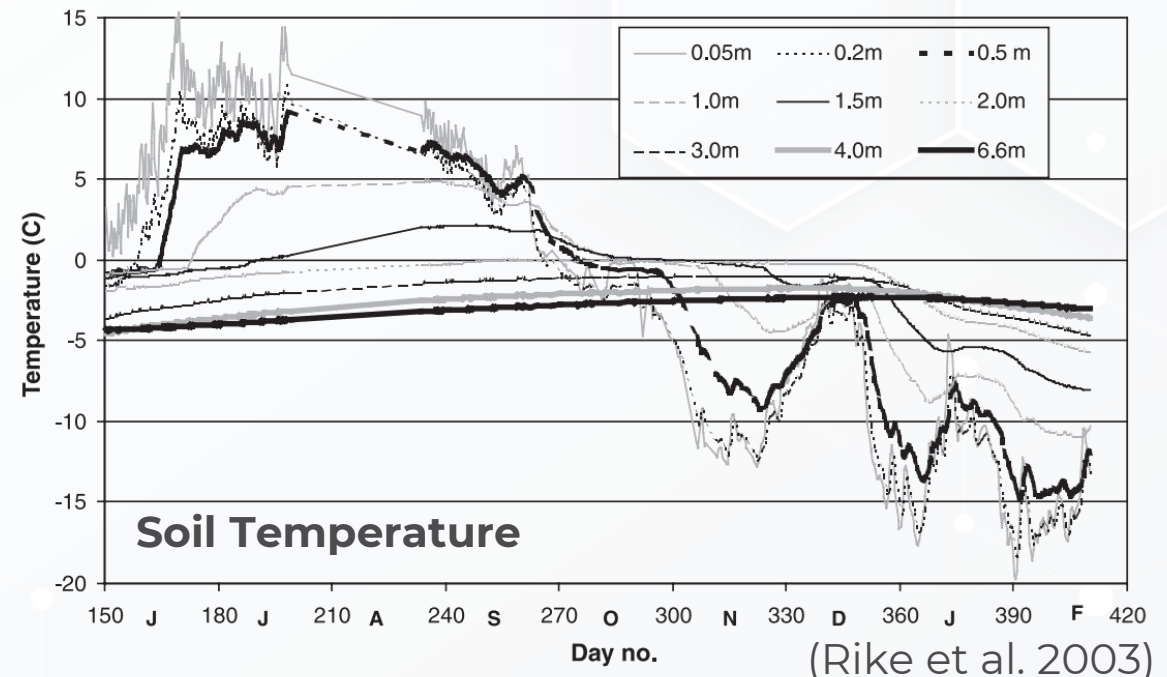
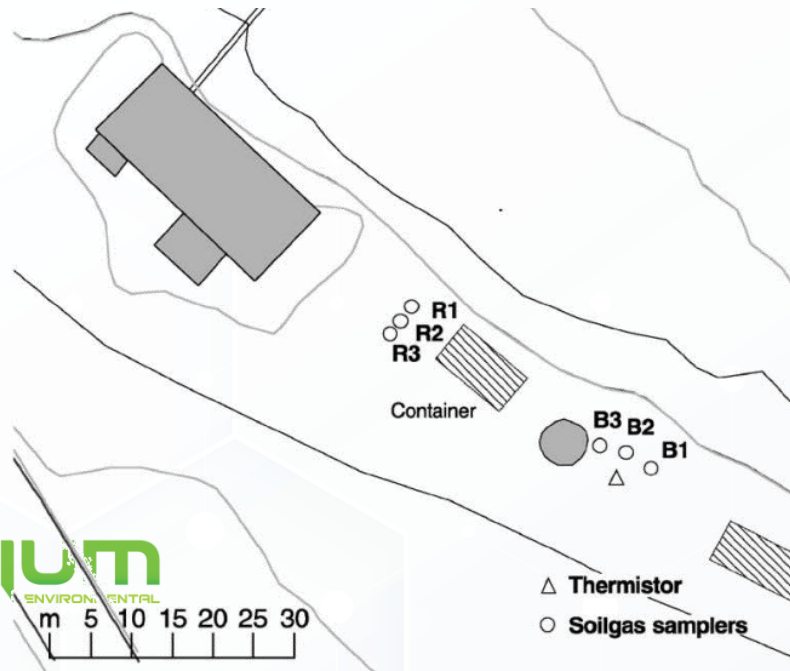
# Pilot Scale Study under Cold Temperature

- Example: Aerobic Bioremediation (Military Site, USA)
  - PHC contaminated site with ~1,300 mg/kg TPH
  - Monitored 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

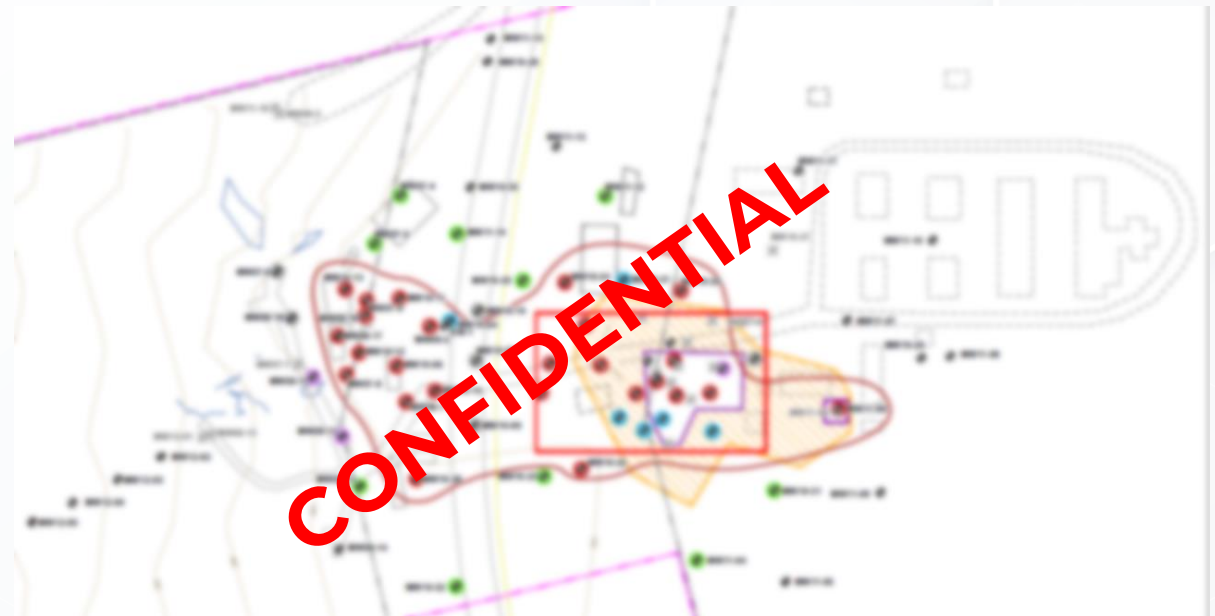
- Example: Aerobic Bioremediation (Longyearbyen, Norway)
  - Oil contaminated site with 205 - 21,500 mg/kg TPH
  - Continuous permafrost zone with annual air temperature of - 6 °C
  - Diesel degraders:  $10^3$  -  $10^5$  MPN / g soil
  - TPH degradation rates: 3.01 - 7.09 mg / kg soil / day



# Field Implementation under Cold Temperature

- Anaerobic Bioremediation (Confidential Site)
  - BTEX and PHC contaminated site
  - DO < 1 mg/L, ORP ~ - 60 mV → **Good condition 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 (Confidential Site)
  - Injected anaerobic microbes along with anaerobic water in January 2023
  - GW temperature 7 - 8°C during/after injection
  - In progress of monitoring





# 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 °C
  - Previous cases have proven
- Adverse temperature effect can be overcome through engineered bioremediation approaches

	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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# Reference

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# Questions?

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