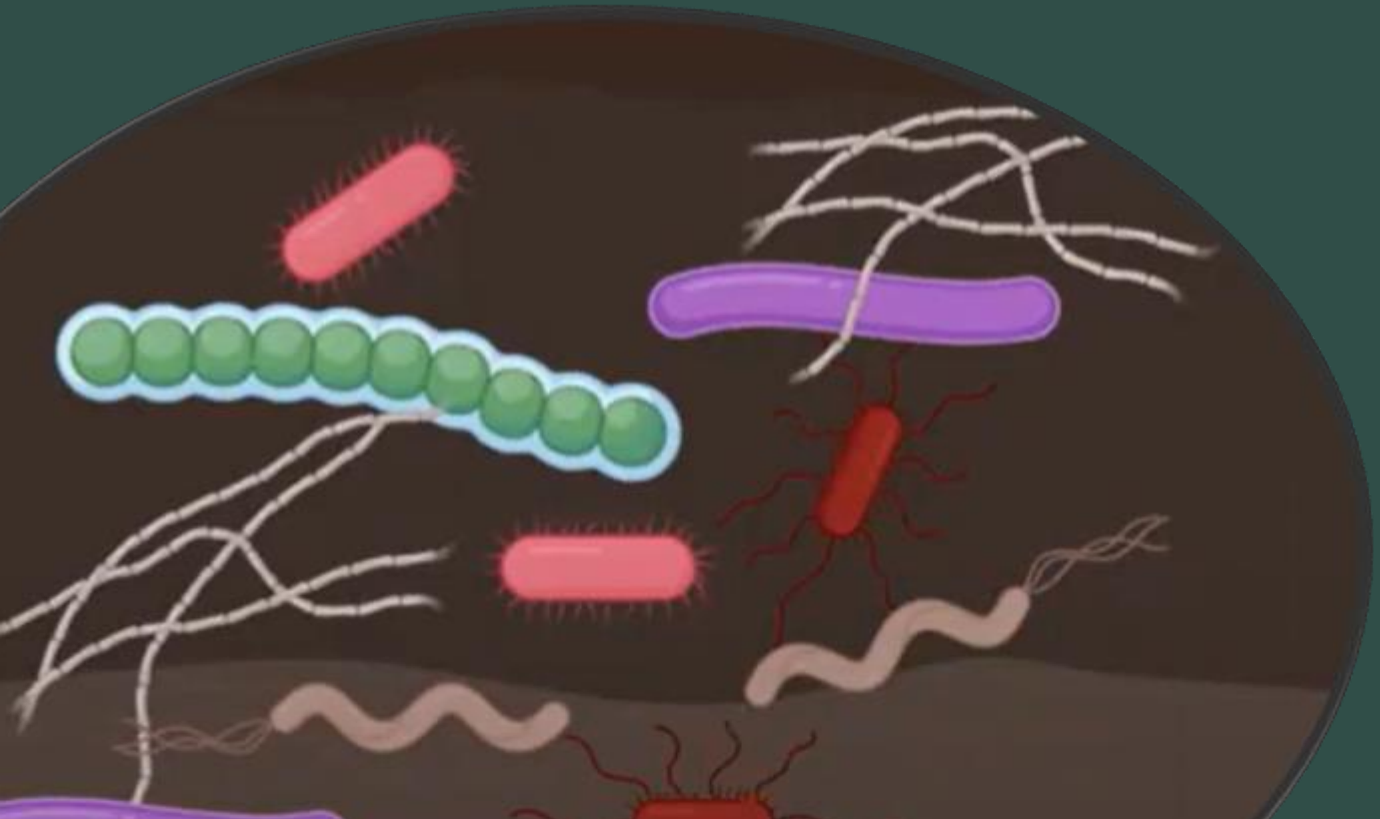
Bioremediation



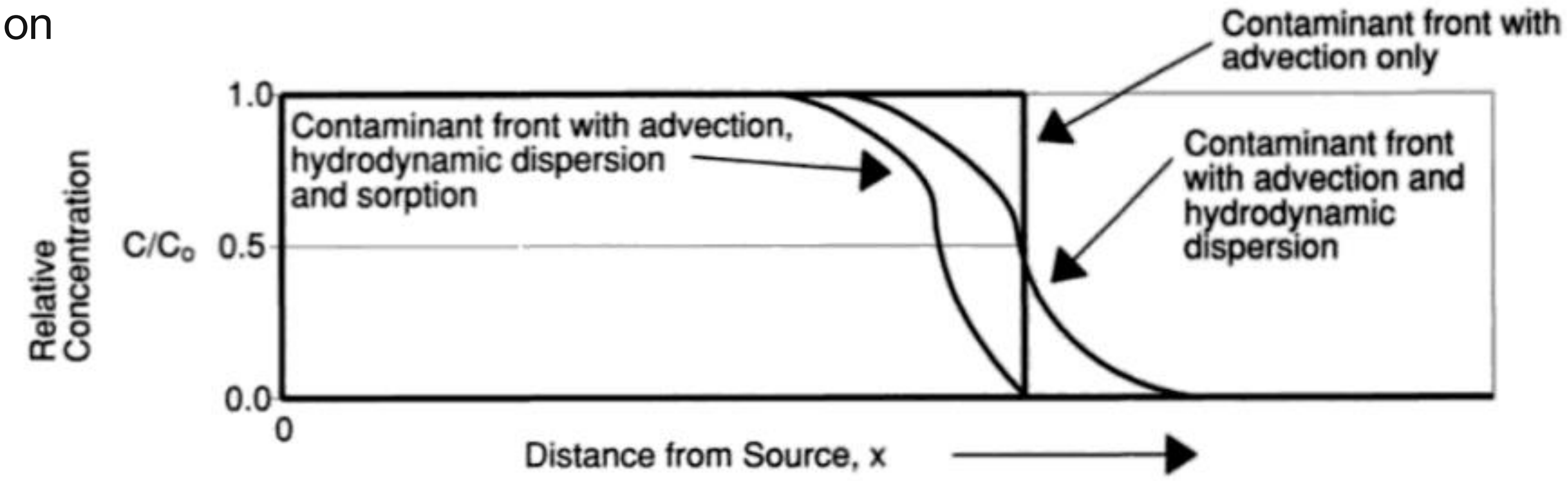
Natural Attenuation Processes

NON-DESTRUCTIVE

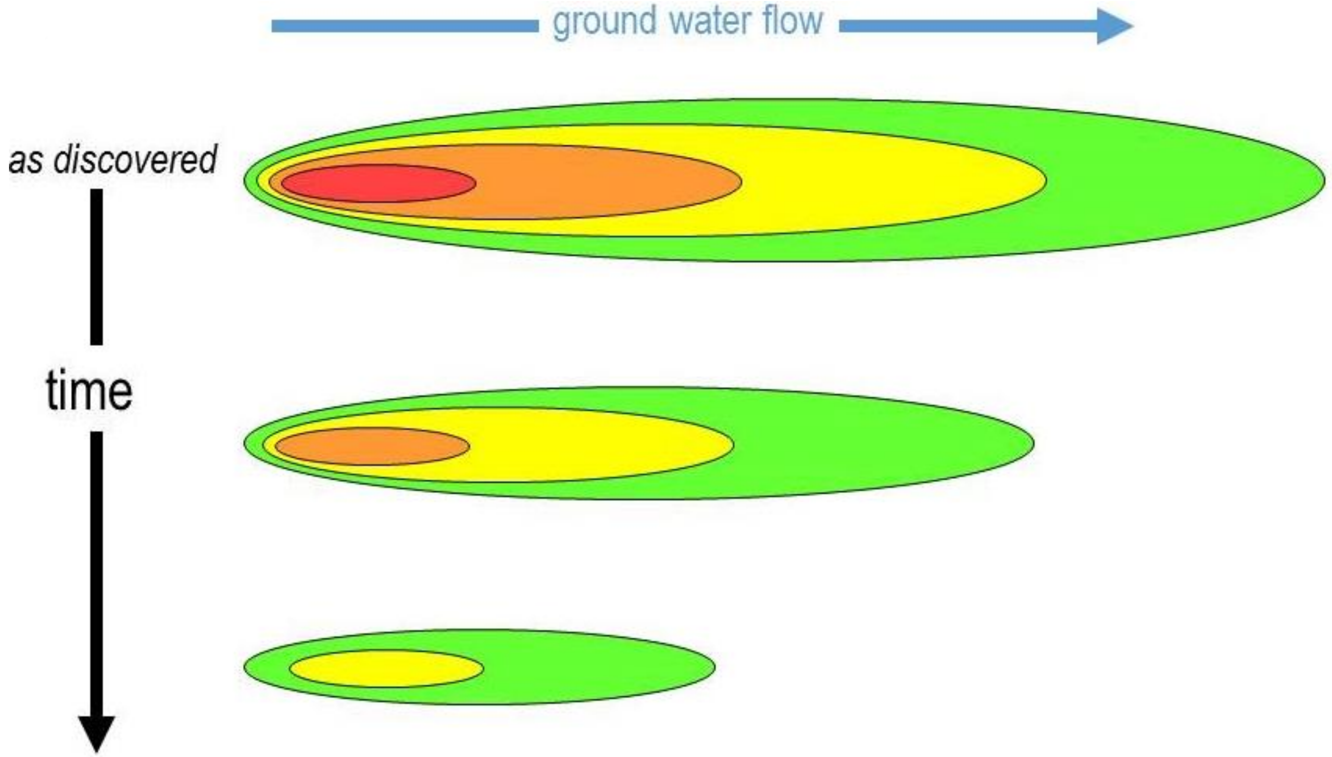
- Hydrodynamic dispersion (mechanical dispersion and diffusion)
- Sorption
- Dilution
- Volatilization

DESTRUCTIVE

- Biodegradation
- Abiotic degradation



Demonstrating Natural Attenuation



- Historical data showing plume stabilization and/or loss of contaminant mass
- Chemical and geochemical data
  - Depletion of electron acceptors/donors
  - Increasing metabolic by-products
  - Decreasing parent compounds
  - Increasing daughter compounds
- Microbiological data showing the occurrence/rate of biodegradation

Environmental footprint, sustainability, accessibility, liability considerations

ISB vs Ex Situ Methods

In situ bioremediation (ISB) typically has a smaller environmental footprint than ex situ or non-biological methods.

Limited Infrastructure Required

In and out quickly, limited permanent systems, low energy usage, easily access treatment areas.

Limiting Liability

Handling/Transporting waste materials inherently comes with increase risk/liability (cradle to grave)

Minimal O&M

Potentially multiple injections, but generally no decades-long O&M

What exactly is bioremediation?

Biostimulation

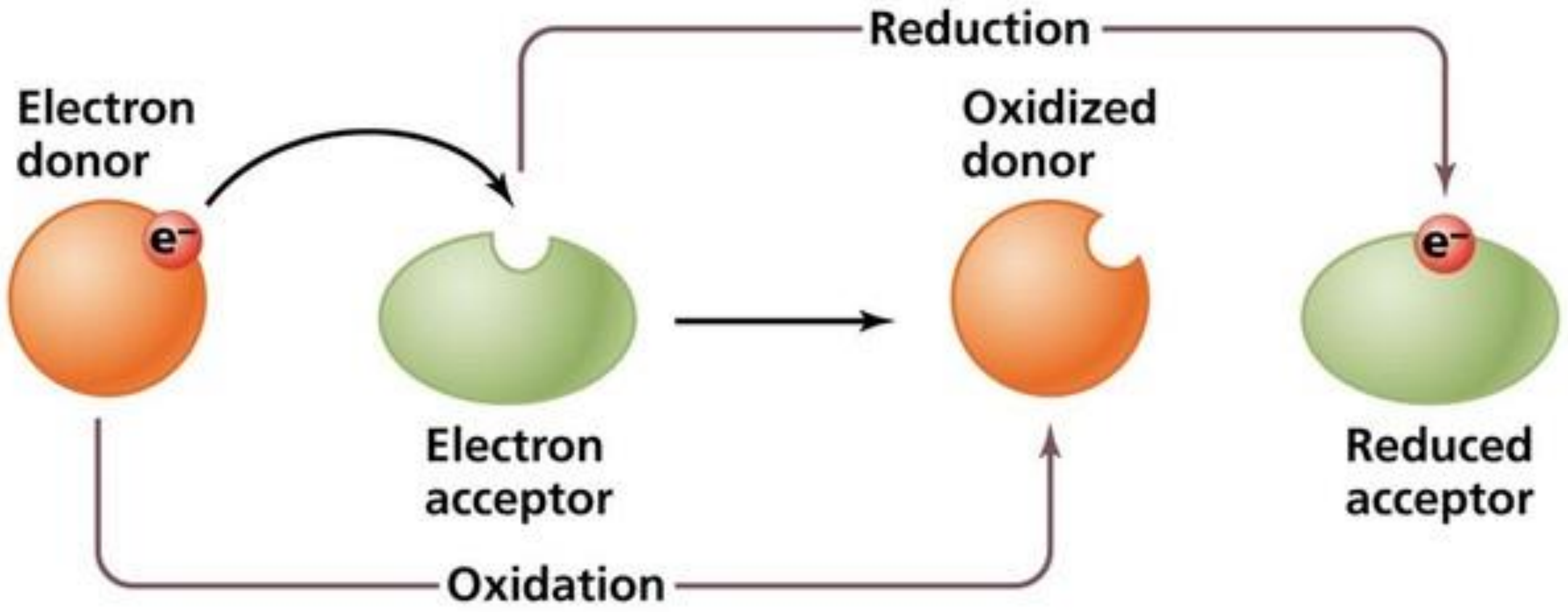
encouragement of indigenous bacterial populations to metabolize target contaminants through the addition of various amendments

Bioaugmentation

adding select strains of bacteria

Bacteria perform coupled oxidation/reduction (redox) reactions to live, and bioremediation exploits these reactions to remove contaminants from contaminated media

Brief Chemistry Lesson...



Applicable Contaminants for Bioremediation

Biodegradable Organic Contaminants

Substance	Aerobic Biodegradation	Anaerobic Biodegradation
Petroleum Hydrocarbons		
Benzene	Yes	Yes
Toluene	Yes	Yes
Ethylbenzene	Yes	Yes
Xylenes	Yes	Yes
Naphthalene	Yes	Yes
1-Methylnaphthalene	Yes	Yes
2-Methylnaphthalene	Yes	Yes
Fuel Additives		
1,2-Dibromoethane (EDB)	Yes	Yes
1,2-Dichloroethane (DCA)	Yes	Yes
Methyl tert-Butyl Ether (MTBE)	Yes	Yes
tert-Butyl Alcohol (TBA)	Yes	Yes
Ethanol	Yes	Yes
n-Butanol	Yes	Yes
iso-Butanol	Yes	Yes
n-Propanol	Yes	Yes

Substance	Aerobic Biodegradation	Anaerobic Biodegradation
Chlorinated Solvents		
Tetrachloroethene (PCE)	No	Yes
Trichloroethene	No	Yes
cis-Dichloroethene	Yes	Yes
trans-Dichloroethene	Yes	Yes
1,1-Dichloroethene	No	Yes
Vinyl Chloride	Yes	Yes
1,1,1-Trichloroethane	No	Yes
1,1,2-Trichloroethane	No	Yes
1,1-Dichloroethane	No	Yes
1,2-Dichloroethane	Yes	Yes
Carbon Tetrachloride	No	Yes
Chloroform	No	Yes
Dichloromethane	Yes	Yes

Summary of Bioremediation Strategies

ISB Strategy	Aerobic	Anaerobic Oxidative	Anaerobic	Aerobic Cometabolism
Key Characteristics	Relies on presence of oxygen	Relies on addition or use of other electron acceptors besides oxygen	Relies on electron donor additions uses contaminants as electron acceptors Anaerobic metabolism includes fermentation, methanogenesis, reductive dechlorination, sulfate- and iron-reducing activities, and denitrification	Relies on addition of cosubstrates for fortuitous degradation of contaminants May be used under aerobic or anaerobic, based on the redox state of the contaminant
Target Contaminants	Petroleum hydrocarbons and some fuel oxygenates Ionic form of metals	Petroleum hydrocarbons present in reducing conditions	Chloroethenes and chloroethanes Perchlorate, Munitions, Chromate, and Nitrate	May be applicable to: PAHs, Explosives, Dioxane, NDMA, PCBs, Pesticides, MTBE, Chloroethenes, Chloroethanes, Chloroform, and methylene chloride

Source: https://www.epa.gov/sites/default/files/2015-04/documents/introductiontoinsitubioremediationofgroundwater_dec2013.pdf

ISB Strategy	Aerobic	Anaerobic Oxidative	Anaerobic	Aerobic Cometabolism
Advantages	Widespread acceptance with documented success for treating target contaminants Aerobic bacteria responsible for degradation are generally ubiquitous in nature	May be applied to highly reduced plumes	Widespread acceptance with documented success for treating target contaminants Documented success in high concentration source material Abiotic degradation often occurs parallel to biological degradation processes	May be able to treat contaminants to low cleanup levels
Limitations	Some petroleum derived plumes are very reduced requiring high doses of oxygen Delivery systems may encounter significant biological fouling	Limited use to date Can be difficult to distinguish from microaerophilic oxidation	Sensitivity to specific range of geochemical conditions May require bioaugmentation with commercially available microbial cultures	Limited use to date in field applications Inhibitory intermediate products can be produced Substrate pulsing may be needed to reduce competitive inhibition between use of substrate and contaminant by the microorganisms

Aerobic bioremediation

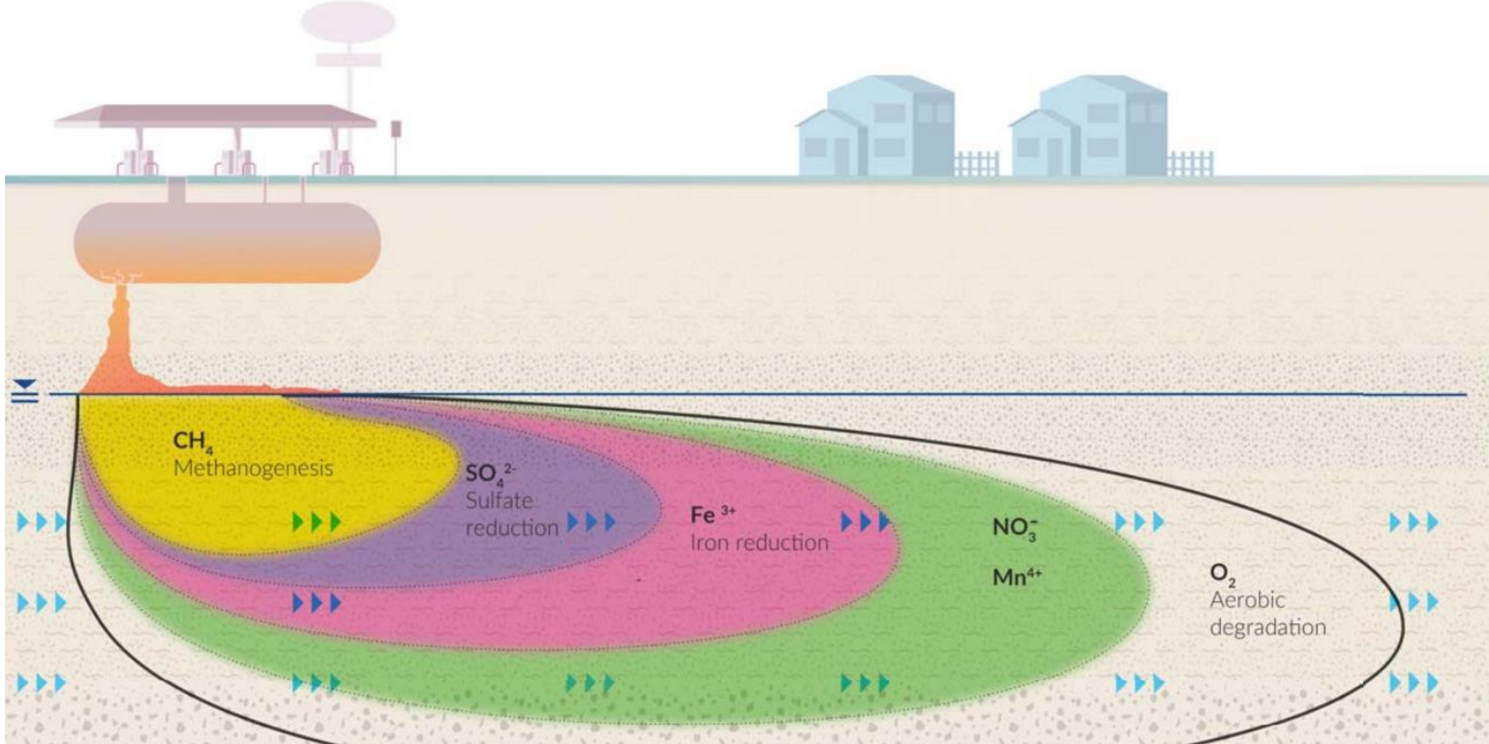
- Aerobic bioremediation most commonly takes place in the presence of oxygen and relies on the direct microbial metabolic oxidation of a contaminant
- Aerobic bioremediation is most effective in treating non-halogenated organic compounds.
  - Many reduced contaminants can be aerobically degraded by aerobic bacteria already present in the subsurface environment.
- Low to moderate weight petroleum hydrocarbons are most readily degradable
- The end products of aerobic respiration are usually carbon dioxide and water
- Intrinsically, bioremediation is more often limited by electron acceptors rather than by nutrients (ammonia, nitrate, phosphate)

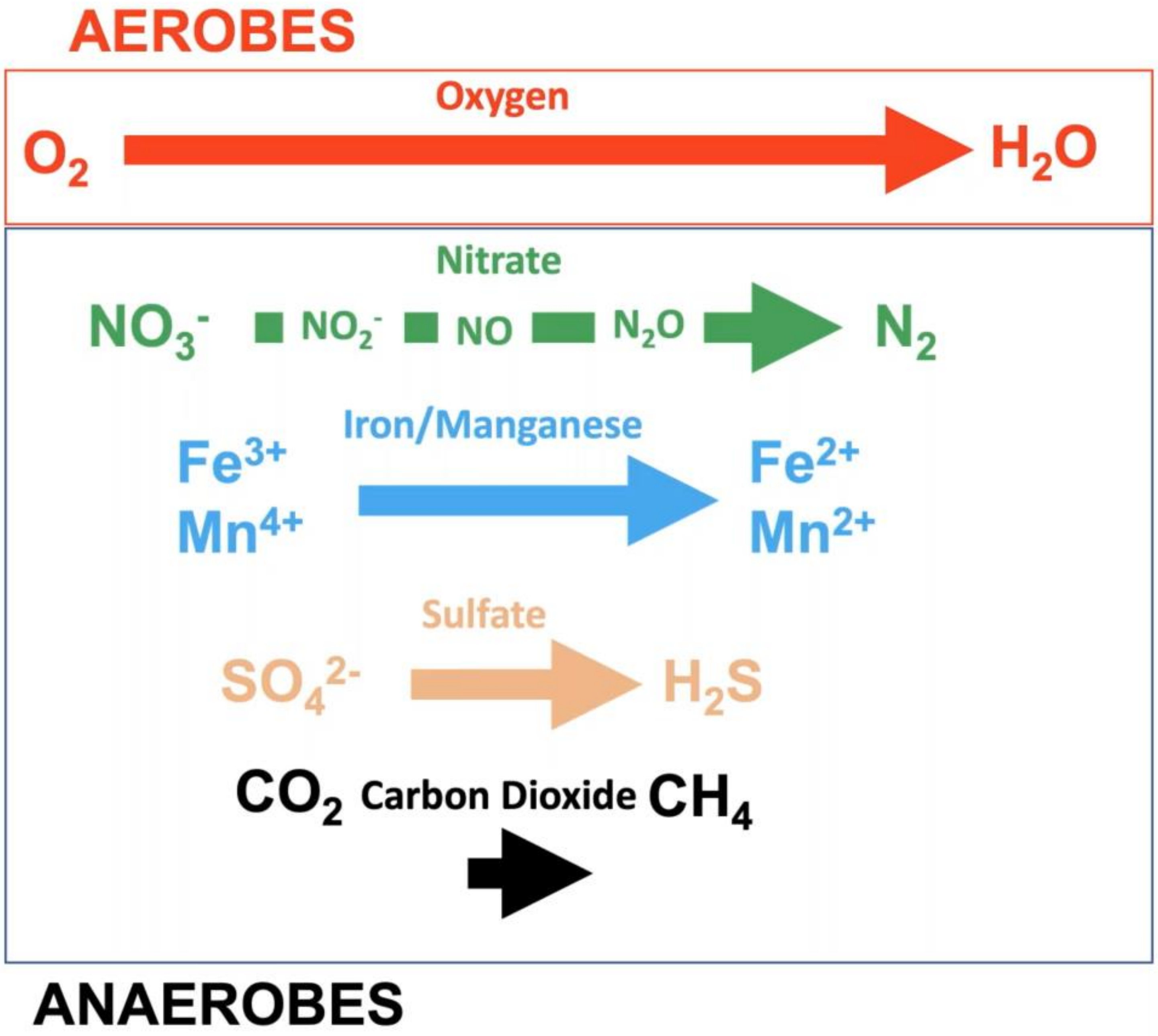
$$C_6H_6 = 7.5O_2 \rightarrow 6CO_2 + 3H_20$$

Anerobic bioremediation

- Anaerobic oxidative bioremediation takes place in the absence of oxygen
  - Other electron acceptors such as nitrate or sulfate for direct microbial metabolic oxidation of a contaminant
- This approach is often applied at petroleum-contaminated sites where oxygen has already been depleted

- Microorganisms will preferentially utilize electron acceptors that provide the greatest amount of free energy during this respiration process
- Aerobic respiration, nitrate reducing, Fe(III) reducing, sulfate reducing, methanogenic respiration

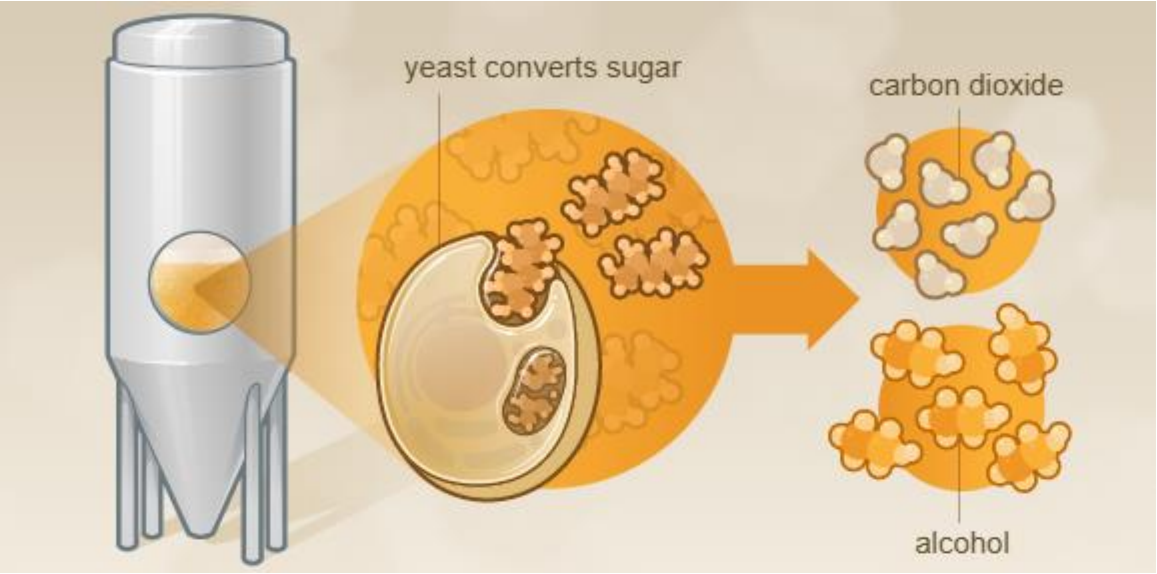




- Microorganisms will preferentially utilize electron acceptors that provide the greatest amount of free energy during this respiration process
- No one organism can reduce all these acceptors
- Can monitor the concentrations of these compounds for diagnostic purposes

Anerobic bioremediation

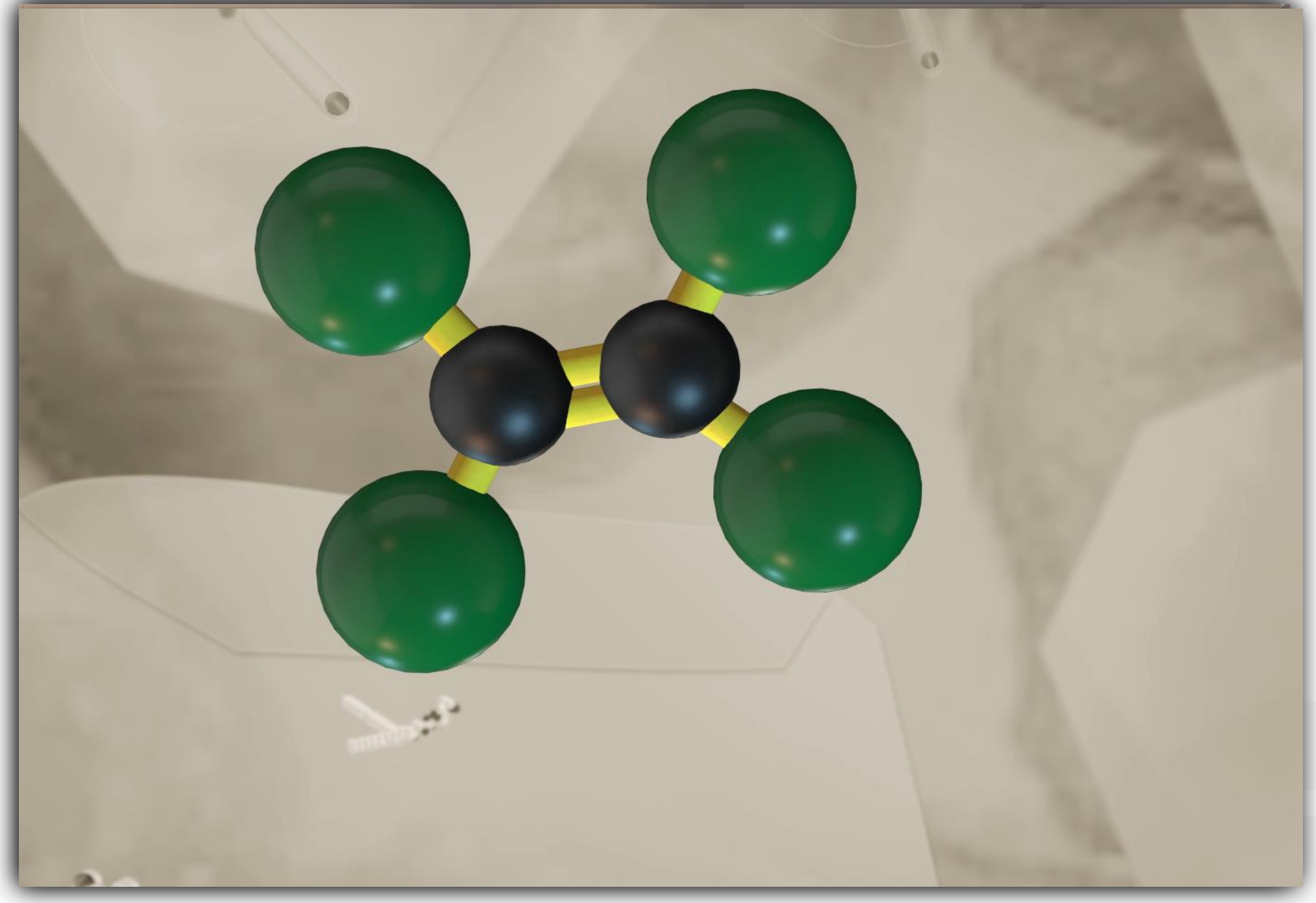
- Anaerobic reductive bioremediation takes place in the absence of oxygen
- Biologically available organic carbon, which may be naturally present or added to stimulate activity
- The organic carbon (electron donor) source, creates and sustains anaerobic conditions by consuming oxygen and other electron acceptors during its biodegradation
- It also promotes the bioreduction of oxidized contaminants such as chlorinated solvents by generating hydrogen through fermentation reactions

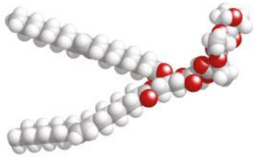


Anerobic bioremediation

- Oxidized contaminants are generally much less susceptible to aerobic oxidation processes
  - They can be reduced by microbes under anaerobic conditions- enhanced reductive dechlorination (ERD) when applied to chlorinated solvents
  - Chlorinated solvent acts as electron acceptor
- In many cases, microorganisms use the oxidized contaminants in a respiratory mechanism and are able to derive metabolically useful energy
- Microbially induced reduction of hexavalent chromium to trivalent chromium may be the most common application of bioremediation to metals

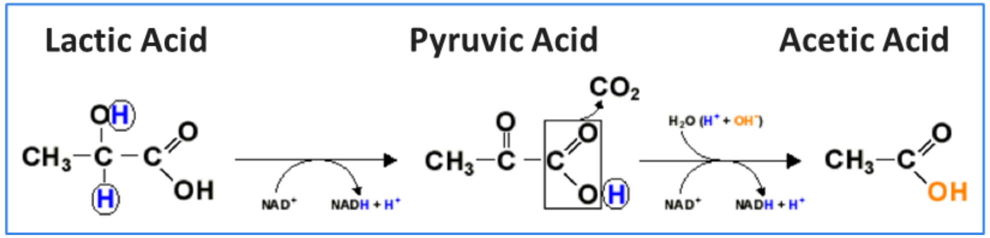
Enhanced Reductive Dechlorination



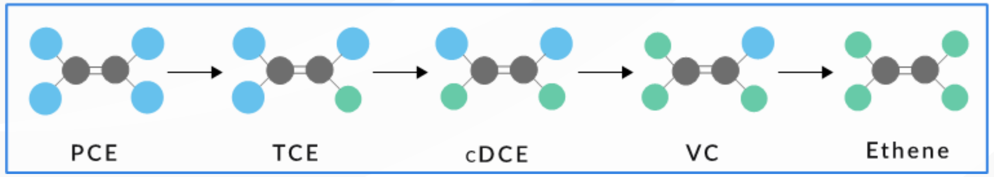


Dissolution

Fermentation



Enhanced Reductive Dechlorination



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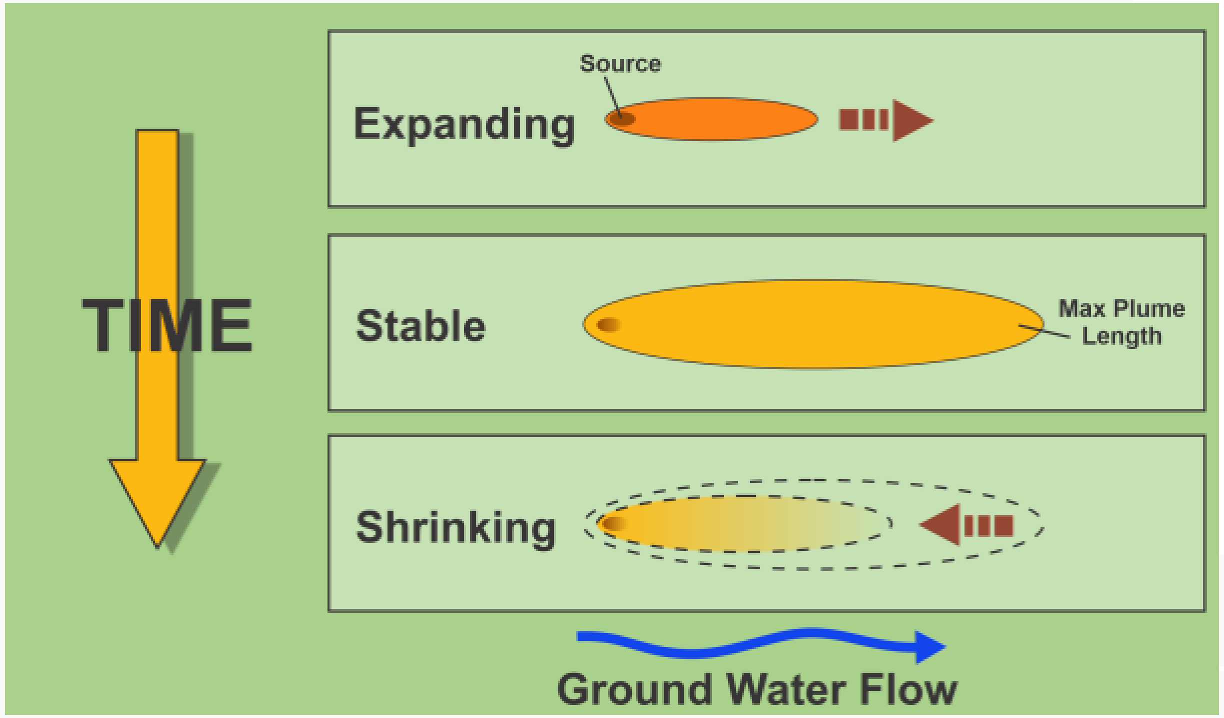
Ideal Site for Enhanced Bio



High Biodegradation Potential

Sites with strong biodegradation potential allow effective breakdown of contaminants by natural or enhanced means.

Accessible Contamination Depth

Contaminant depth should be reachable by remediation technologies to ensure all affected zones are treated.

Optimal pH and Sulfate Levels

Suitable pH and sulfate levels support microbial activity essential for successful ERD remediation.

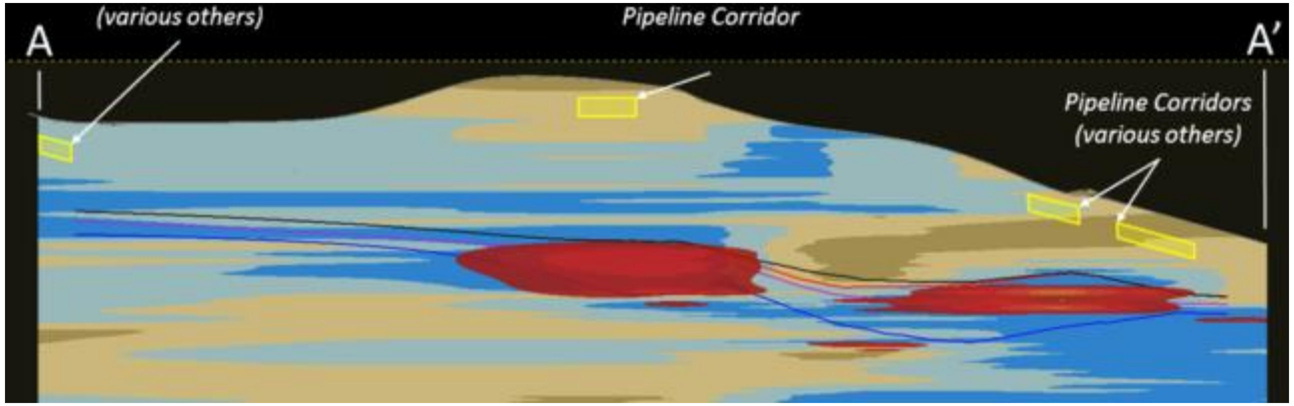
Site Size Considerations

The overall site size influences the scope and strategy for implementing any in situ remediation processes.

Insert Case Study - TBD

Data Needs: Before, During, and After Bioremediation

Conceptual Site Model is Key



Geological Setting Importance

Recognizing geological heterogeneity is critical for success

Hydraulic Conductivity Measurement

Hydraulic conductivity varies greatly; slug tests, pumping tests, and vertical profiling, passive flux measurements provide complementary data.

Porosity Types and Significance

Primary, secondary, and effective porosity influence contaminant mass estimation and amendment delivery efficiency.

Anthropogenic Conditions

Reworked soils, preferential pathways, pumping conditions, etc.

Mass Flux

Barriers Are Highly Sensitive To Mass Flux

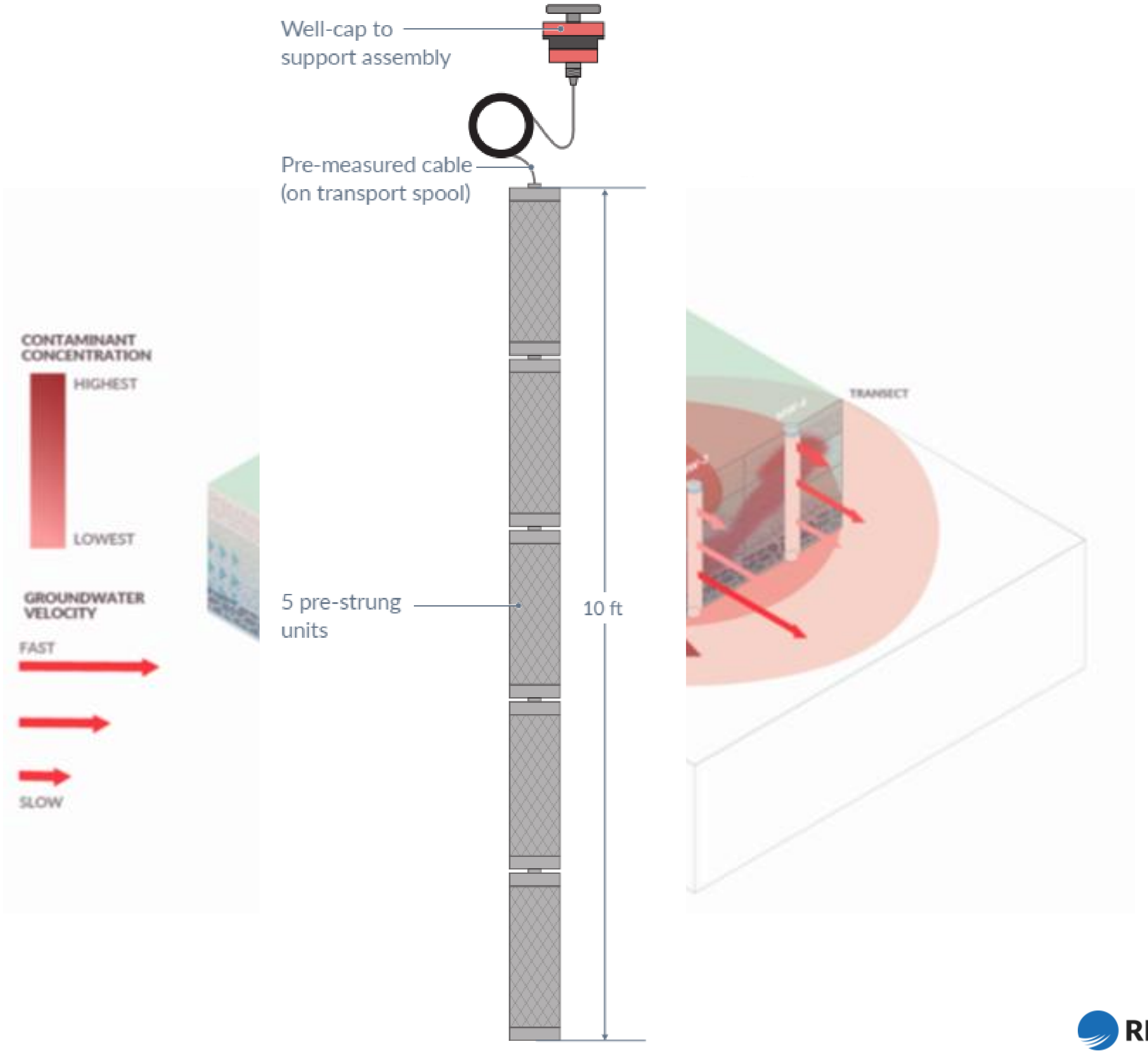
- Rule-of-Thumb: ~80% of the mass flows through 20% of the aquifer
- Wrong mass flux results in:
  - Barrier punch through - failure
  - Over- or under-treatment

Passive Flux Measurement Devices

Easy-to-use devices that vertically delineate contaminant mass flux and groundwater velocity within existing monitoring wells

- Used to optimize dosing

FluxTracer®
Flux Mapping Tool



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Baseline Data

pH, ORP, conductivity

Alkalinity

Oxygen, iron, manganese, nitrate, nitrite, sulfate

Methane, ethane, ethene, carbon dioxide

TOC

BOD

Chloride

Microbial Analysis

Performance monitoring indicators and program design

Importance of Performance Monitoring

Measuring remedial performance is essential for optimization and achieving long-term site cleanup goals.

Aerobic Bioremediation Indicators

Key indicators include oxygen concentration, redox conditions, and contaminant levels measured in the field.

Anaerobic Bioremediation Indicators

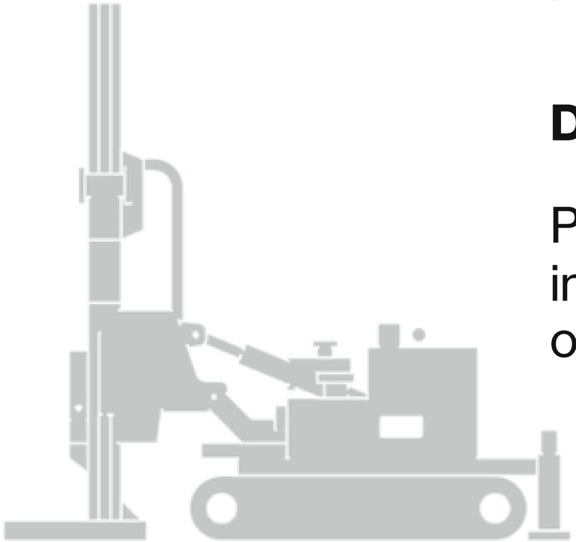
Indicators include electron acceptors, organic carbon, contaminants, and target bacterial populations tracked via qPCR.

Monitoring Program Design

Monitoring frequency varies; initial frequent sampling tapers to less frequent over time to track trends accurately.

Implementation Logistics for Bioremediation

Treatability studies: bench and pilot testing



Bench/Treatability Studies

Bench tests evaluate if remediation is feasible, cost-effective, and scalable before full-scale deployment. Goals of testing need to be well defined.

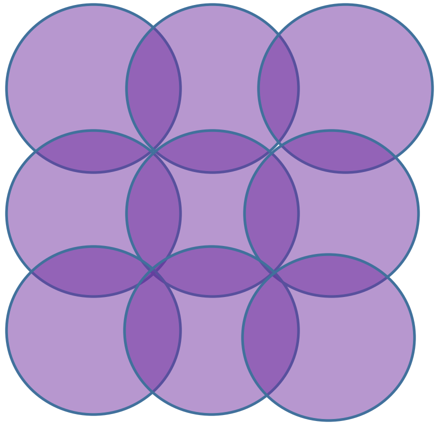
Pilot Testing Methodology

Pilot tests involve small-scale field injections and monitoring to optimize amendments and microbial responses.

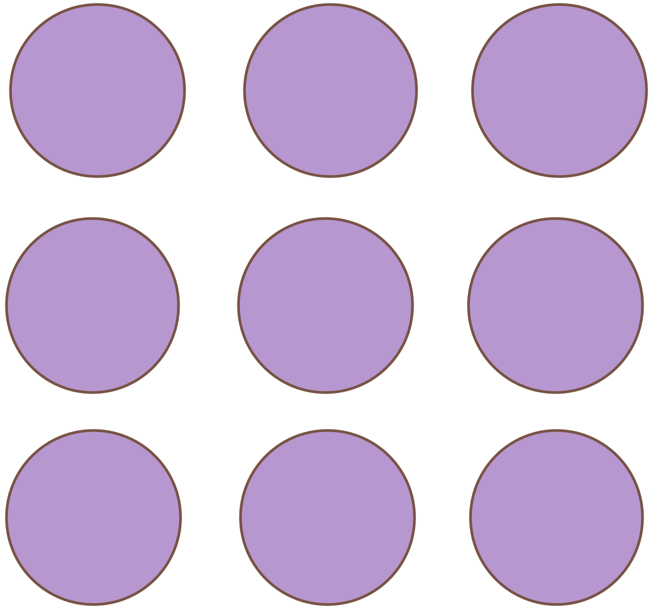
Design and Scalability Considerations

Pilot tests guide full-scale design including injection spacing, amendment dosage, and operational logistics.

Design Geometry Considerations



GOOD



BAD

Grid Spacing Trade-offs

Closer spacing increases drilling costs but can reduce overall injection volumes. Grid applications provide direct source area treatments.

Biobarriers for Plume Control

Passive biobarriers are used to contain large dissolved plumes and prevent off-site migration at cost-effective rates.

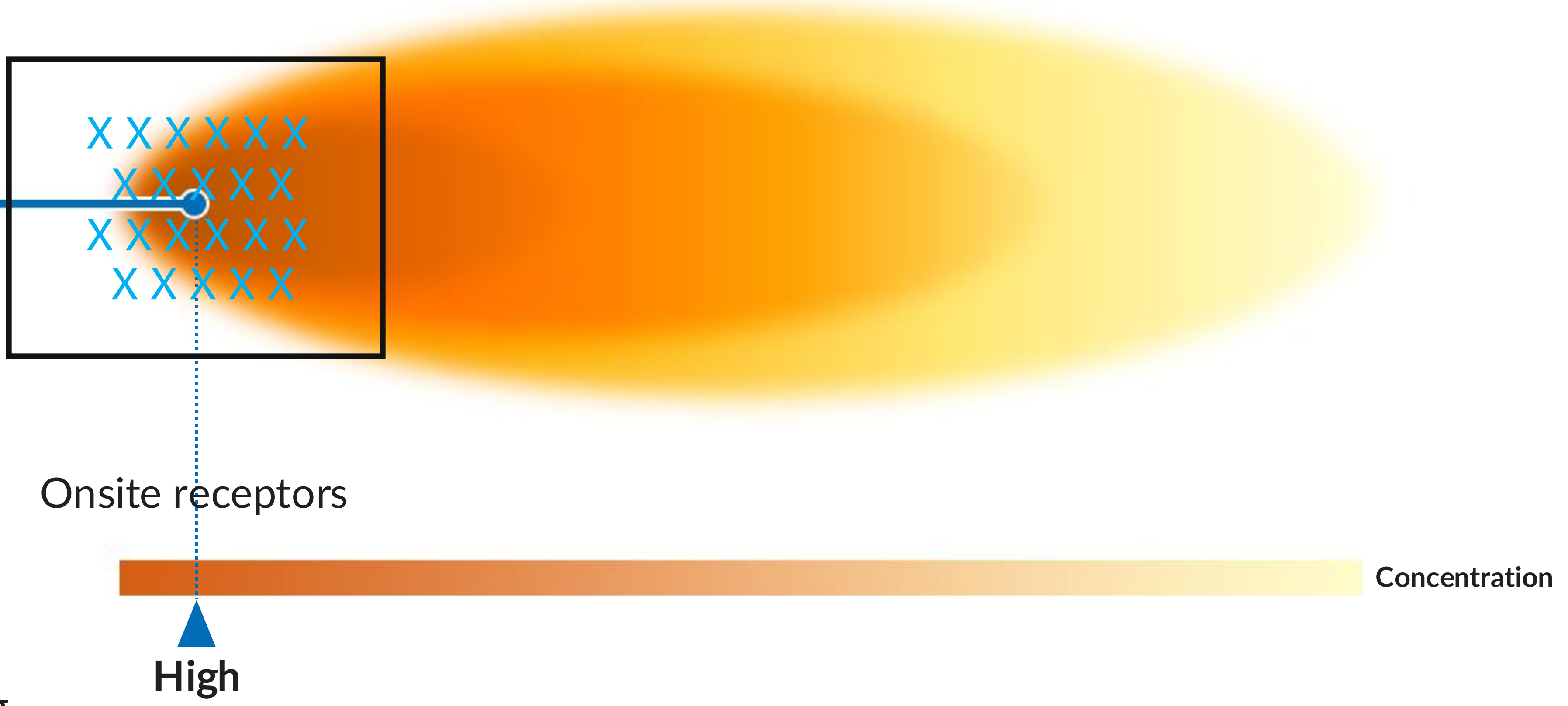
Circulation Systems

Circulation systems enhance amendment distribution and hydraulic control, enabling treatment under restricted site conditions.

Source Area

Challenges

1. Residual DNAPL
2. Sorbed mass
3. High dissolved concentrations
4. Diffused mass in low permeability zones
5. Vapor risk
6. Plume generation
7. Requires rapid and long-term mass reduction



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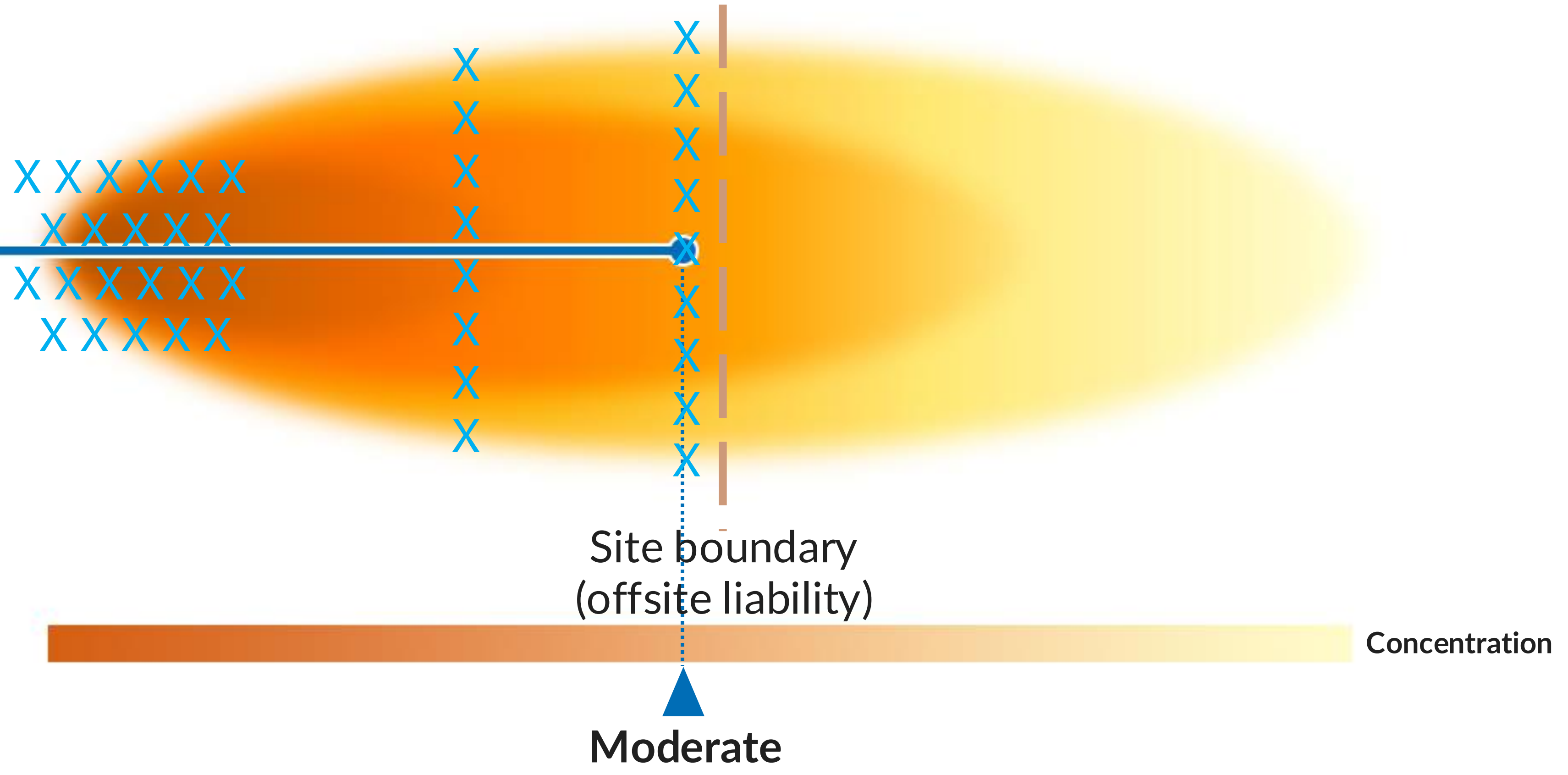
Mid-plume

Challenges

1. Moderate dissolved concentrations
2. Diffused mass in low permeability zones
3. Movement through flux zones
4. Off-site liability
5. Requires elimination of risk to downgradient receptors



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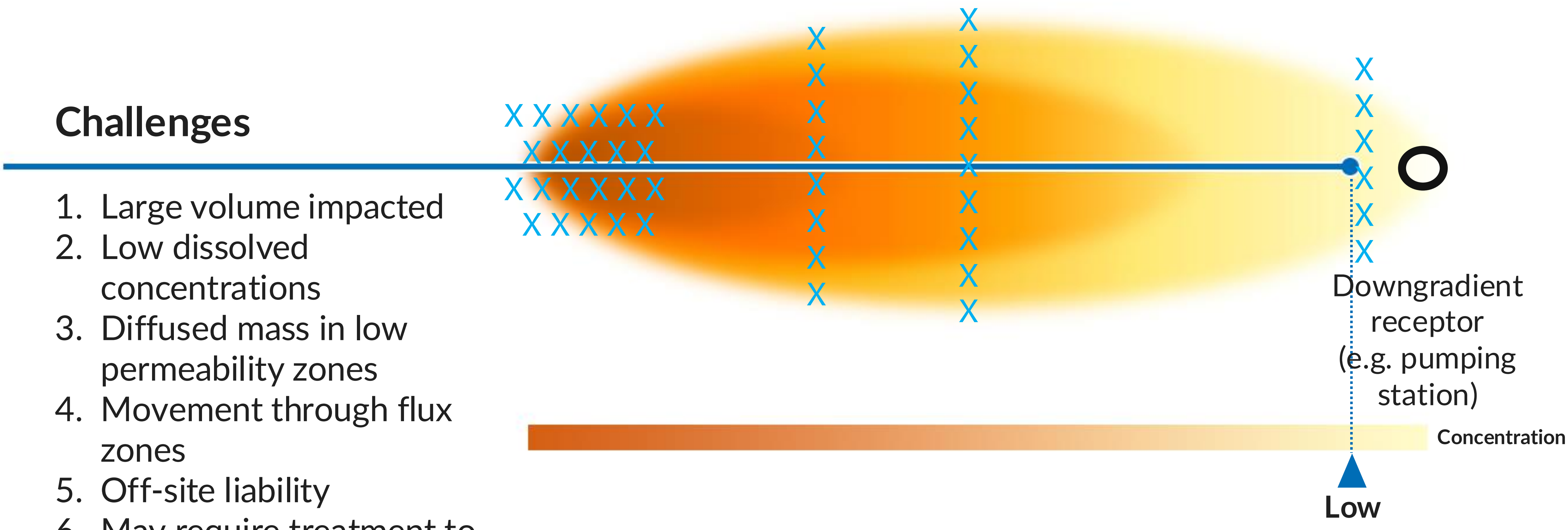
Distal-plume

Challenges

1. Large volume impacted
2. Low dissolved concentrations
3. Diffused mass in low permeability zones
4. Movement through flux zones
5. Off-site liability
6. May require treatment to very stringent remedial targets



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Application

Choice of Injection Tooling

Monitoring during injection of Injection pressures, flow rates, well seals, leaks, pressure changes, flow rate changes, volumes of injectate/water, substrate breakthrough at monitoring points (ROI) or at preferential pathways

GW Quality: pH, ORP, DO, conductivity, temp, depth to water

Consideration for unwanted byproducts (ex: methane)

Combined Approaches: Activated Carbon and Bioremediation

Combined Remedies

- All remediation technologies have strengths and weaknesses.
- These are different from one technology to another.
- Employing technologies in suitable combination can enable strengths to be combined and weakness overcome.
- This in turn can increase efficiency, improve performance, and thereby save time, money and resources.

Sorbent/Bioremediation Combination

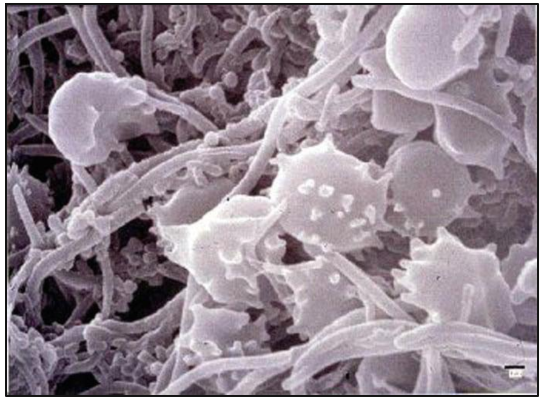
- A highly dispersive, injectable sorbent and microbial growth matrix

- Sorbent
  - Rapid drop in dissolved-phase contaminant concentration
  - Immediate risk-reduction

- Microbial growth matrix
  - Accelerated bio-destruction of sorbed mass
  - Ability to secure clean-up to much lower targets

Bio Basics: Availability and Threshold Concentrations

- Bacteria live on surfaces – biofilms
  - Think 'sink plug-hole slime' or 'dental plaque' rather than 'tadpoles'



- They have to wait for their growth substrate (food) to come to them – they do not hunt
  - They must therefore either sit on / in the food source (rotting vegetables)
  - Or wait for it to dissolve and come to them in solution (groundwater bioremediation)

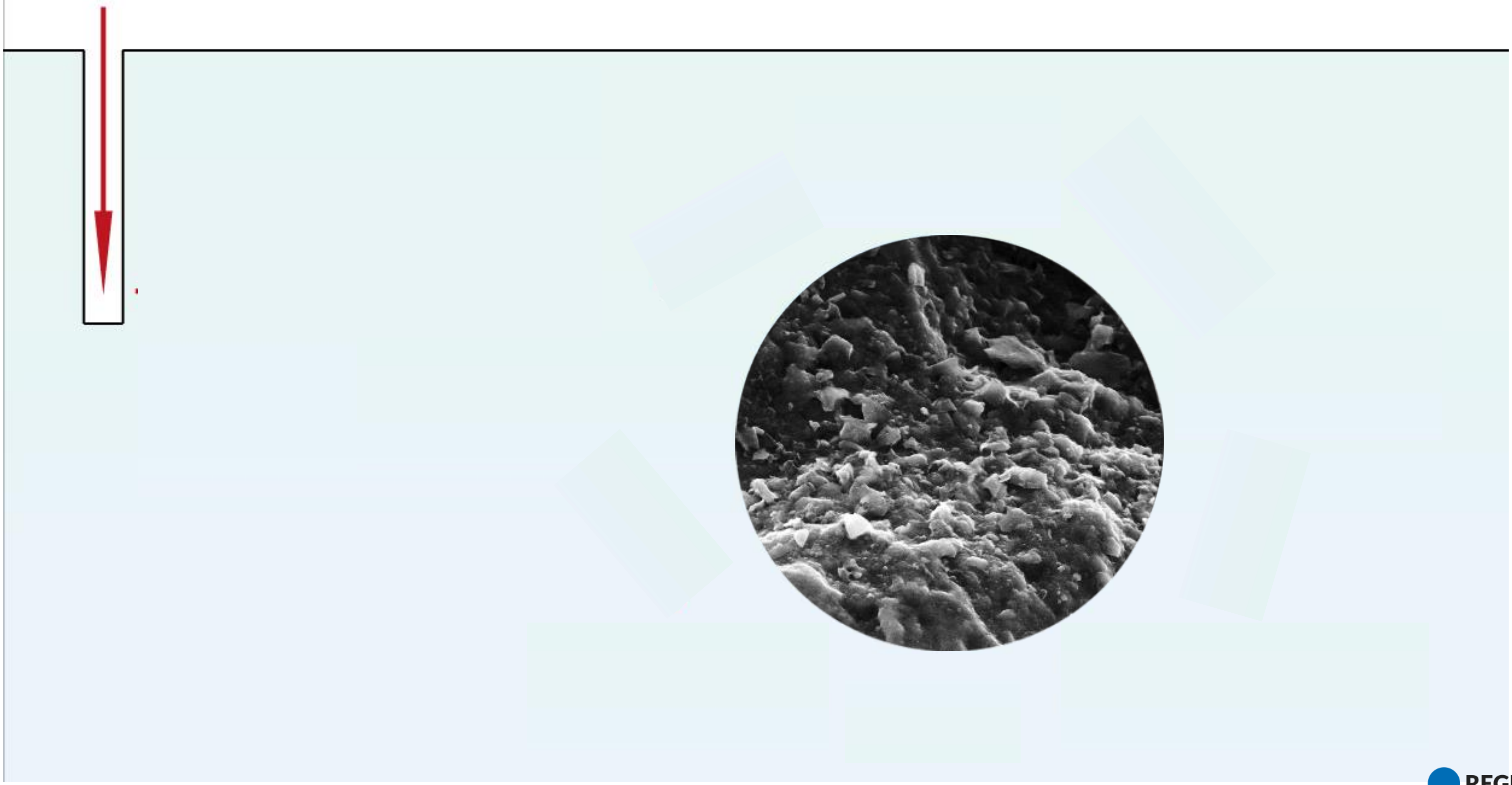
- As the plume is progressively cleaned up, the contaminant concentration drops
  - The rate that substrate comes to the bacteria reduces
  - The rate that bacteria can therefore degrade it reduces – first order kinetics / half-life degradation

- Furthermore, below a certain concentration, the rate can slow dramatically
  - Threshold concentrations (S_{min}) for microbial growth – sufficient energy for activity – 'starvation' boundary
  - The threshold is variable but typically in the low µg/L range – therefore not relevant for every site
  - This step change slow-down is in addition to first-order diminishing returns

Activated Carbon

- Used to further increase bioremediation performance
  - Reduced treatment times
  - Achieve very low target concentrations (low µg/L range)
- Can be co-applied with electron donor/acceptor technologies
- Can be used in low level plumes with less than ideal geochem
- Used as a standalone treatment
  - Where natural donor/acceptor supply is adequate
  - E.g. migrating plume management – back-diffusion management

Injection



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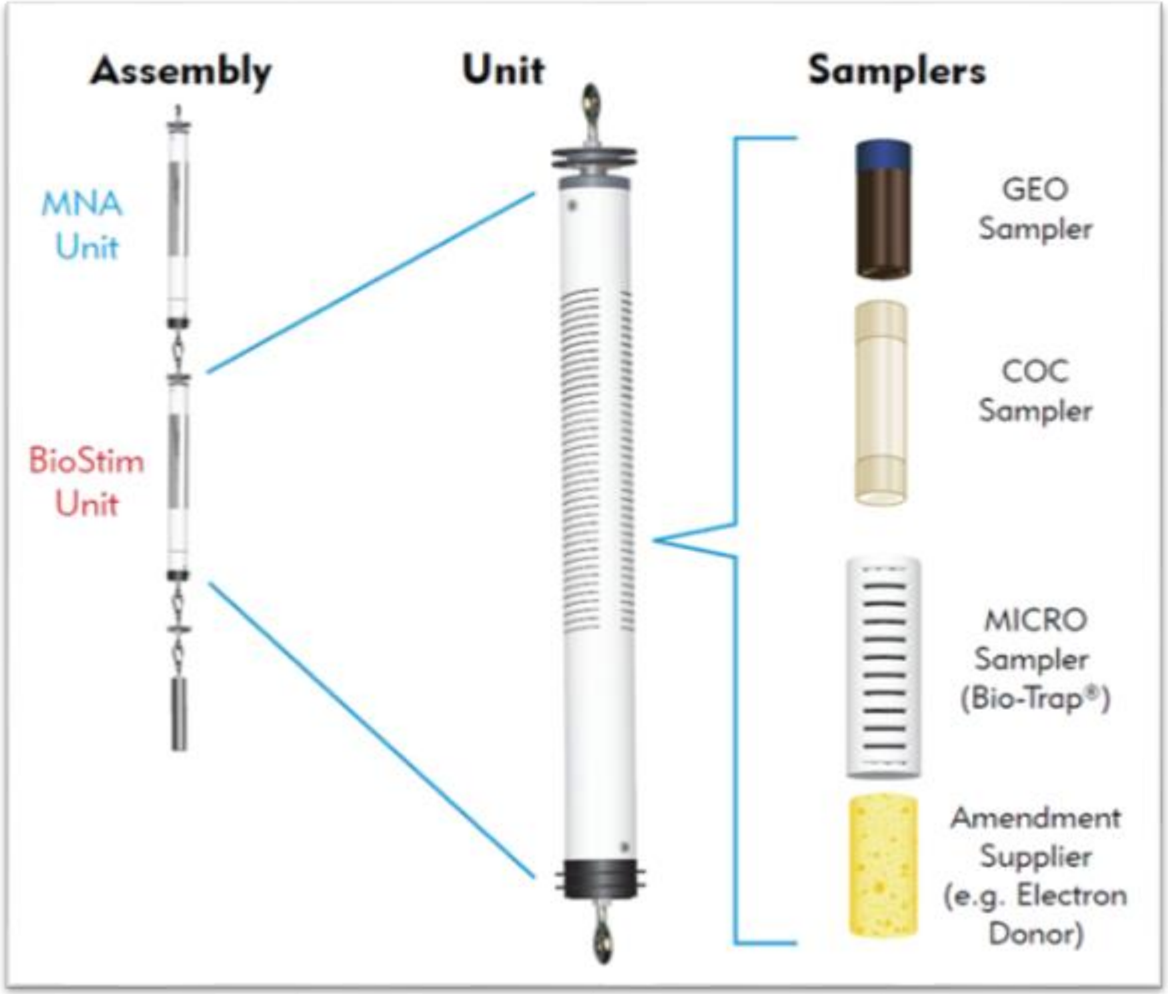
Insert Case Study - TBD

Advanced Tools in Bioremediation

Use of molecular biological tools and advanced site characterization



Molecular Biological Tools (MBTs)

qPCR, qPCR arrays, next gen sequencing, CSIA, SIP

High Resolution Site Characterization (HRSC)

HRSC improves conceptual site models by using detailed sampling to reveal contaminant distribution and subsurface heterogeneities. DPT uses tools like cone penetrometer testing, electrical conductivity probes, and hydraulic profiling for real-time data collection.

Contaminant Detection Technologies

Technologies such as Laser Induced Fluorescence and Membrane Interface Probe support detection of contaminants in subsurface environments.

3D Visualization

Software tools, Geostatistical Kriging

Recommendations and Lessons Learned

Maintenance requirements

Amendment Re-application

Regular re-application of electron acceptors or donors is sometimes necessary.

Geochemical and pH Adjustment

Confirming/adjusting geochemical conditions and pH in groundwater ensures biological populations thrive for successful remediation.

Well Maintenance Challenges

Well fouling from mineral precipitation and biological growth may require cleaning and chemical treatments to maintain functionality.

Monitoring and Performance

Effective monitoring programs help determine when additional amendments or maintenance are needed as well as determining when transition to true MNA.

Aquifer matrix diffusion and contaminant rebound mechanisms

Heterogeneous Aquifer Layers

zones with different permeability affecting groundwater flow patterns and remediation efficiency

Matrix Diffusion and Contaminant Storage

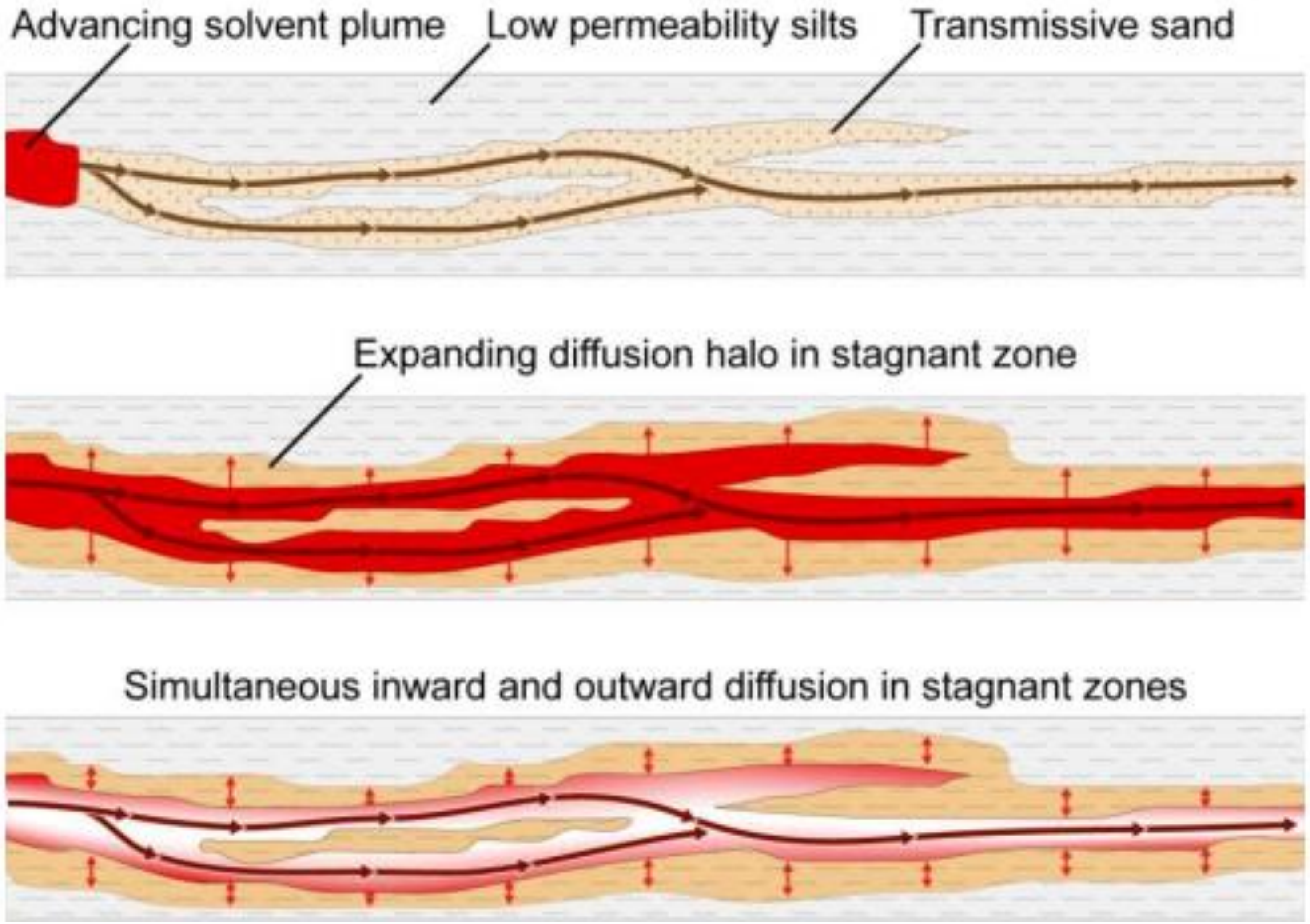
Contaminants diffuse from high permeability zones into less permeable matrix zones, storing contamination over time

Contaminant Rebound Effect

During remediation, contaminants diffuse back into permeable zones from matrix, causing concentration rebound

Impact on Cleanup Dynamics

Matrix back-diffusion prolongs cleanup times, especially in dual porosity bedrock and sedimentary aquifers



Our Process

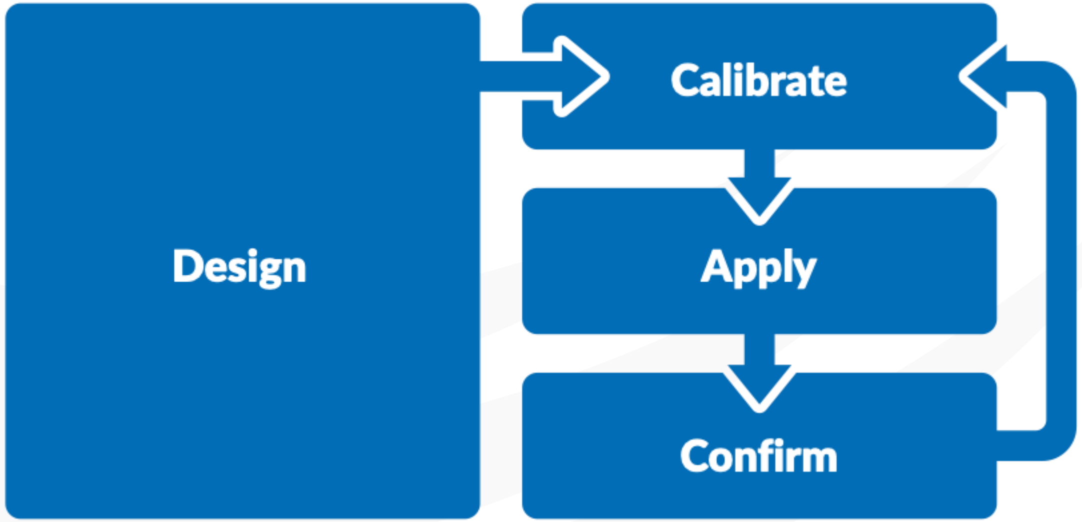
Design field ready remedial approach in collaboration with our clients

Calibrate the design to match field conditions and injection dose response

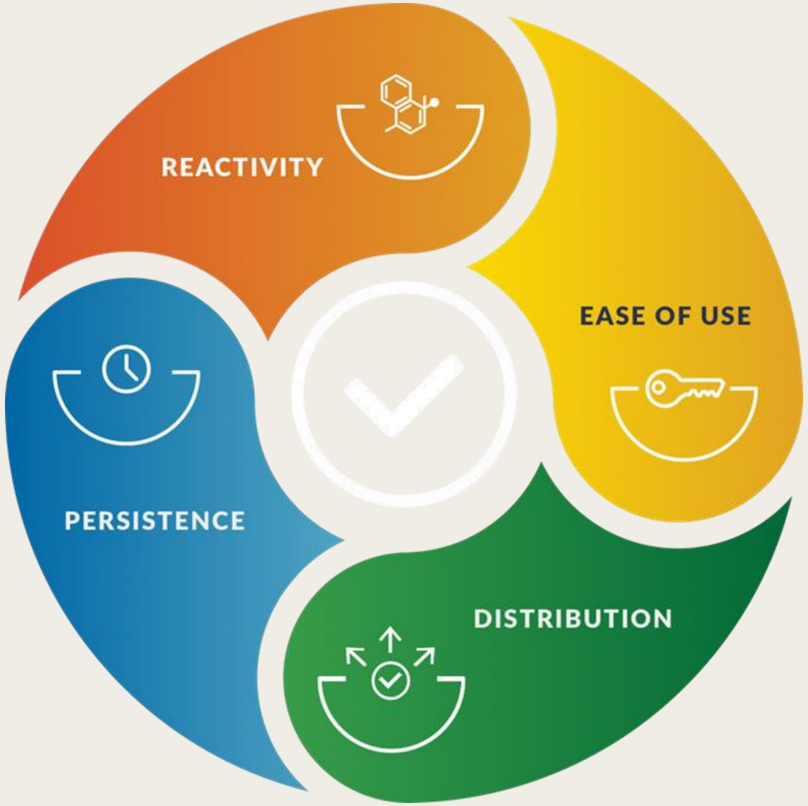
Apply the design with high efficiency using the safest operating procedures

Confirm the application is achieving the desired distribution throughout the target treatment zone

Performance Driven Process



Conclusions



Versatile Bioremediation

Bioremediation provides a flexible and effective method to treat groundwater contamination safely and sustainably.

Site-Specific Understanding

Successful bioremediation requires detailed knowledge of site conditions and contaminant characteristics.

Planning and Monitoring

Careful planning and continuous monitoring ensure effective treatment and adaptation to changes during bioremediation.

Emerging Tools

Utilizing new technologies and tools enhances bioremediation efficiency and effectiveness in groundwater treatment.