provectus
ENVIRONMENTAL PRODUCTS™

Private Webinar: Overview of Applied Environmental Biotechnologies

TBD LOCATION, TBD DATE

Jim Mueller – Freeport, IL

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12/19/2014

Presentation Outline

- Introduction to Provectus Environmental Products, Inc.
- Environmental Biotechnologies
  - Provect-CH4™ Methane Inhibitor
  - ABC-CH4™ Liquid, Antimethanogenic ERD Reagent
  - Provect-IR™ Solid, Antimethanogenic ISCR Reagent
  - Provect-IRM™ Antimethanogenic ISCR/Heavy Metal Immobilization Reagent
  - AquaBlok-CH4™ Antimethanogenic Reactive Cap
  - Provect-OX™ Self-Activating ISCO / Enhanced Bioremediation Reagent
- ISCO or ISCR?
- Services and Offerings

My Background

- B.S., M.S. SIU Carbondale – 1983/1985
- Ph.D. Clemson University -1988
- Post Doctoral Studies US EPA GBERL (1988 – 1991)
- SBP Technologies, Inc.→ RF Weston (1991 to 1997)
- Dames & Moore → URS (1997 to 2002)
- Malcolm Pirnie, Inc. (2002 to 2003)
- WR Grace → Adventus Americas, Inc. → FMC Corporation → Peroxychem/JPM (2003 to April, 2014)
- Provectus Environmental Products – May, 2014 (acquired patents on CH4 inhibitors, ISCR and ISCO)

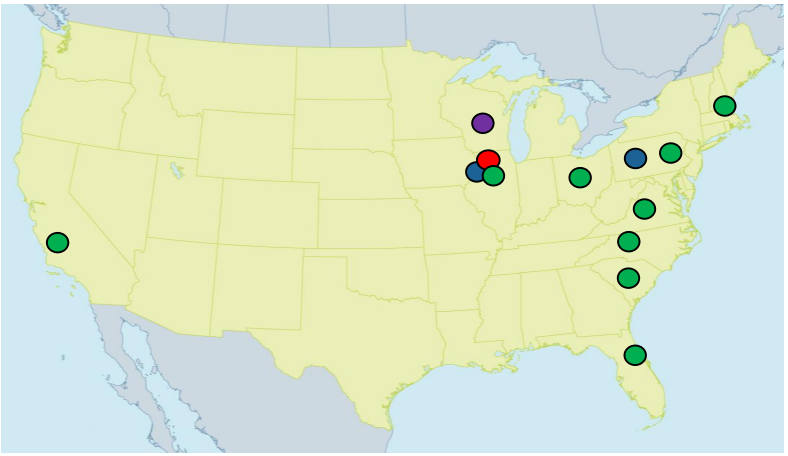
Overview of Provectus' Patent Estate

Application / Patent Number	Title	Filling Date	Issue Date
7,129,388 and 7,531,709	Method for Accelerated Dechlorination of Matter; Parts 1 and 2.		10/31/2006 and 05/12/2009
7,828,974	Method for the Treatment of Ground Water and Soils Using Dried Algae and Other Dried Mixtures		10/09/2010
8,147,694	Method for the Treatment of Ground Water and Soils Using Mixtures of Seaweed and Kelp		04/03/2012
8,766,030	Utilization of Ferric Ammonium Citrate for In-Situ Remediation of chlorinated Solvents		01/30/2014
13/785,840 and 14/36,632 CIP 14/268,637	Method for Inhibition of methane production during anaerobic reductive dechlorination	03/05/2013 05/02/2014	
13/866,158	Use of encapsulated substrates to control the release rates of organic hydrogen donors	04/19/2013	
13/891,934 and CIP 14/268,629 PCT/US14/36,642	Chemical Oxidation and Biological Attenuation Process for the Treatment of Contaminated Media	05/10/2013	
62/024,640	Method and Composition for Inhibiting Methanogenesis During In Situ Sediment Treatment	07/15/2014	
Filed	Inhibition of methane and hydrogen sulfide production in anaerobic digester animal farms, landfills, sediments and sewer systems	08/01/2014	
Filed	Method and Composition for Inhibiting Heavy Metal Methylation During In Situ Remedial Actions	10/15/2014	

5 issued patents; 8 Pending Patents (October, 2014)

Provectus Environmental Products

- Small business
- Guaranteed Performance and PFP contracting with strategic partners
- Safer, more effective, more efficient remedial actions



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Warehousing Offices Affiliate Offices Affiliate Lab

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  - **Provect-CH4™ Methane Inhibitor**
  - ABC-CH4™ Liquid, Antimethanogenic ERD Reagent
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What is a Methanogen?

- Methanogens are microorganisms that produce methane
- Methanogens are Archaea (Woese and Fox, 1977) and hence, from a genetic perspective, *Dehalococcoides ethenogenes* are as different from methanogens as you are.
- Methanogens are often dominant as compared to DHC spp. and acetogens: averaging 2% to 15% of all soil microbes (Bates, *et. al.,* 2011)

  Even at biostimulated populations of DHC rising to $>10^8$ cells/L Archaea populations can be orders of magnitude greater in number
- Methanogens are important members of synergistic, fickle anaerobic communities = we need some

What is The Problem With Methanogens?

- **Cost and Efficiency Issues**: Production of methane is a direct indication that hydrogen generated from the electron donor amendments was used by methanogens instead of the target microbes (*e.g., Dehalococcoides spp.*), substantially reducing application efficiency.

Constituent	Groundwater Concentration (mg/L)	Molecular Weight (g/mol)	Moles of H_2 to Reduce Mole Analyte	Moles of H_2 Acceptor In Treatment Area
Contaminant Electron Acceptors (To End Product Ethene)				
Tetrachloroethene (PCE)	10.0	165.8	4	1,393
Trichloroethene (TCE)	7.0	131.4	3	364
cis-1,2-Dichloroethene (cDCE)	0.0	96.9	2	0
Vinyl Chloride (VC)	0.0	62.5	1	0
			Complete Dechlorination (Soil+Groundwater) Subtotal	**1,757**
Native Electron Acceptors				
Dissolved Oxygen	9.0	32	2	199
Nitrate (as Nitrogen)	9.0	62	3	682
Sulfate	50.0	96.1	4	736
Fe^{+2} Formation from Fe^{+3}	20.0	55.8	0.5	63
Mn^{+2} Formation from Mn^{+4}	10.0	54.9	1	64
			Baseline Geochemistry Subtotal	**1,745**
Hydrogen Waste for Methane Formation				
Methane Formed	20.0	16	4	**1,769**
			Initial Treatment Area Hydrogen Usage	**5,271**

Even in a highly oxidized setting with relatively high total concentrations of PCE and TCE, generating just 20 mg/L of methane constitutes **greater than 33%** of the total amendment consumption based on moles of H_2.

What is The Problem With Methane?

- ***Potential* Health and Safety Issues (*in Some Situations*)**:

✓ Methane is considered to be a major greenhouse gas.

✓ Methane is explosive, with an LEL of 5% and an UEL of 15%.

✓ Microbial fermentation processes can lead to accumulation of methane in soil gas subsequently impacting indoor air, accumulating in basements, under slabs/foundations and/or migrating along utility corridors substantially reducing application efficiency.

✓ In the presence of an active methanogenic community, some studies suggest that heavy metals can be more readily methylated.

✓ In the presence of Cl resulting from dechlorination reactions, some studies suggest that chloromethanes can be generated.

Example sites where methane production was problematic

- ✓ Subsurface fires next to an industrial facility at a site in the Midwest USA immediately following the application of a conventional ISCR reagent;
- ✓ Generation of up to 23% methane in soil gas immediately adjacent to a public church in North Carolina (with sustained methane production >LEL for 8 to 9 months) from an excavated area treated with conventional ISCR reagent;
- ✓ Requirement to install an air sparge/SVE contingency system at a site in northern Indiana due to methane accumulation associated with an application of emulsified oil;
- ✓ Changes in aquifer flow dynamics and in activation of an *in situ* injection system due to methane production and extensive biomass generation following repeat applications of molasses for ERD at a site in Rio De Janeiro, Brazil; and
- ✓ Delayed occupancy of a newly developed, high-rise residential complex in Sao Paolo, Brazil due to presence of elevated methane in soil gas following the use of conventional ISCR reagent.

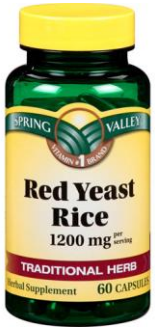
What is The Problem With Methane?

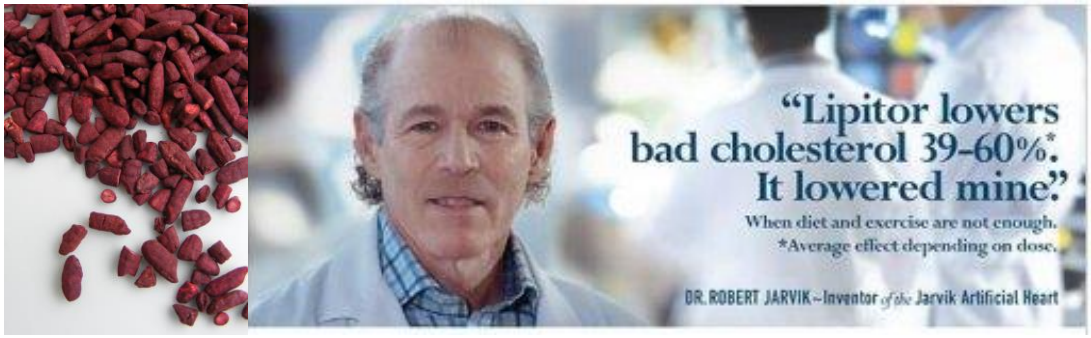
- **New and Emerging Regulatory Issues**:

- ✓ State specific regulations for methane in groundwater have been promulgated, with others pending for soil gas and indoor air.
- ✓ For example, current regulations for methane in groundwater vary from *ca*. 10 ppm to 28 ppm CH_4 (IDEM, 2014).
- ✓ Notably, several ERD projects which intended to use liquid carbon (emulsified oils) sources have failed to receive regulatory approval due to issues associated with excessive production of methane during previous technology applications (Personal Communications - States of CA, MN, FL, NC).
- ✓ As a result, many remedial practitioners proactively design contingencies for conventional ERD/ISCR implementation in the event that methane exceeds a threshold level ranging from 1 ppm to 10 ppm in groundwater.
- ✓ These contingencies often entail expensive and extensive systems for capturing methane in soil gas/vapor via SVE systems and subsequently treating the vapors.

What is Red Yeast Rice (RYR) Extract?

- RYR extract is a substance extracted from rice that has been fermented with a yeast called *Monascus purpureus*.
- RYR extract contains a number of natural statins - most importantly, Monacolin K - otherwise known as Lovastatin® / Lipitor® /etc.
- In addition to Monacolin K, RYR also contains 9 other statins, mono-unsaturated fatty acids, vitamins and other nutrients that will effectively stimulate anaerobic bacteria.
- RYR is used as a food coloring, food additive and preservative, and is widely consumed directly by humans.

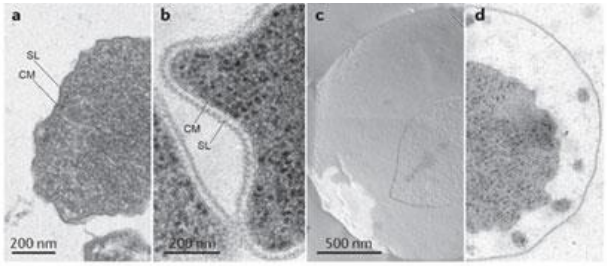


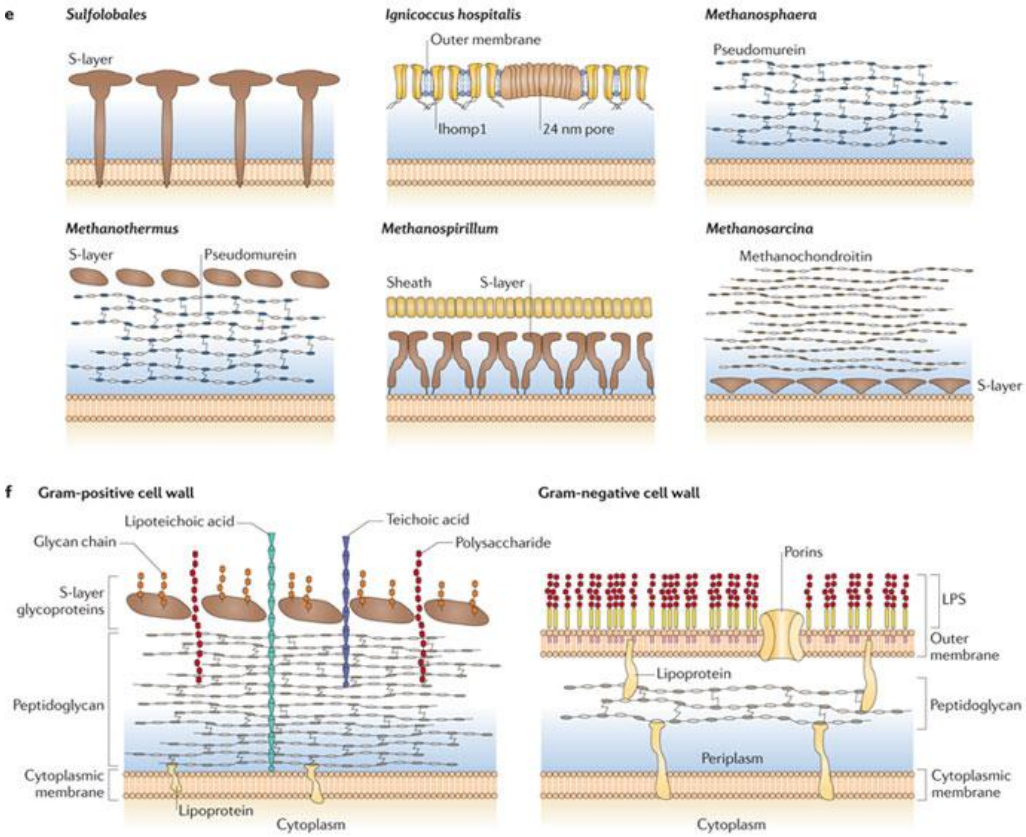


Lovastatin is a registered trademark of Merck; Lipitor is a registered trademark of Pfizer

How Does RYR Control Methanogens?

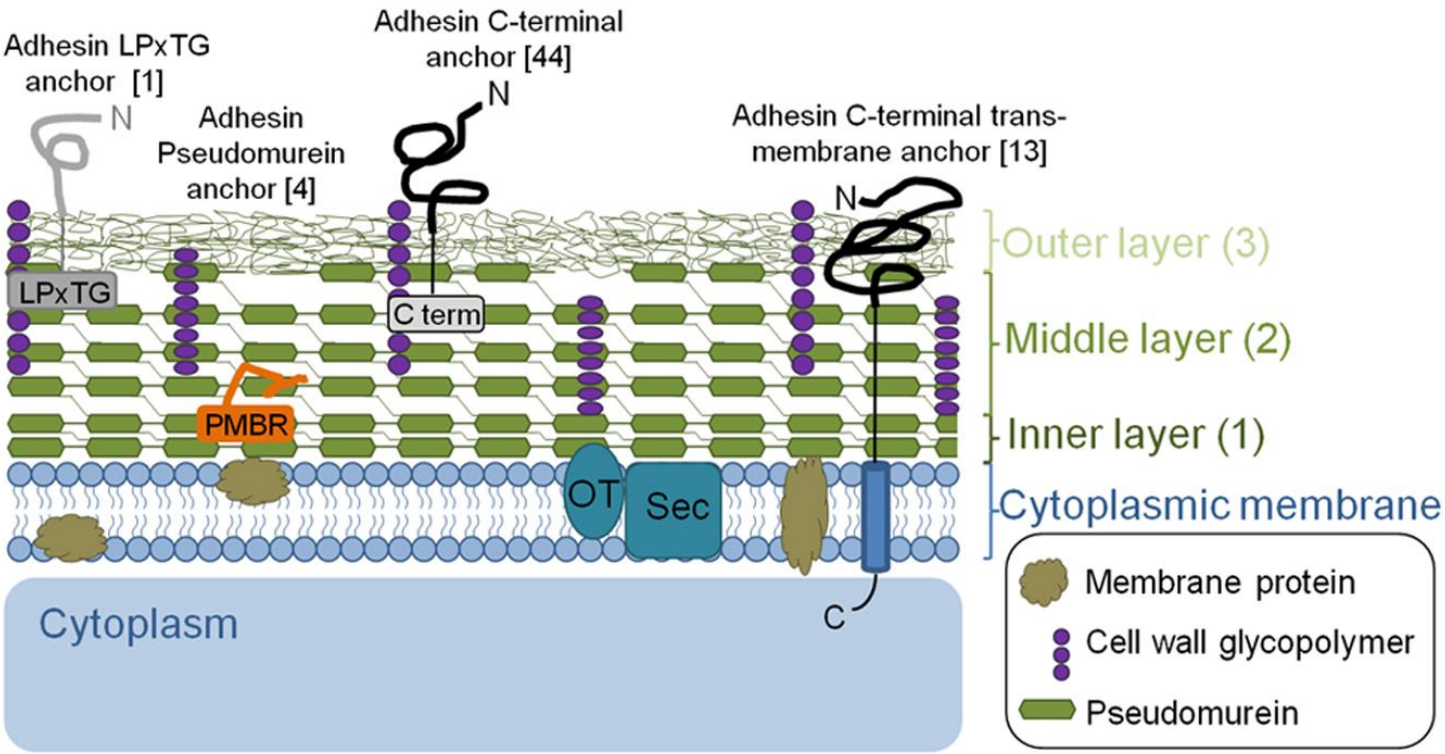
- Bacteria cell walls contain peptidoglycan (murein).
- Methanogens cell walls contain pseudomurein.
- Pseudomuerin is biosynthesized via activity similar to that of 3-hydroxyl-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which is a key enzyme in the cholesterol biosynthesis pathway in humans (Alberts *et al.*, 1980).





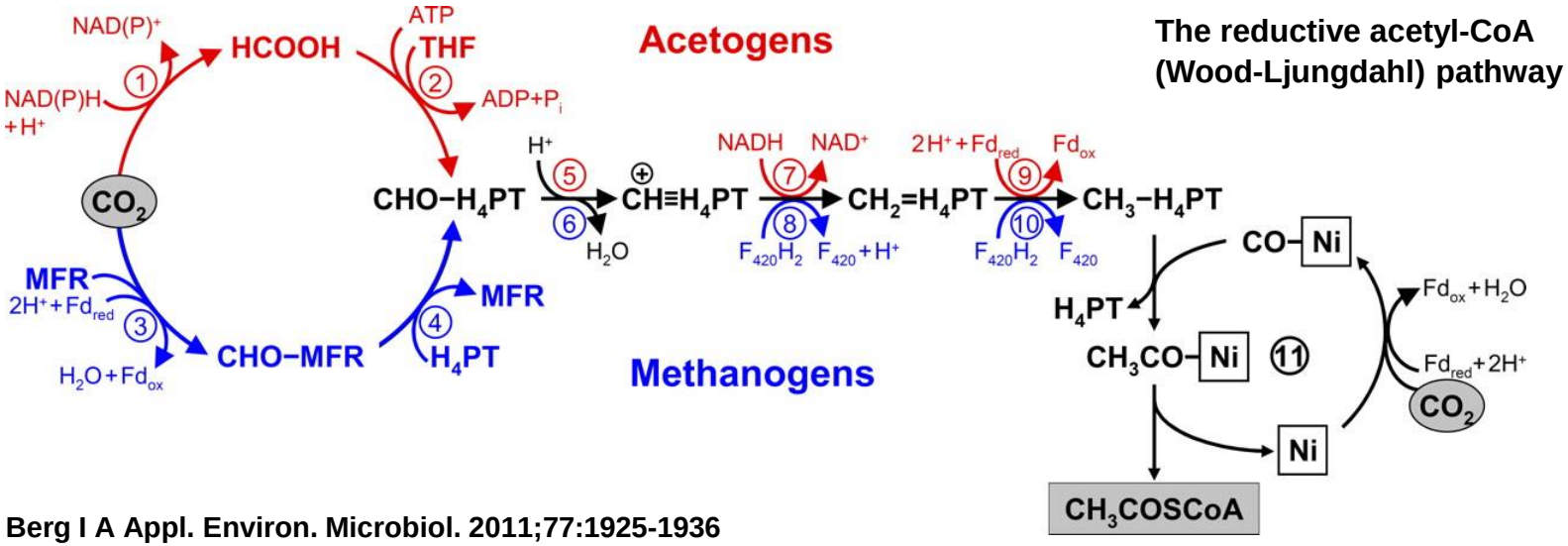
How Does Provect-CH4 Control Methane?

- In the presence of a Monacolin K and other statins in Provect-CH4™ HMG-CoA reductase is inhibited, pseudomurein ( ) biosynthesis pathway is interrupted, and methanogens are restricted from growth, development and proliferation.



How Does Provect-CH4™ Control Methane?

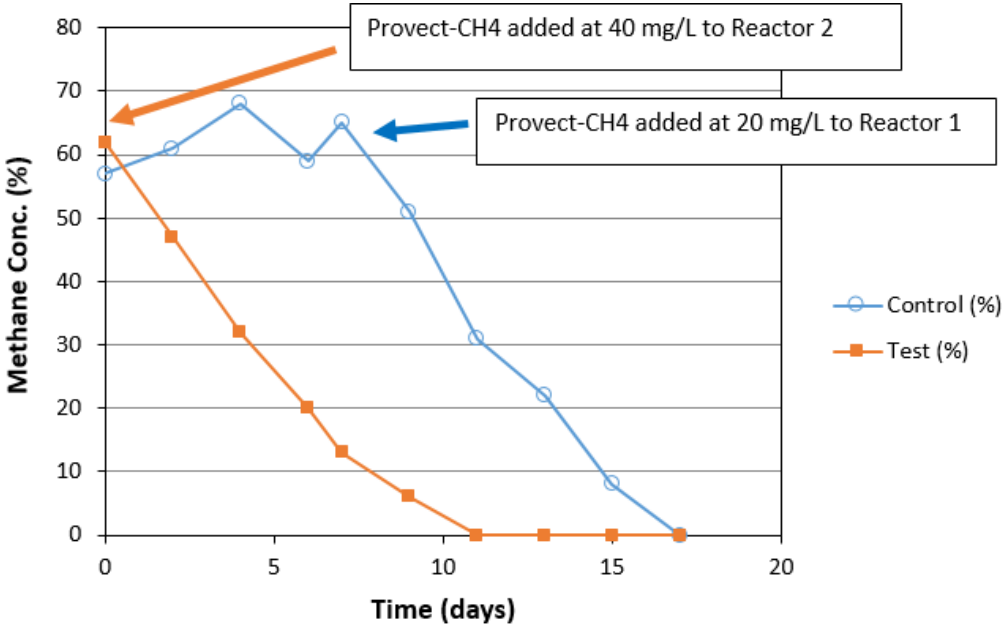
- Monacolin K and other statins also interrupt F420 enzyme synthesis in methanogens and they are further restricted from growth and proliferation.
- Because Archaea are so different from other microbes, these inhibitory effects are selective to methanogens and are not observed in microbes that are typically associated with: i) catabolism of organic contaminants (such as various *Pseudomonas spp*.) and/or, ii) halo-respiration/biodegradation of chlorinated solvents (such as various *Dehalococcoides spp*).



Berg I A Appl. Environ. Microbiol. 2011;77:1925-1936

Proof of Concept

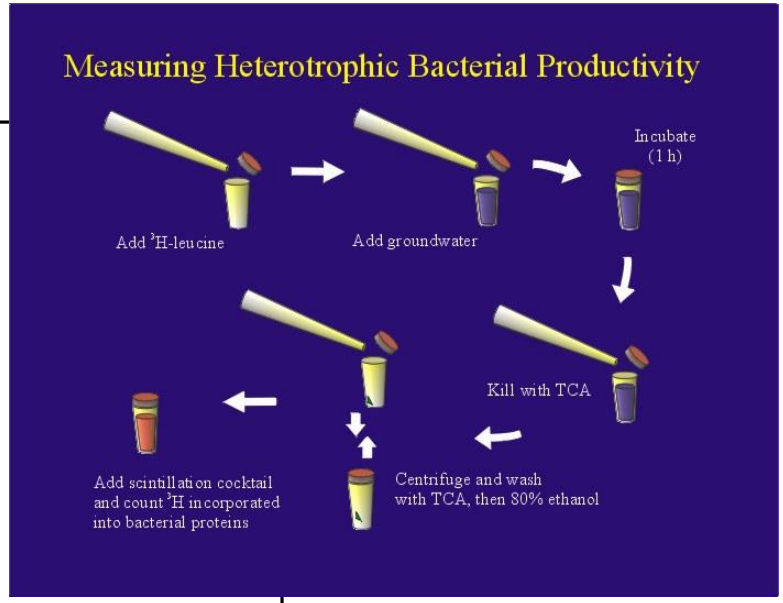
- >90% reduction in methane production within 10 days
- Current recommended field application dosing of 50 ppm for source areas and 100 ppm for PRBs / excessive CH4 area.
- Longevity site specific, estimated at 3 to 6 months (critical time)

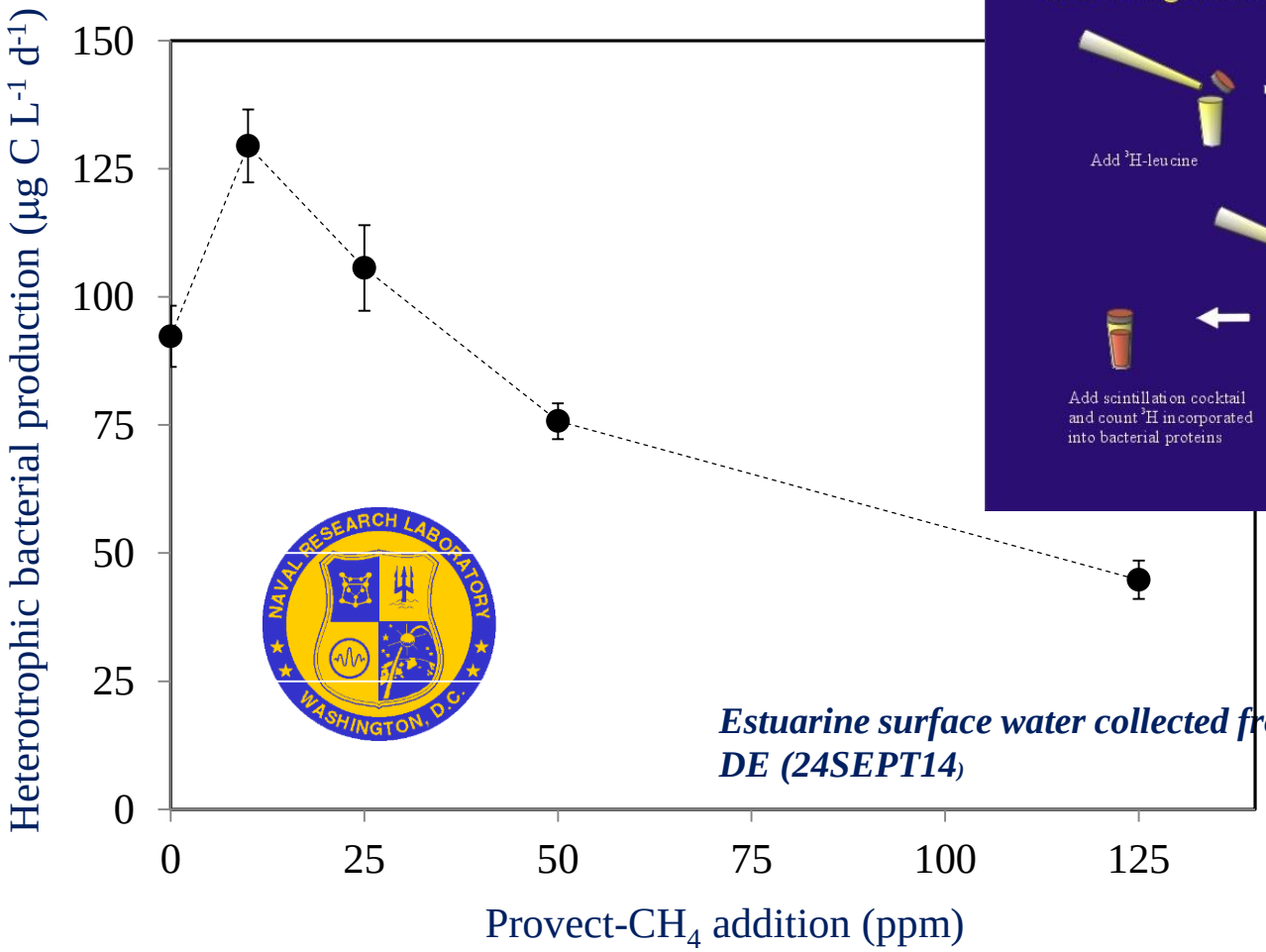


Does not impede DHC inoculants (SDC-9)

Treatment	qPCR Day 0	qPCR Day 25
control	9,840	18,400
P-IR 1.0	65,900	51,100

Effect on Heterotrophic Bacterial Assemblage



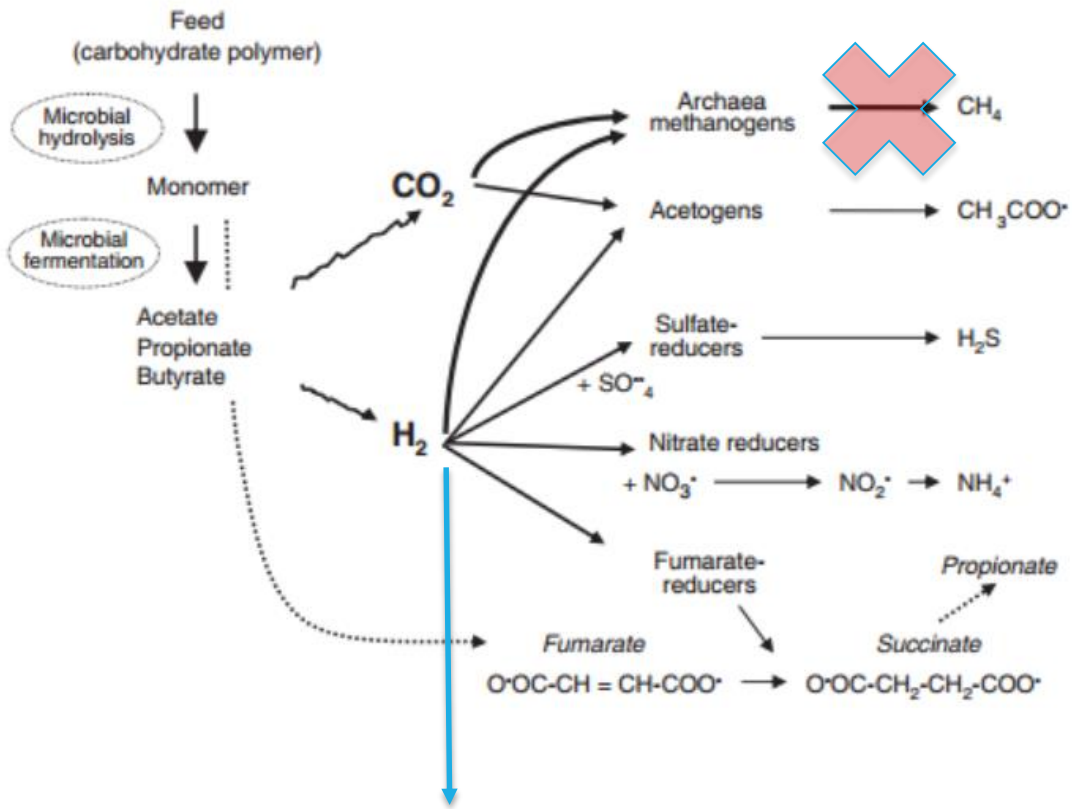


Estuarine surface water collected from Canary Creek, Lewes, DE (24SEPT14)

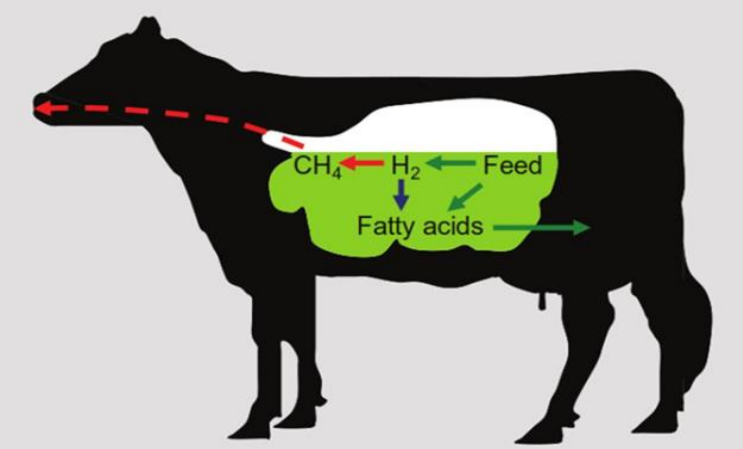
The amino acid ^{3}H-leucine is incorporated into heterotrophic bacterial biomass (macromolecules like protein, DNA, and lipids) at a rate that is proportional to the growth rate of the natural assemblage. This rate is converted to µg of carbon L^{-1} day^{-1} that the assemblage is metabolizing to give you an upper bound for the amount of intrinsic bioremediation that is occurring at a site.

What is the Fate of H2 in the Absence of CH4 Production?

Figure 1 Schematic microbial fermentation of feed polysaccharides and H_2 reduction pathways in the rumen.



Dehalogenators



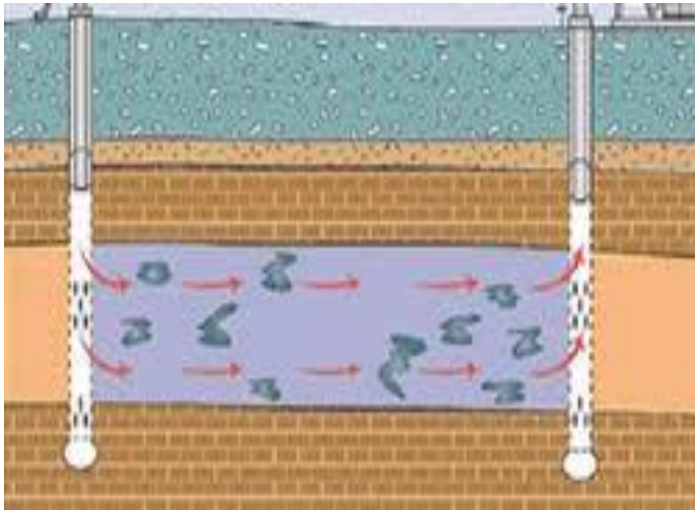
Feed is normally fermented in the rumen to produce hydrogen (H_2) and, importantly, the volatile fatty acids that form a major part of the animal's nutritional needs [green arrows]. Methanogens use hydrogen to form methane (CH_4) which exits the rumen into the atmosphere [red arrows]. When the methanogens are inhibited, the activity of homoacetogens could form volatile fatty acids from the hydrogen instead [blue arrow].

♦ RYR extract has been used in the cattle industry for decades to manage rumen microbiology and control methane production in cows (Henderson *et al*., 2010).

Henderson et al.,2010. Morgavi et al., 2010.

What is Provect-CH4™ Methane Inhibitor / ERD Supplement?

- Proprietary combination of Red Yeast Rice (RYR) extract specially selected for the environmental industry
- Cold water soluble powder that is safe and easy to handle
- Packaged and sold in 55.1 lb (25 kg) drums
- Used as an ERD Supplement; component to ABC-CH4™ and Provect-IR™ and AquaBlok-CH4™
- Multiple patents pending




Chris

Provect-CH4™ Case Study

- Former Dry Cleaner Site near Atlanta, GA
  - Combinations of sodium lactate, ethyl lactate, emulsified oils, and ZVI added in 2004 (3), 2005 (4), 2006 (2) - legal issues and delays - 2013 (1)
  - Residual PCE, TCE and c-DCE concentrations required additional treatment
  - Excessive CH4 production previously noted
- Repeat ABC applications in July, 2014
  - 2,500 lbs (250 USG) ABC added via 3 DPT points proximal to MW-4
  - 2,500 lbs ABC (250 USG) + 37 lb Provect CH4 added via 3 DPT points proximal to MW-207 (targeted 50 to 75 ppm within the PRB zone)

Well Head and Dissolved Gas Analysis (6 weeks post treatment)

Dissolved Gas Analysis (Method RSK 175) = 35% CH4 compared to MW-4

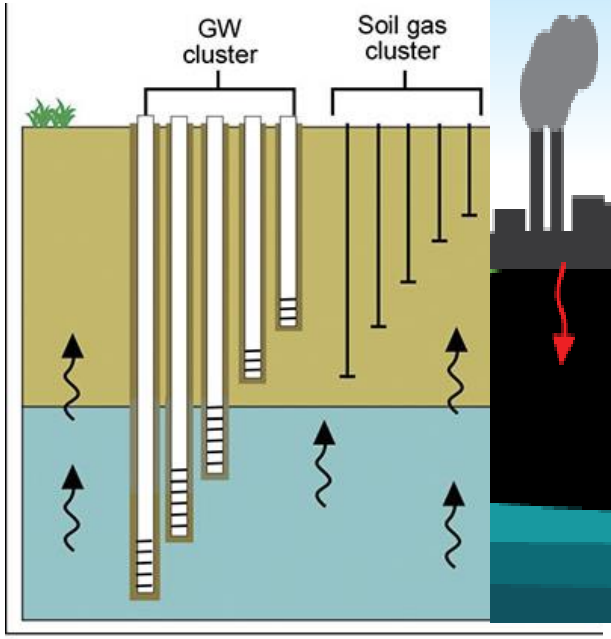
Well Location	Pre-injection (ppm)		6 weeks Post-Injection (ppm)	
	CH4	PCE	CH4	PCE
MW-4	13.7	170	10.2	--
MW-207s	11.8	1,200	4.2	--

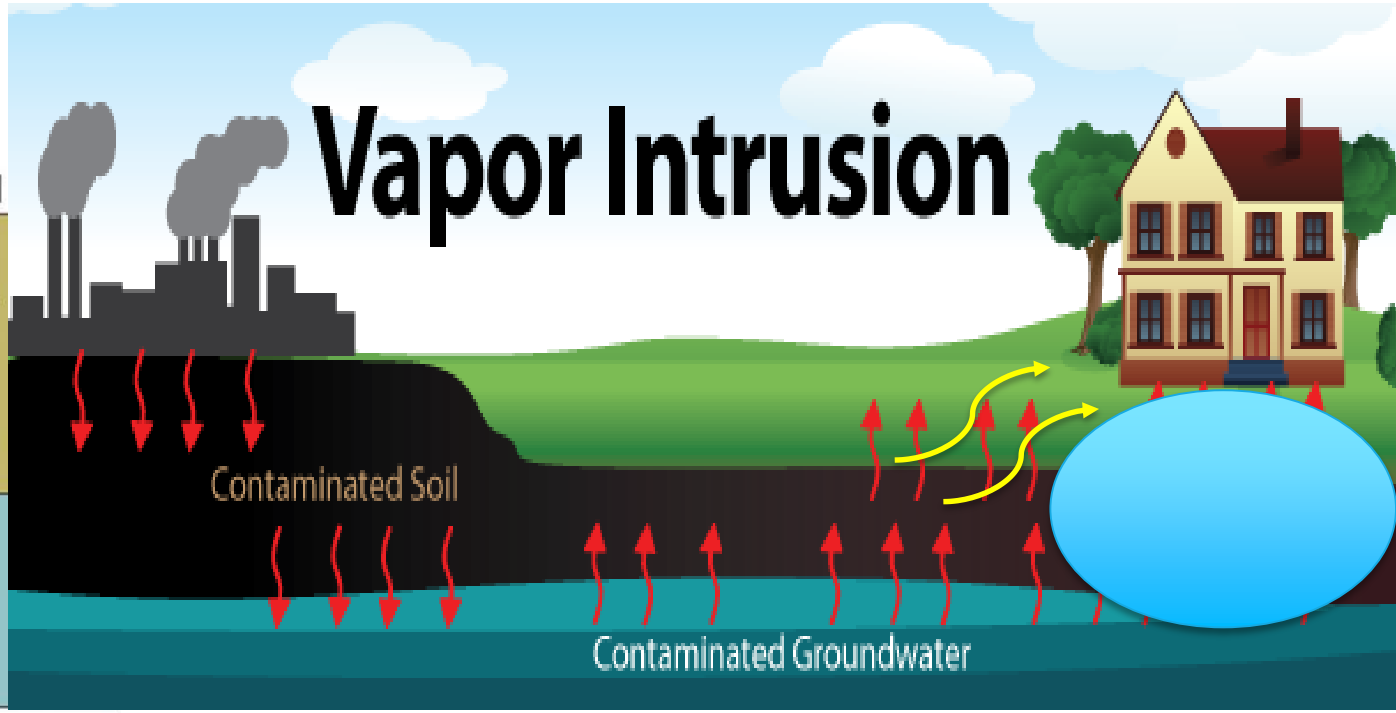
- **Thermo/Foxboro TVA-1000B PID/FID Analyzer (PID sensitive to 2,000 ppm CH4; FID sensitive to 50,000 ppm CH4)**
- **LandTec GEM5000 Landfill Gas (LFG) Meter (infrared detector calibrated to 15% methane**

Well Head Gas Analysis = Provect CH4 = >98% less CH4

Well Location	CH4 PID (ppm)	CH4 FID (ppm)	CH4 TGA %	CO2 %	O2 %	Balance (N) Est. %
MW-4	ABC Only					
0 min	297	>50,000	34.8	65.2	0.0	0.0
5 min	439	>50,000	35.6	61.0	0.2	3.2
MW-207s	Provect CH4 added					
0 min	82	Out of range	0.5	1.0	12.7	85.8
5 min	41	1,599	0.4	0.7	20.2	78.7

CH4 - How to Monitor, Where to Monitor?





Provect-CH4™ What Does it Cost?

Treatment Zone Dimensions		
Width of targeted zone (perpendicular to gw flow)	266	ft
Length of targeted zone (parallel to gw flow)	266	ft
Depth to top of treatment zone	20	ft
Depth to bottom of treatment zone	45	ft
Treatment zone thickness	25	ft
Calculated Volume	1768900	ft3
Methane Inhibitor Calculations		
Estimated Porosity	30	%
Calculated impacted liquid	530,670	ft3
Targeted RYR Conc in GW	50	ppm
Methane Inhibitor for Project	1,656	lbs
ROUND TO 55 lb units (25 kg drums)	30	
Unit Price as ERD Supplement (VOLUME DISCOUNTS APPLY)	$35/lb	1,650 lbs
TOTAL (excludes shipping)	**$57,750**	

- Calculated at least 30% less ERD Amendment required = cost offsets.
  - 90,000 lbs ERD required x $1.50/lb = $135,000
  - Can use 30% less ERD = 67,500 lbs x $1.50/lb = $101,250 (saves $33,750)
  - Requires 1,650 lbs Provect-CH4 x $35/lb = $57,750 → $159,000 (adds $24,000 or *ca*. 15%)
- Avoids need for contingency planning; Safety and Efficacy assurance

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ABC-CH4™ Liquid, Antimethanogenic ERD Reagent

- Provect-CH4 can be used to supplement myriad organic amendments / H donors:
  - Oils
  - Lecithins
  - Emulsified oils
  - Sugars (lactate, dextrose, glucose)
  - Various “hydrogen release compounds
  - Plant-based carbon sources (*e.g.,* cellulose and hemicellulose)
  - Carbon + ZVI conventional ISCR amendments

- Performance and effectiveness of such applications may vary based on myriad parameters

- Proactive v. Reactive: To provide a controlled, engineered product that minimizes these variables, we developed ABC-CH4™ in collaboration with Redox-Tech, LLC (operating since 2000).

What is Anaerobic BioChem (ABC®)?

- 60% Water Soluble Carbon consisting of
  - Glycerin
  - Ethyl Lactate – green solvent
  - Fatty Acids – all dissolved
- Dipotassium phosphate for micronutrients and pH buffering
- Potash or bicarbonate for pH control

REDOX TECH, LLC

"Providing Innovative In Situ Soil and Groundwater Treatment"

What is ABC-CH4™

- Includes Provect-CH4 to achieve min. 50 ppm RYR in GW
- UNIQUELY CONTROLS METHANE PRODUCTION
  - Safer
  - More efficient (can use at least 30% less)

ABC-CH4™ Advantages

- Liquid ERD amendment
- Long lasting (2+ years) but water soluble so large volume of chase water not required
- Lower injection pressures
- Does not require hydrolysis of oils to release fatty acids
- No emulsion breaking potential
- No soap formation from bringing pH up to high
- Demonstrated buffering
- ABC-CH4 is formulated for each site specific application

REDOX TECH, LLC

"Providing Innovative In Situ Soil and Groundwater Treatment"

ABC-CH4™ Application Guidelines

- List price $1.80 / lb + shipping includes methane inhibitor
- VOLUME DISCOUNTS APPLY
- Sold in 5 gallon pails, 55 gallon drums or 275 or 325 gallon totes
- Orders can be routed through Redox-Tech, LLC
  - High permeability requires more ABC with more Oleic acid
  - Not recommended for total chlorinated solvent concentrations less than 50 ppb if not adding DHC
  - ORP less than -175 mV is commonly achieved
  - Loading rates are 0.04 to 0.1 pounds per cubic foot of aquifer (both aqueous and solid phase)
  - Loading rate depends upon ORP and contaminant levels
  - DKP and buffer depends upon contaminant levels and background pH
  - DHC added about 5% of the time

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  - ERD Plus 1,650 lbs Provect-CH4 x $35/lb = $57,750 → $159,000 (adds $24,000 or *ca*. 15%)
  - 67,500 lbs ABC-CH4 x $1.80/lb = $121,500 **($13,500 less than ERD alone)**
- Avoids need for contingency planning; Safety and Efficacy assurance

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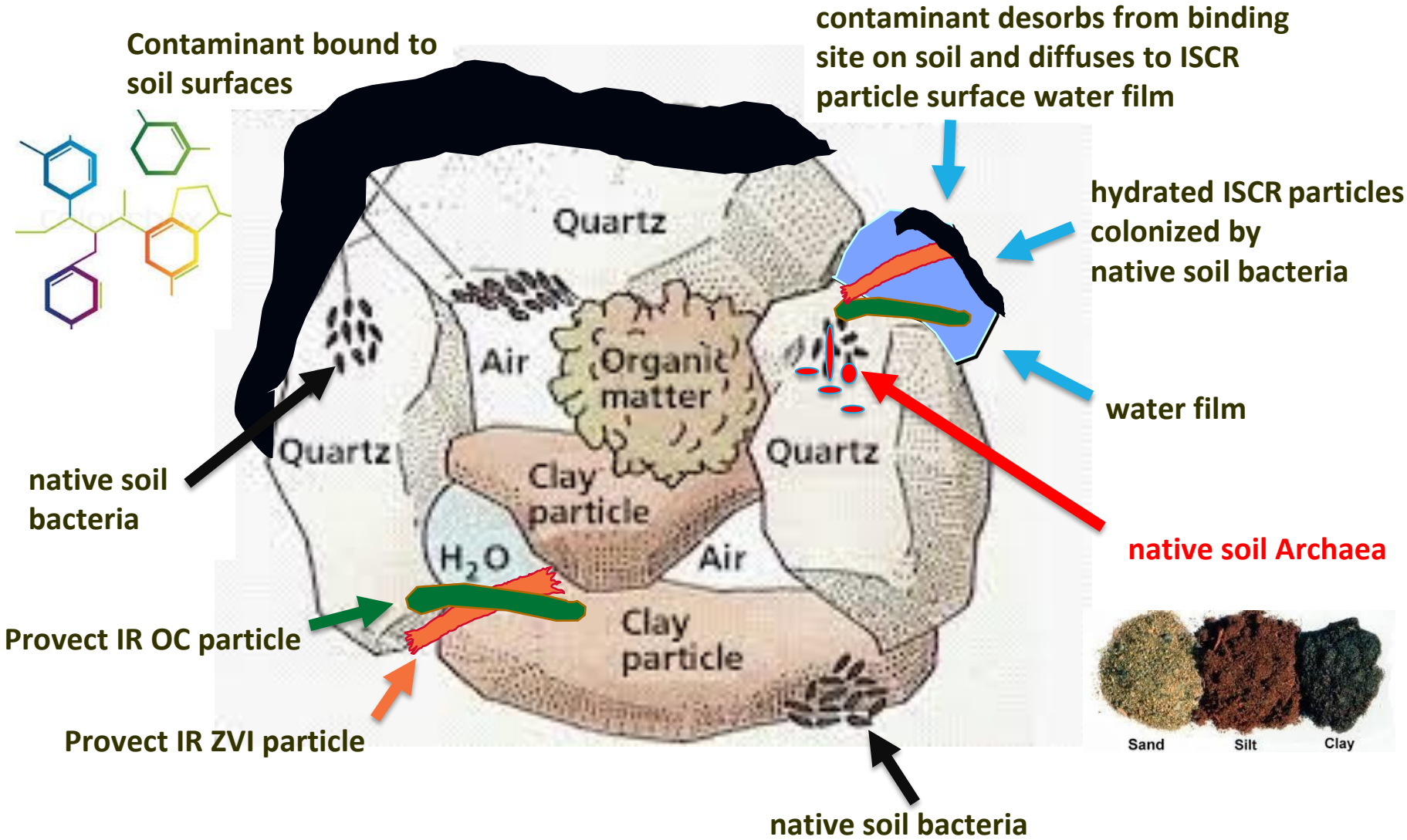
What is *In Situ* Chemical Reduction (ISCR)

- In 2004, ISCR was defined as "a synergistic process that combines biotic + abiotic reactions and creates highly reducing, electron-rich conditions" (Mueller and Brown, 2004)
- ISCR is **not** enhanced anaerobic bioremediation/ERD

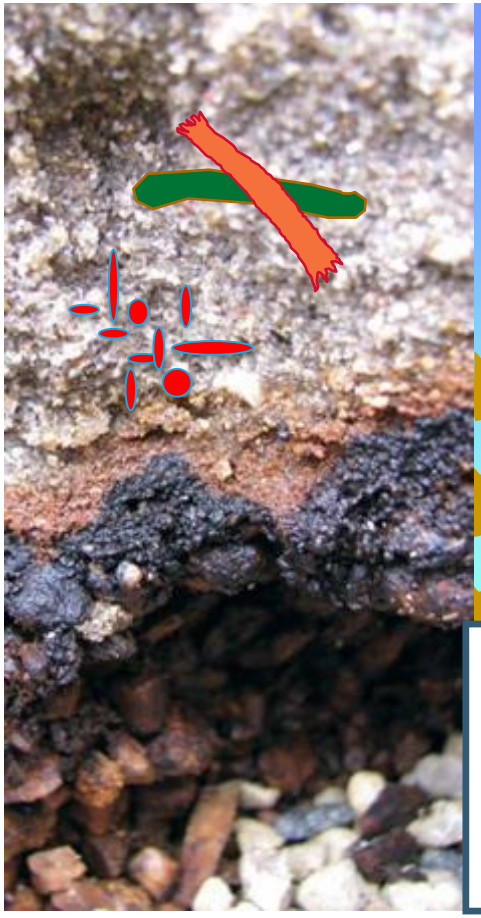
Process	Amendments
Enhanced Anaerobic Degradation / ERD	**Molasses, emulsified vegetable oils, sodium lactate, polylactic acid, whey, simple H release compounds, ABC**
***In Situ* Chemical Reduction / ISCR**	**Provect-IR™, ABC+, EHC®, DARAMEND®**
Antimethanogenic ISCR Reagents	**Provect-IR®, Provect-IRM®, Aquablok®-CH4, and to some degree ABC-CH4™**

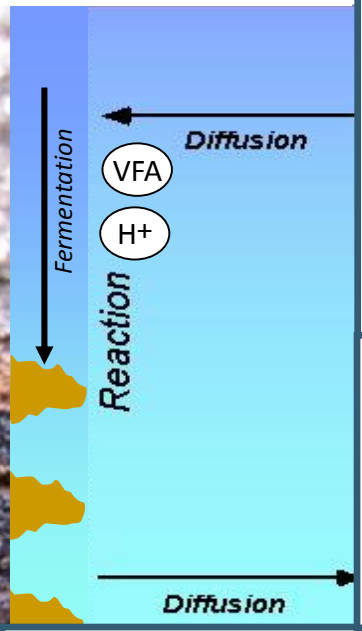
Conventional ISCR Reagents (*ca.* 2003)



Mueller et al, 2003; 2004; 2005; 2006 etc.

Carbon Fermentation + ZVI Corrosion = ISCR Multiple Reaction Mechanisms





Production of organic acids (VFAs): electron donors for reduction of COIs, O_2, NO_3, SO_4

- By preventing basification, reduces precipitate formation on ZVI surfaces to increase rate of iron corrosion /H_2 generation / reactivity

ZVI Reactions: H_2 and Fe^{+2} and generation

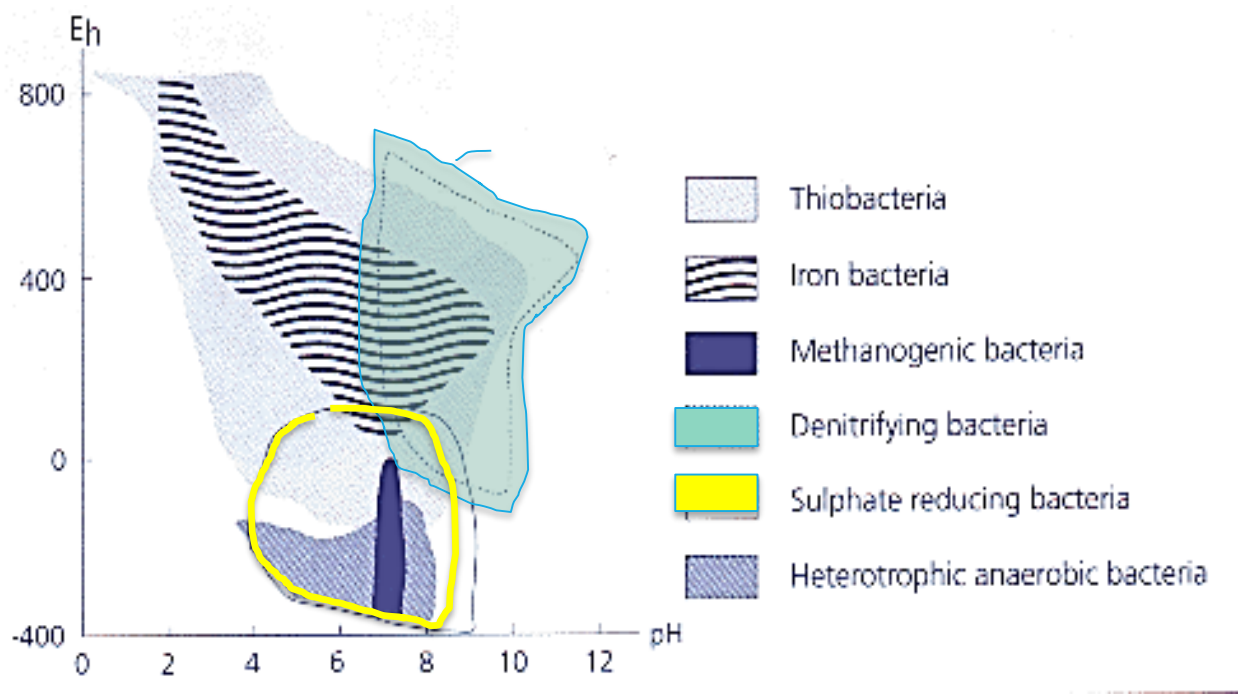
$$Fe^0 \rightarrow Fe^{2+} + 2e^-$$
$$2H_2O \rightarrow 2H^+ + 2OH^-$$
$$2H^+ + 2e^- \rightarrow H_{2(gas)}$$
$$R\text{-}Cl + H^+ + 2e^- \rightarrow R\text{-}H + Cl^-$$

ISCR = thermodynamic conditions for dechlorination:

- Combined oxygen consumption from carbon fermentation and iron oxidation → Strongly reduced environment (-250 to -500 mV)
- High electron/H^+ pressure

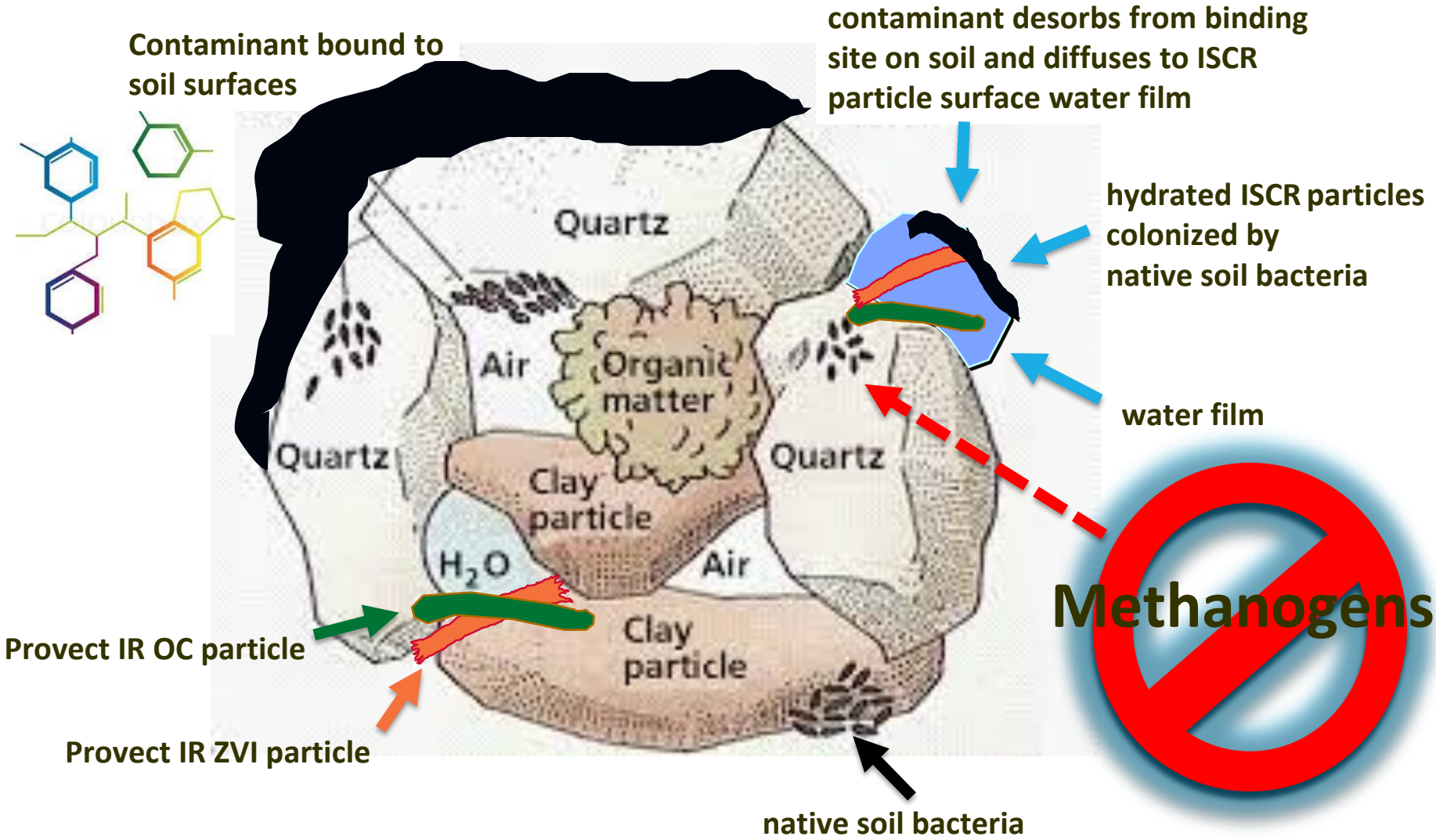
Idealized Eh pH Ranges for Microbial Growth

Microbe	Doubling Times
Dehalococcoides spp.	24 to 48 hours
Methanogens with cytochromes	10 hours
Methanogens without cytochromes	1 hour



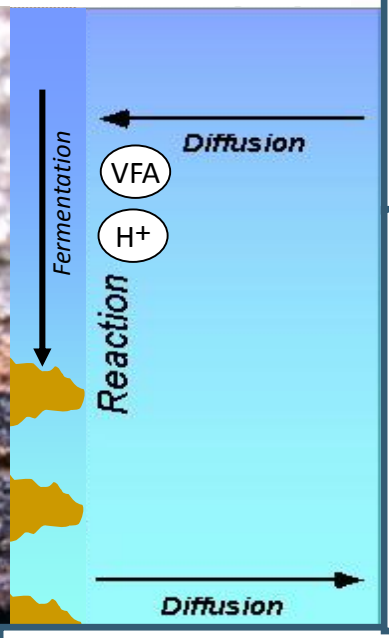
Zajic, 1969. Sigma Aldrich

Provect-IR™ Solid, Antimethanogenic ISCR Reagent



Mueller et al, 2014a, 2014b

Potential Benefits of Antimethanogenic ERD/ISCR Reagents



More Efficient:

- Calculated minimum 30% more efficient use of hydrogen donor (Mueller *et al*, 2014)
- Allows for slower-growing acetogens and DHC-type microbes an opportunity to compete

Safer Remedial Actions:

- Elevated methane concentrations (>1,000 ppm) can exceed current and pending regulations of < 10 to <28 ppm in groundwater and/or 0.5% v/v methane in soil gas (*e.g.*, 10% of the LEL) and/or indoor air regulations (methane is flammable between 5 and 15% v/v)

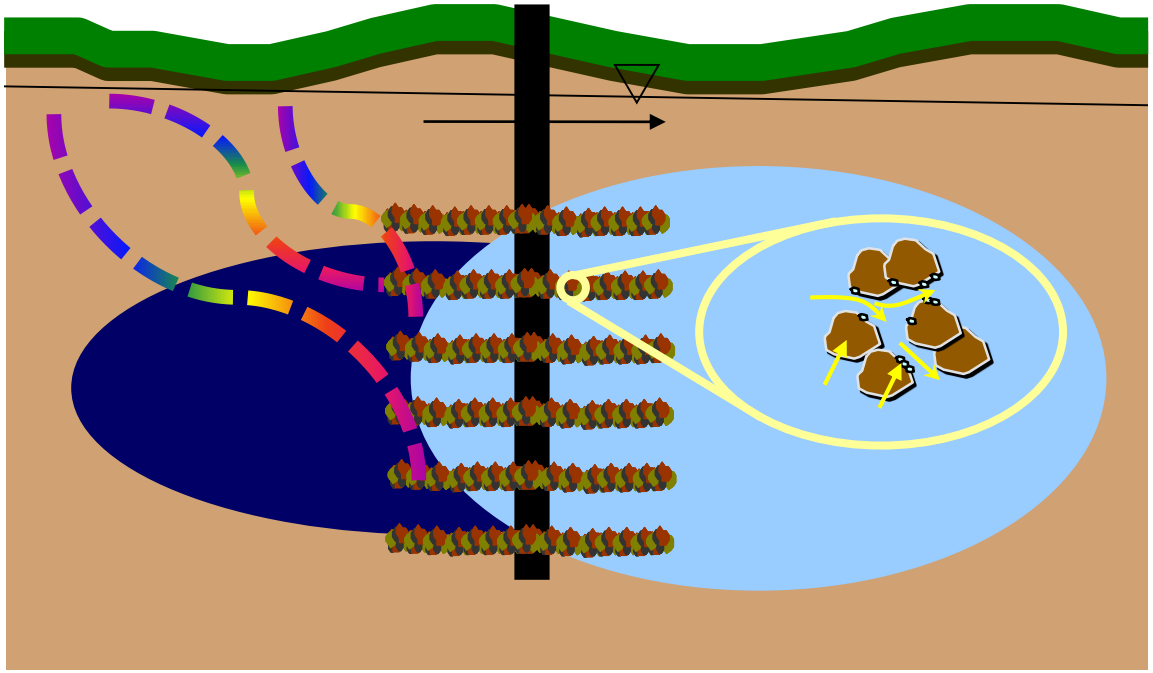
Other Potential Benefits:

- Avoids regulatory reporting (DOI?) and Contingency planning (AS/SVE gas)
- Minimizes potential for methylation of Hg and other heavy metals
- Minimizes potential for secondary COI issues

Provect-IR™ Solid, Antimethanogenic ISCR Reagent

- Multiple, Complex, Hydrophilic, Timed-Release organic carbon source (plant materials, Kelp, Ca Propionate) @ 390 g H donor / lb product
- 10% (wgt) Small (ave.10 µm) ZVI particles = 25 ft surface area / lb
- Integrated Vitamins, minerals and nutrients (yeast extract) specially selected for anaerobes
- Chemical oxygen scavenger to maintain ZVI
- Package in 50 lb bags or 2,000 lb supersacs.

Subsurface Injection of Slurried Solids



Figures adapted from Mueller et al, 2012 Redox-Tech's Third Biennial Southeastern In Situ Soil and Groundwater Remediation Conference

Provect-IR™ What Does it Cost?

Component / Feature	Provect-IR™	Others
Multiple, Complex, Mixed-Release Organic Hydrogen Donors, namely Kelp plus specially selected organic plant matter and readily fermentable proprionate	YES	NO
Integrated Methanogenic Inhibitor (patents pending)	YES	NO
Chemical Oxygen Scavenger to maintain reduced ZVI during Mixing and Injection	YES	NO
Integrated Vitamins, Micro-Nutrients and Macro-Nutrients selected for Enrichment of Indigenous Anaerobes, namely yeast extract that contains nitrogenous compounds, carbon, sulfur, trace nutrients, vitamin B complex and other important growth factors, which are essential for the growth of diverse microorganism	YES	NO
Calcium Propionate Primer for Enrichment of Indigenous Anaerobes	YES	NO
Modified formulations for heavy metal immobilization	YES	YES
ZVI Component Average Particle Size (micron) current values assumed = not confirmed by vendors	15	100 75 50
% by weight ZVI	10	40
Square Feet of ZVI Surface per Pound of Product -Displayed values are based on estimated micron average size	25	15 19 30
Grams of Organic Hydrogen Donor/ Pound of Product	390	272
Price Ranges * based on information found in published documents (non confidential, public, readily available)	$1.50 to $2.35/lb	<$2.00 to $2.65/lb

Provect-IR™ How Does it Compare?

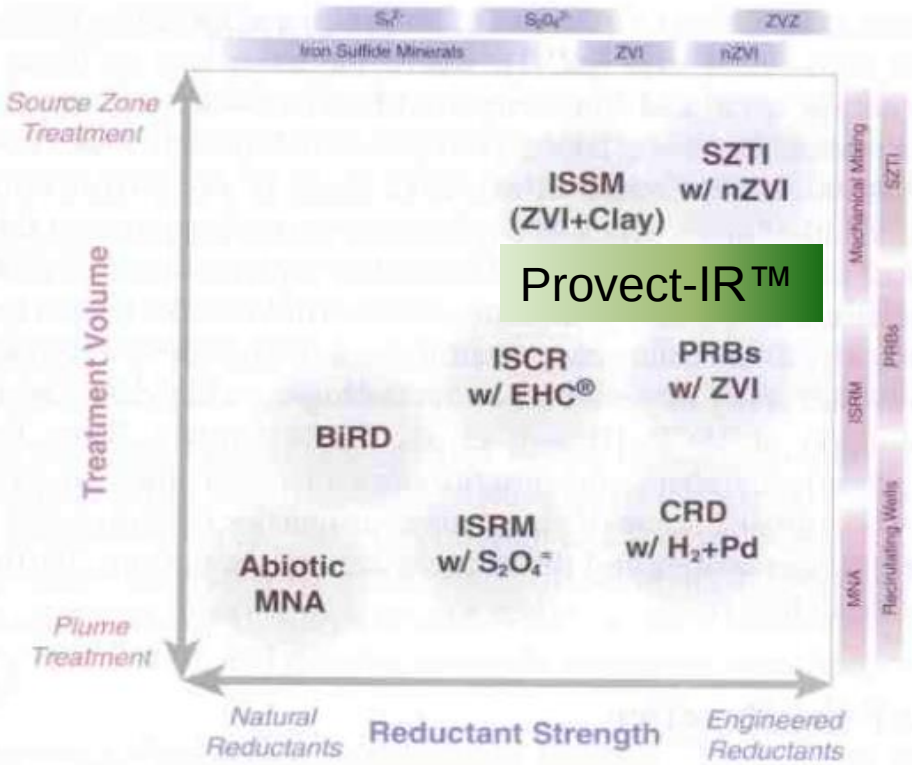


Figure 10.1. Map of ISCR technologies in current practice. The *horizontal dimension* represents the continuum from naturally occurring and/or mild reductants (Fe^{II} and $S^{II-/I-}$ containing minerals) to the generally strong chemical reductants used in fully engineered systems (Fe^0 and Zn^0). The *vertical dimension* represents the various modes of application, from emplacement of reductants to intercept plumes (PRBs) to injection of reductants that target the source zone (nZVI). Acronyms used to identify the specific technologies are defined in the following subsections.

Modified from Tratnyek et al., 2014

Provect-IRM™ Antimethanogenic ISCR Reagent for Heavy Metal Stabilization

Contaminant	Treatment Mechanisms in the ISCR zone
As (III, V)	Reductive precipitation with oxidized iron minerals. Precipitation as As sulfide and mixed Fe-As sulfide
Cr(VI), Mo(VI), Se(IV,VI), U(VI)	Reductive precipitation with oxidized iron minerals and adsorption to iron oxides.
Me^{2+} (Cu, Zn, Pb, Cd, Ni)	Metal cations precipitate as sulfides, following stimulated heterotrophic microbial sulfate reduction to sulfide. Adsorption to iron corrosion products (e.g.; iron oxides and oxyhydroxides).

Provect-IRM Inhibits Metal Biomethylation

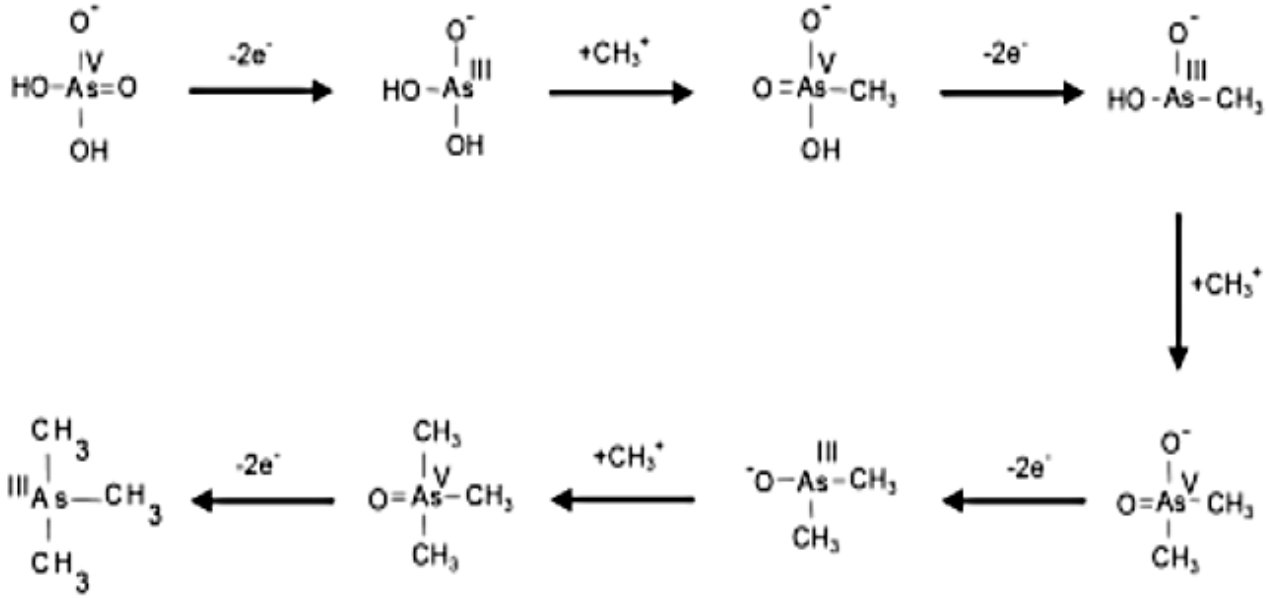
- With the possible exception of Pb almost all Group IV, V and VI elements can be biomethylated (Bentley and Chasteen, 2002).
- Methylmetal(loids) are usually volatile and more toxic than their inorganic counterparts due to increased water solubility and hydrophobicity (*e.g.,* methylmercury).
- Microorganisms are primarily responsible for the biosynthesis of organo-metals (Challenger, 1945), and the activity of methanogens is a main source of their production (Michalke, *et al*., 2006).

Volatile Methylmetal(loids) produced by Growing Cultures of Methanogens (Archaea).

	Metal/metalloid					
	As	Bi	Se	Te	Sb	Reference
Methanobacterium formicicum	AsH_3, CH_3AsH_2, $(CH_3)_2AsH$, $(CH_3)_3As$, X	BiH_3, CH_3BiH_2, $(CH_3)_2BiH$, $(CH_3)_3Bi$	$(CH_3)_2Se$, $(CH_3)_2Se_2$	$(CH_3)_2Te$	SbH_3, CH_3SbH_2, $(CH_3)_2SbH$, $(CH_3)_3Sb$	Michalke et al., 2000
Methanobrevibacter smithii	CH_3AsH_2, $(CH_3)_2AsH$, $(CH_3)_3As$	CH_3BiH_2, $(CH_3)_2BiH$, $(CH_3)_3Bi$	$(CH_3)_2Se$, $(CH_3)_2SeS$, $(CH_3)_2Se_2$, X	$(CH_3)_2Te$	$(CH_3)_3Sb$	This study
Methanococcus vanielli	CH_3AsH_2, $(CH_3)_2AsH$, $(CH_3)_3As$, X	CH_3BiH_2, $(CH_3)_3Bi$	$(CH_3)_2Se$, $(CH_3)_2SeS$	$(CH_3)_2Te$	$(CH_3)_3Sb$	This study
Methanolacinia paynteri	n.d.	$(CH_3)_2BiH$, $(CH_3)_3Bi$	$(CH_3)_2Se$, $(CH_3)_2SeS$, $(CH_3)_2Se_2$, X	$(CH_3)_2Te$	$(CH_3)_3Sb$	This study
Methanolobus tindarius	n.d.	$(CH_3)_3Bi$	$(CH_3)_2Se$, X	$(CH_3)_2Te$	CH_3SbH_2, $(CH_3)_3Sb$	This study
Methanoplanus limicola	$(CH_3)_3As$	$(CH_3)_3Bi$	$(CH_3)_2Se$, $(CH_3)_2SeS$, $(CH_3)_2Se_2$, X	$(CH_3)_2Te$, X	$(CH_3)_3Sb$	This study
Methanosarcina barkeri	AsH_3, X	$(CH_3)_3Bi^*$	$(CH_3)_2Se$, $(CH_3)_2Se_2$	n.d.	$(CH_3)_3Sb$	Michalke et al., 2000
Methanosarcina mazei	$(CH_3)_3As$	$(CH_3)_3Bi$	$(CH_3)_2Se$, $(CH_3)_2Se_2$	$(CH_3)_2Te$	$(CH_3)_3Sb$	This study
Methanosphaera stadtmanae	$(CH_3)_2AsH$, $(CH_3)_3As$	CH_3BiH_2, $(CH_3)_2BiH$, $(CH_3)_3Bi$	$(CH_3)_2Se$, $(CH_3)_2SeS$, $(CH_3)_2Se_2$, X	$(CH_3)_2Te$	$(CH_3)_3Sb$	This study
Methanothermobacter thermautotrophicus	AsH_3	n.d.	n.d.	n.d.	$(CH_3)_3Sb$	Michalke et al., 2000

Provect-IRM™ Safer, More Effective

- Provect-IRM limits the number and activity of methanogens hence the targeted metal contaminants are more able to participate in the desired stabilization reactions.
- Moreover, the overall toxicity of the site is not increased via the generation of methylmetal(loids) as a consequence of the treatment process (example – biomethylation of arsenate).

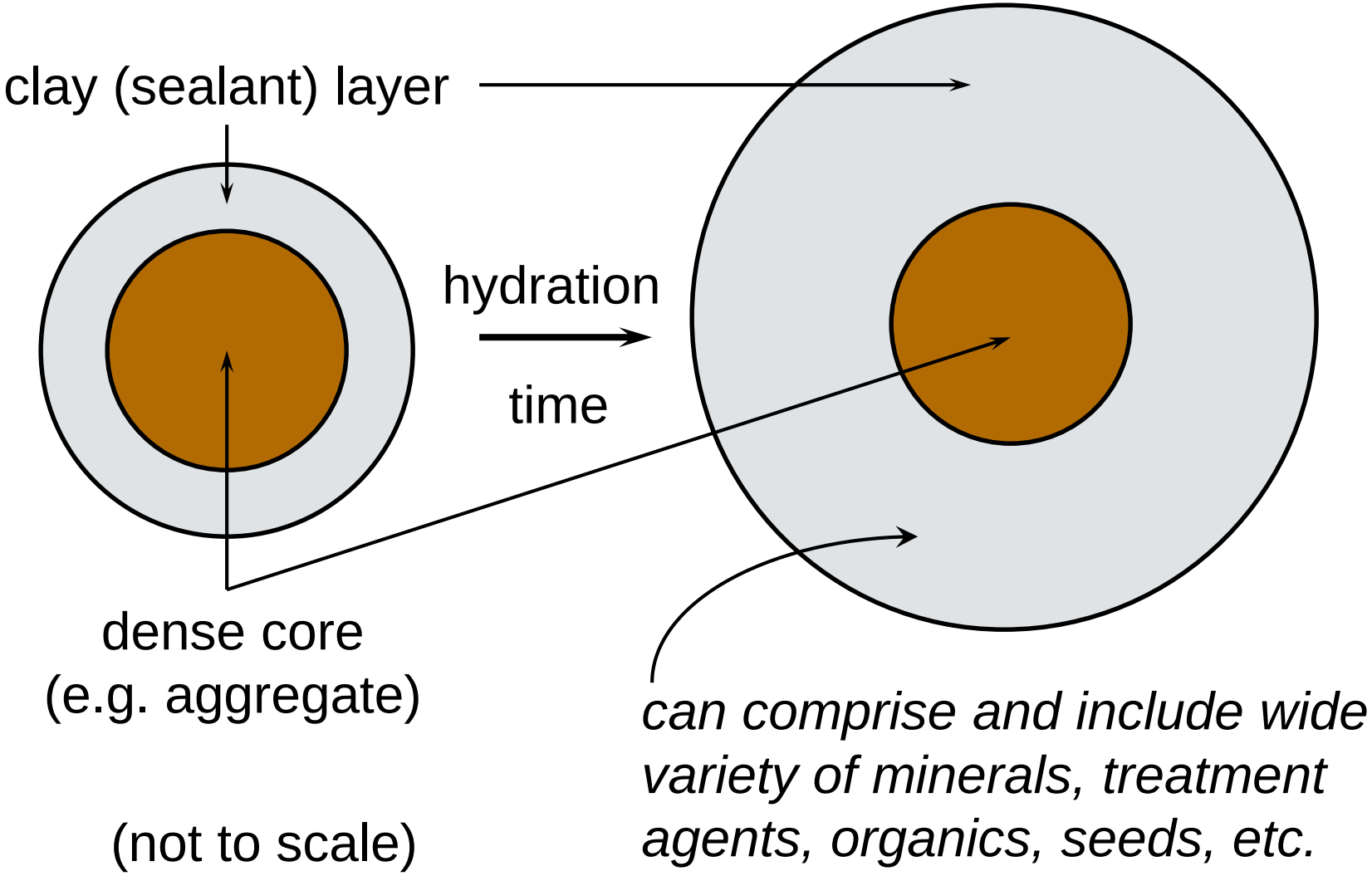


Challenger mechanisms for biosynthesis or Arsenate (Challenger, 1945)

Presentation Outline

- Introduction to Provectus Environmental Products, Inc.
- Environmental Biotechnologies
  - Provect-CH4™ Methane Inhibitor
  - ABC-CH4™ Liquid, Antimethanogenic ERD Reagent
  - Provect-IR™ Solid, Antimethanogenic ISCR Reagent
  - Provect-IRM™ Antimethanogenic ISCR/Heavy Metal Immobilization Reagent
  - **AquaBlok-CH4™ Antimethanogenic Reactive Cap**
  - Provect-OX™ Self-Activating ISCO / Enhanced Bioremediation Reagent
- ISCO or ISCR?
- Services and Offerings

AquaBlok-CH4™ Antimethanogenic *In Situ* Capping Technology



Basic Product Behavior in Water

AquaBlok Placement Methods

Evidence that Recontamination is Not Due to Flux Through the Cap

Split-core from Section A (2.5 yrs after placement)

New sediment Deposits

AquaBlok Clean Cap Layer

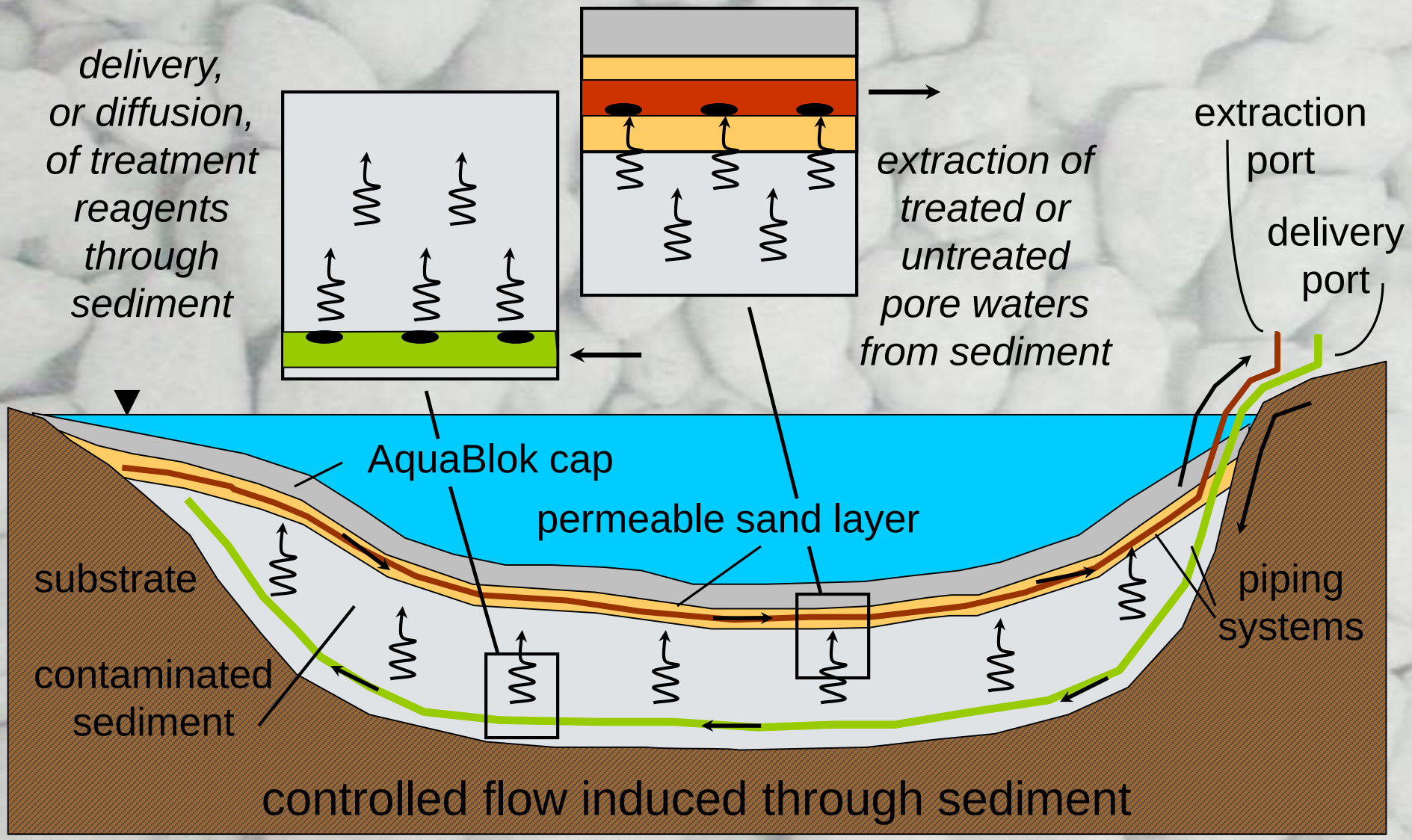
Discrete boundary

Contaminated Sediment

Issues with Methane Generation

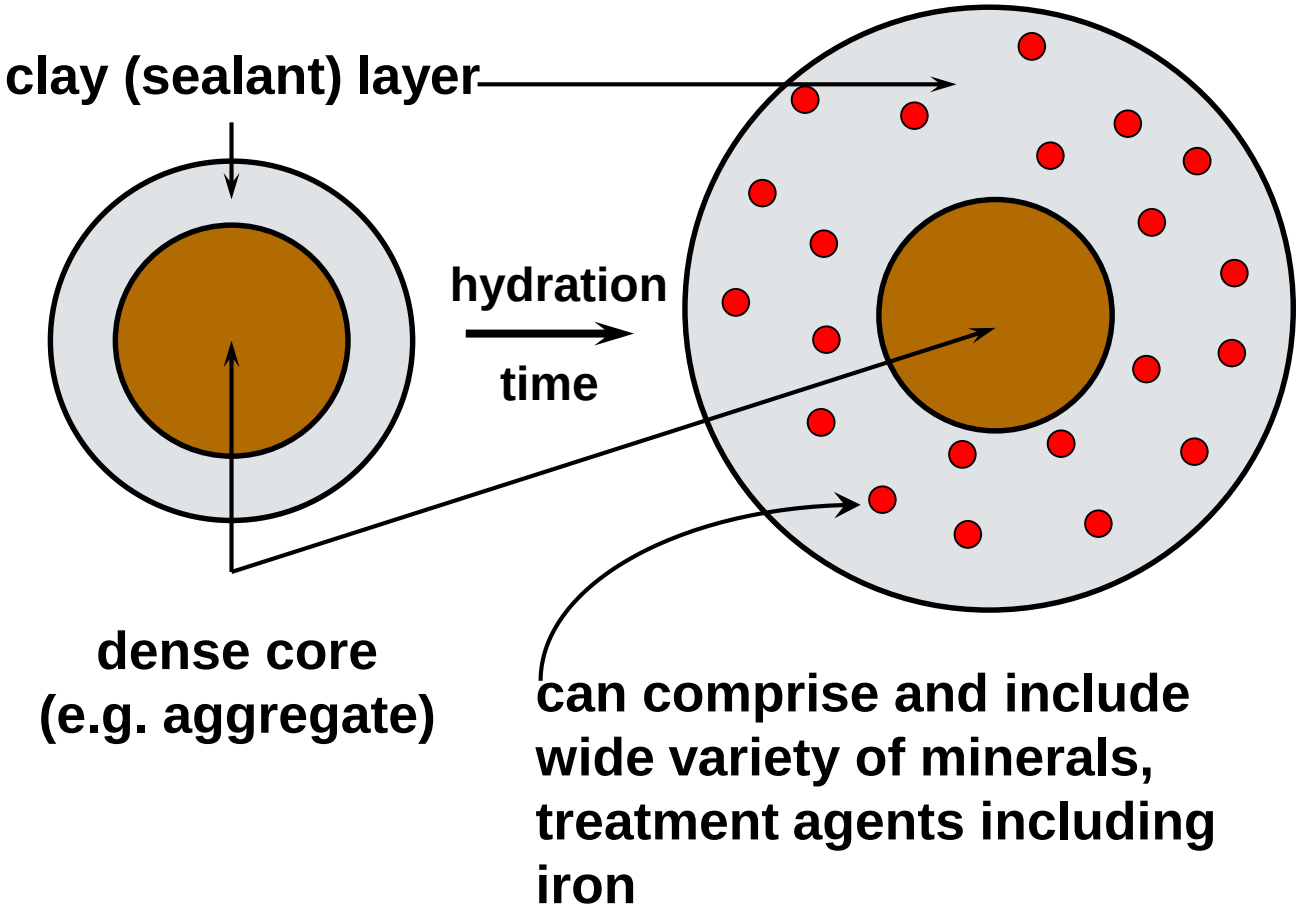
- Typically there is a short term stimulation of methanogenic /microbial activity as a result of disturbing sediments, etc
- Methane gas ebullition causes cap breaching and induced migration = sheen
- Can result in the generation of methylmetal(loids)

Facilitating *In Situ* Sediment Treatment



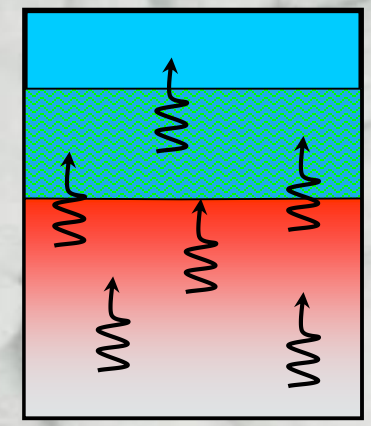
(not to scale)

AquaBlok-CH4™ Particle

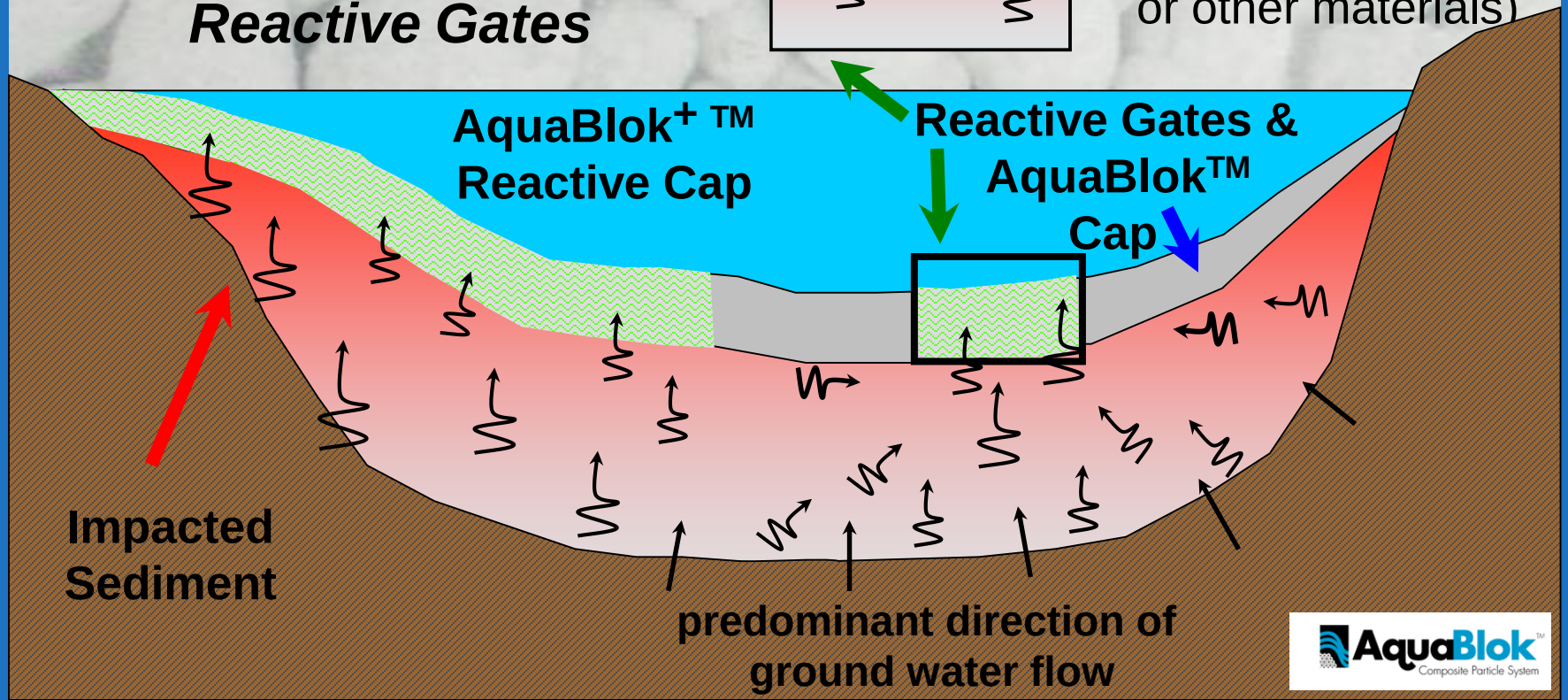


Horizontal Reactive Barrier (hPRB) for *In Situ* Sediment Treatment

Contiguous Reactive Cap or funneling *of contaminant-bearing sediment pore waters beneath low-permeability cap through* ***Reactive Gates***

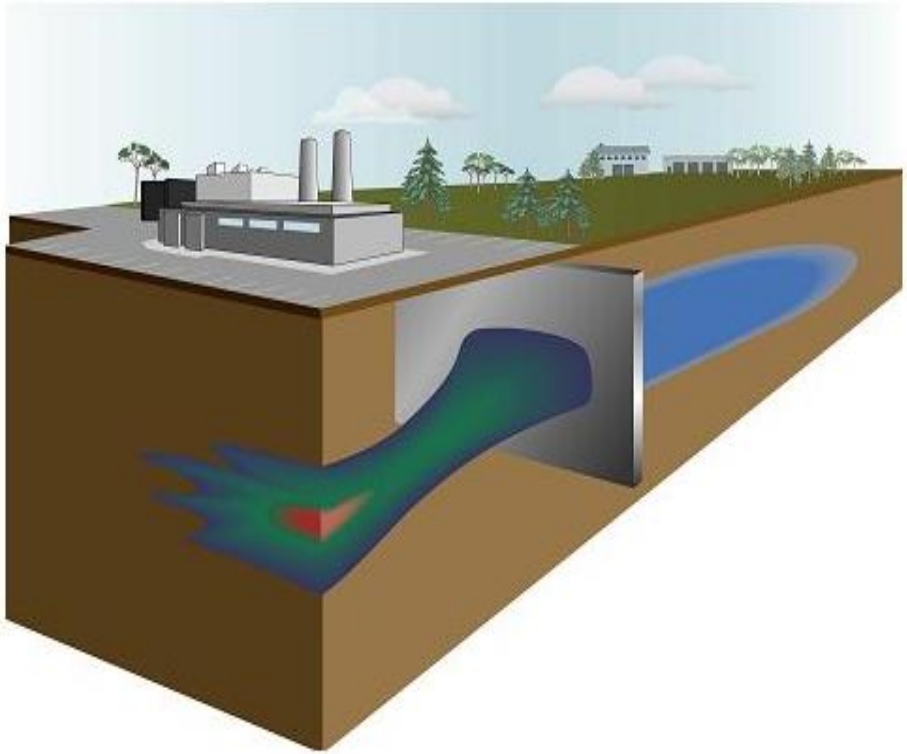


higher-permeability treatment "**gates**" (includes reactive medium, ZVI, buffering agents, microbes, or other materials)

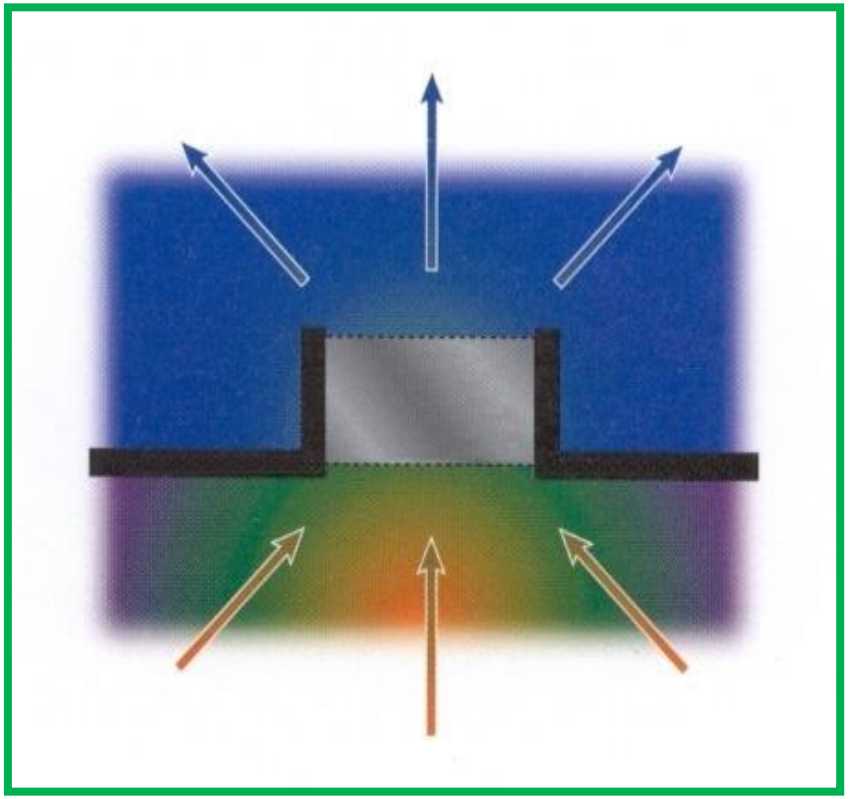


Vertical PRB Configurations

Continuous PRB



Funnel and Gate



- Mitigates ebullition issues
- Reduces potential for methylation of metals (Hg)

Presentation Outline

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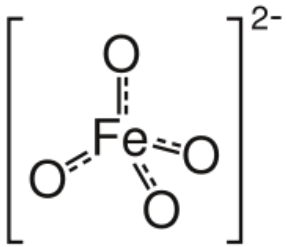
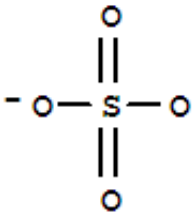
  - Provect-IRM™ Antimethanogenic ISCR/Heavy Metal Immobilization Reagent
  - AquaBlok-CH4™ Antimethanogenic Reactive Cap
  - **Provect-OX™ Self-Activating ISCO / Enhanced Bioremediation Reagent**
- ISCO or ISCR?
- Services and Offerings

What is Provect-OX™?

- Sodium Persulfate + Ferric Oxide ISCO Reagent
  - Chemical Oxidation via Sulfate (SO4•) Radical
  - Chemical Oxidation via Ferrate (Fe6+•) Radical
- Enhances Biological Attenuation via Sulfate and Iron Reduction Processes
- Terminating Reaction Results in Pyrite: An Abiotic Reactive Particle with similar Kinetics to ZVI (BiRD / Pseudo-ISCR)
- Easily Transitions from Oxidation to Biological Attenuation to Abiotic Mineralization
- Safely Handled Catalyzed Process without the Hazards of Extreme Activators Caustics





Provect-Ox™
Coupled Oxidation / Bioremediation

- $1.80 / lb includes activator

- >$1.50 / lb persulfate only

- Safely Handled
- Can be all in one bag
- Uses Fe^{+3} as activator
- Conserves Oxidant
- Formation of Reactive Ferrate Species
- Enhances Bio attenuation
- Encourages the Formation of Pyrite
- Prevents H2S Formation
- Utilizes both iron and sulfate reducing bio processes

- When Chelated Fe^{+2} used as activator EDTA Consumes the Oxidant
- When Caustic is used as activator
  - Short Lived Reaction
  - H_2S Formed
  - Secondary plumes / metals
  - Can generate extreme heat
  - Handling and Safety issues

Does Not Promote Bioattenuation

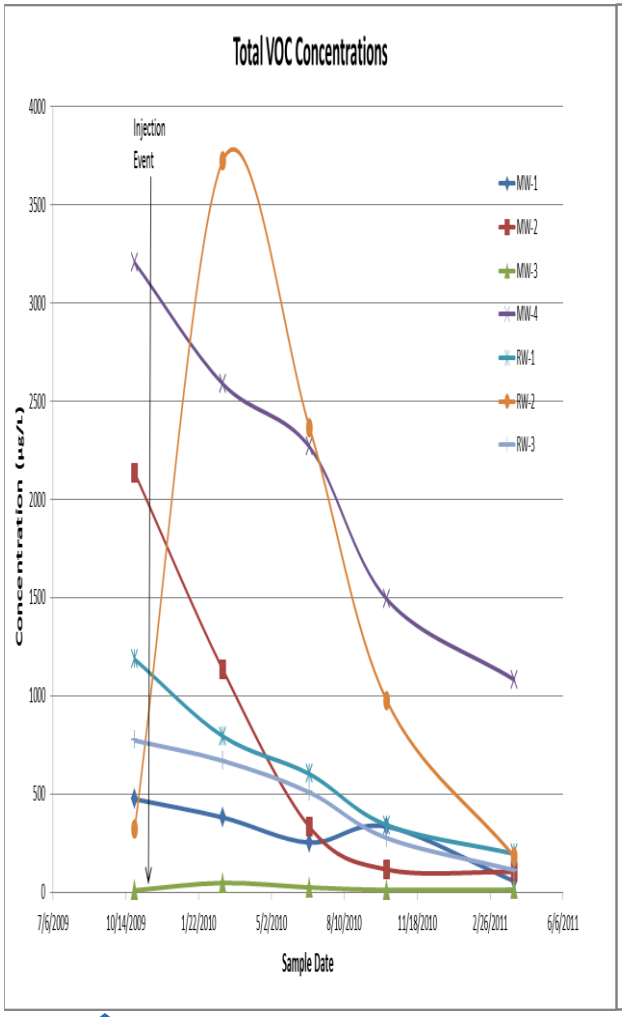
Provect-Ox™ Field Case Study – NJ Site

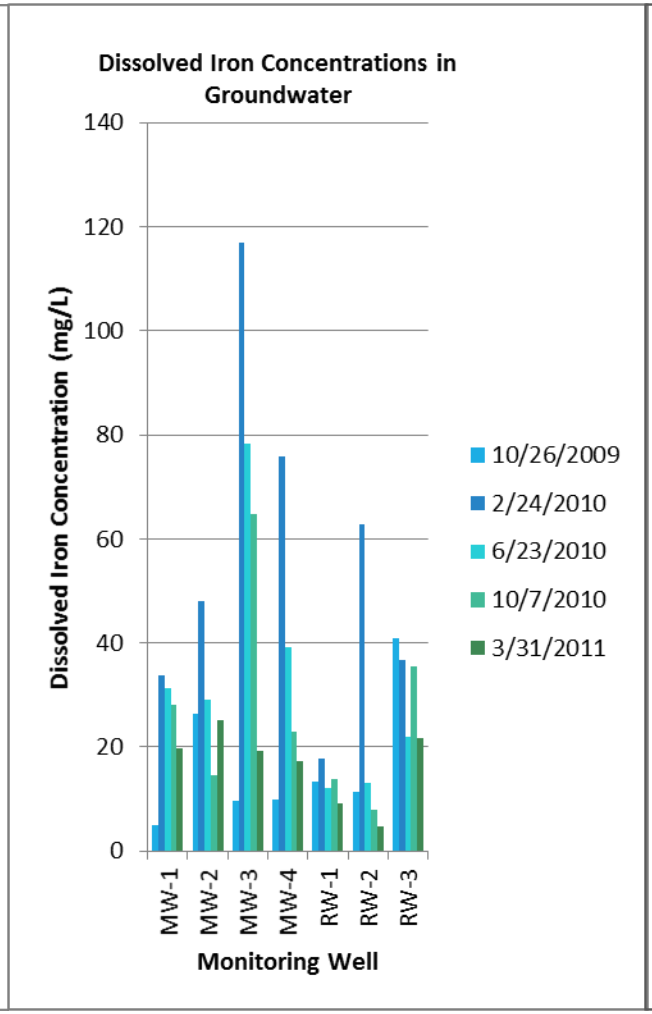
- 4,392 lbs injected
- 75 usg / 35 dpt
- <$8,000 material cost

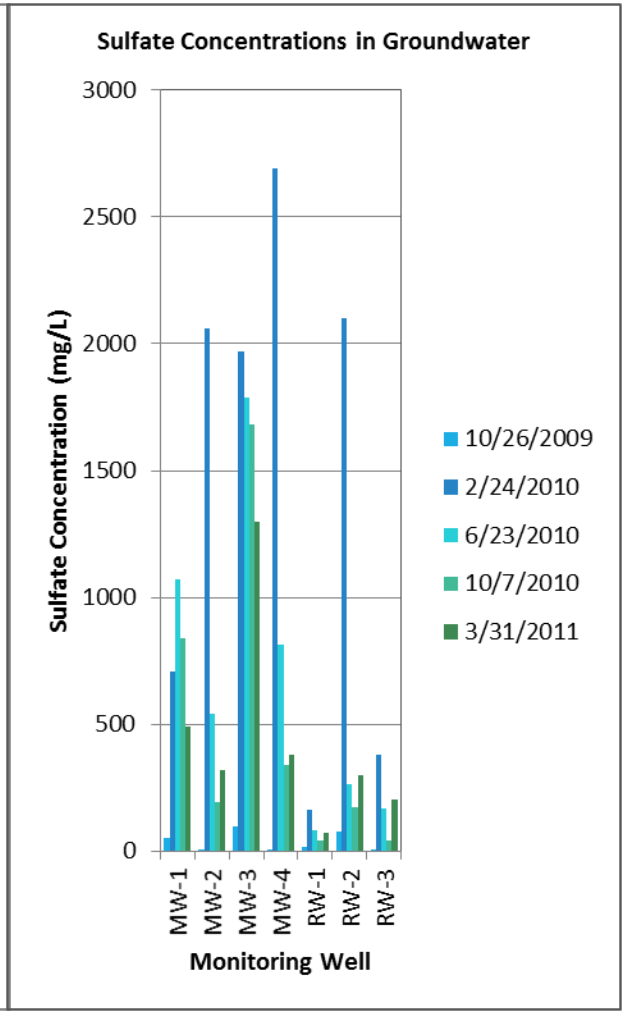
	MW-2				
Sampling Date	06/20/2013	10/02/2013	11/26/2013	02/28/2014	05/28/2014
pH	7.27	6.88	6.89	6.86	7.43
ORP (mV)	-14	+220	+86	+55	+292
D.O. (mg/L)	2.17	0.76	0.90	0.85	0.83
Conductivity (mS/cm)	0.97	3.44	1.52	2.38	1.55
Temperature (°C)	17.7	20.4	17.0	12.2	14.3
Groundwater Elevation (ft)	94.05	90.43	88.86	92.93	93.70
Sulfate (mg/L)	56.6	1,510	266	980	332
Total Iron (mg/L)	0.377	2.01	0.149	0.089	0.160
Dissolved Iron (mg/L)	0.249	1.83	0.0097	ND	ND
Benzene (ppb)	ND	7.20	43.4	ND	ND
Toluene (ppb)	ND	2.33	0.38	ND	ND
Ethylbenzene (ppb)	ND	4.08	ND	ND	ND
Total Xylenes (ppb)	ND	21.04	3.16	ND	ND

Provect-Ox™ Field Case Study

Demonstrated Sulfate and Iron Utilization as Terminal Electron Acceptors







Oxidation OR Reduction, How to Decide

Is in-situ chemical oxidation or in-situ chemical reduction the best option for your site? What factors should be considered to make such a decision?

By **Jim Mueller, Ph.D.,** The Adventus Group, and **Dick Brown, Ph.D.,** ERM

Many chlorinated hydrocarbon compounds (CHCs) can be degraded via oxidative or reductive processes.[1] Accordingly, various in-situ chemical oxidation (ISCO) technologies using oxidizing agents such as hydrogen peroxide (Fenton's chemistry), permanganate,[2] ozone or activated persulfate[3], have been developed to remediate impacted environments. Each of these oxidants and their activators offer unique features, and they can be very effective on a varying range of CHCs.

Variations on the ISCO theme such as in-situ stabilization using modified permanganates[4,5] or surfactant-assisted oxidation[6,7] offer potential enhancements for managing known source areas containing phase-separated hydrocarbon, or non-aqueous phase liquids.

Depending on the site-specific conditions, however, in-situ chemical reduction (ISCR) may represent a more effective remedial strategy (See **Figure 1**). ISCR has two main branches, zero-valent iron and dual-valent iron. An ISCR approach using a combination of zero-valent iron and controlled release carbon can generate environmental conditions that facilitate the chemical reduction of various contaminants.[8,9] The salient characteristics

COIs Treated by Technology

ISCR

- **Chlorinated Solvents**
  - PCE, TCE, cDCE, 11-DCE, VC
  - 1122-TeCA, 111-TCA, 12-DCA
  - CT, CF, DCM, CM
- **Pesticides**
  - Toxaphene, Chlordane, Dieldrin, Pentachlorophenol
- **Energetics**
  - TNT, DNT, RDX, HMX, Perchlorate
- **Heavy Metals including**
  - As, Cr, Pb, Zn, Cd, Ni

ISCO

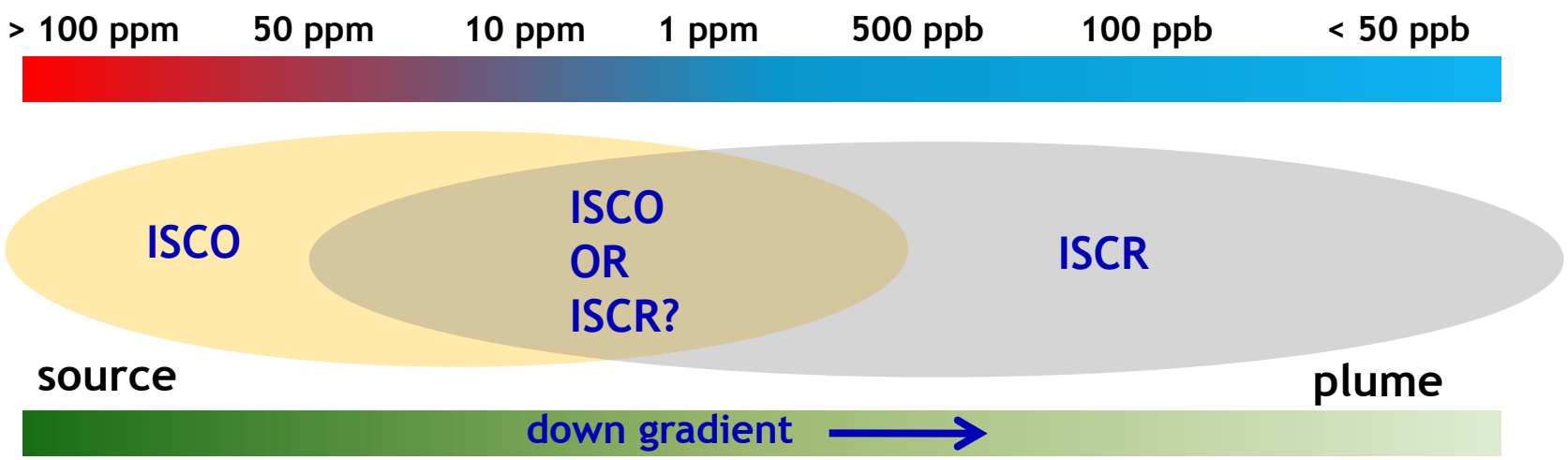
- **Chlorinated Solvents**
  - PCE, TCE, cDCE, 11-DCE, VC
  - 1122-TeCA, 111-TCA, 12-DCA
  - CT, CF, DCM, CM
- **Pesticides**
  - Chlordane, Dieldrin, Pentachlorophenol
- **Energetics**
  - TNT, DNT, RDX, HMX, Perchlorate
- **Petroleum Hydrocarbons**
  - BTEX, TPH, Diesel

Contaminant Type

COIs	ISCO	ISCR	
	Provect-OX	ZVI	Provect-IR Provect-IRM
Chlorinated Ethenes	Yes	Yes	
Chlorinated Ethanes Chlorinated Methanes	Yes	Not 1,2-DCA, CA, DCM, CM	Yes
OC Pesticides	Yes	Yes	
Organic Explosives	Yes	Yes	
TPH GRO and DRO	Yes	No	
Mixed CVOCs and TPH	Yes	No	
Metals (Cr, Pb, Cu, As, Ni)	Emerging	Yes	
Mixed CVOCs and Metals	Emerging	Yes	
Mixed TPH and Metals	Sequential Treatment		

Battelle, 2010, 2011. Pollution Engineering News, 2008. FRC, 2012

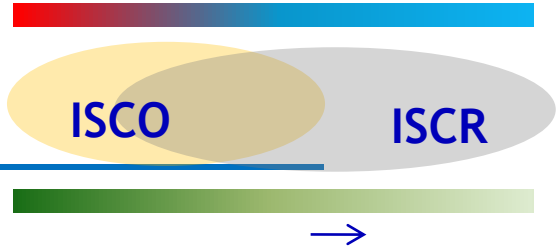
Contaminant Concentration



Media	COI Concentrations	ISCO	ISCR
Soil	Up to 10 mg/kg	Yes	Yes
	> 10 mg/kg	Yes	Maybe
	NAPL	Yes	No*
Groundwater	ppb Level <1,000 ppb	No**	Yes
	ppm Level 1 ppm to 100 ppm	Yes	Yes
	High ppm Level, >100 ppm	Yes	No*

Battelle, 2010, 2011. Pollution Engineering News, 2008. FRC, 2012

Remedial Application Strategy



	ISCR	ZVI	ISCO
PRB Applicability	Yes (lifetime of 3 to >7 yrs)	Yes - (Ideal for managing long-term sources)	Typically not applicable (short longevity) EMERGING SRT
Widespread Plume Treatment	More cost efficient at lower COI concentrations	Several PRBs possible	May not be cost effective at low concentrations
Source Treatment	• Generally not applicable for pooled-NAPL areas* • Can apply to residual NAPL	Yes	Yes
Source with NAPL		Iron or iron / clay can be mixed into NAPL zones	Oxidants have shown success on NAPL

*** Based on time and cost**

Battelle, 2010, 2011. Pollution Engineering News, 2008. FRC, 2012

Site Logistics / Costs

Conditions	Favors ISCO?	Favors ISCR?
Proximity to sensitive receptors	Maybe	Yes
Limited access for injections	Maybe	Yes
Amendment compatibility with subsurface utilities	Maybe	Yes
Health and Safety concerns from handling and injecting the amendment	Maybe	Yes
Relatively short time available for treatment	Yes	Maybe
Injection depths > ca 100 ft bgs	Yes	Maybe/ERD

For cases where treatment can be done in one application of either amendment, ISCO and ISCR costs will be comparable

For sites where multiple applications are required to meet remediation goals:

- ISCR costs can be higher (long-term monitoring and mobilization for injections).
- ISCO costs can be lower (short time between applications, utilize previously installed injection wells).

Battelle, 2010, 2011. Pollution Engineering News, 2008. FRC, 2012

Services Offered

- Complimentary Site Evaluation
  - Technology Selections
  - Conceptual Remedial Designs
  - Material Cost Estimates
- Complimentary review of quarterly field performance data for 1 year with every site application
- Laboratory Treatability Studies
- Turn-Key, Pay-for-Performance Contracting Options
- Project Specific Guarantees and Warranties

Thank You. Questions Welcomed.

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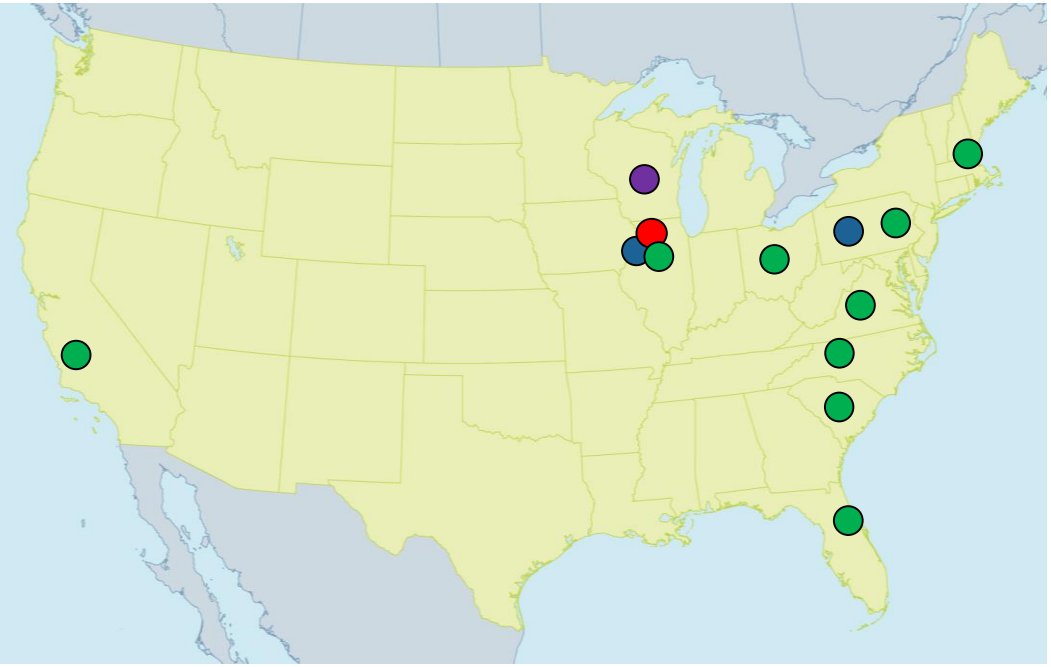

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Chloromethane Issues?

Reactions with halogens

Methane reacts with halogens, given appropriate conditions:

$$X_2 + UV \rightarrow 2\ X\bullet$$
$$X\bullet + CH_4 \rightarrow HX + CH_3\bullet$$
$$CH_3\bullet + X_2 \rightarrow CH_3X + X\bullet$$

where X is a halogen: fluorine (F), chlorine (Cl), bromine (Br), or iodine (I). The mechanism for this process is called free radical halogenation. It is initiated with UV light or some other radical initiator. A chlorine atom is generated from elemental chlorine, which abstracts a hydrogen atom from methane, resulting in the formation of hydrogen chloride. The resulting methyl radical, $CH_3\cdot$, can combine with another chlorine molecule to give methyl chloride (CH_3Cl) and a new chlorine atom.[11] Similar reactions can produce dichloromethane (CH_2Cl_2), chloroform ($CHCl_3$), and, ultimately, carbon tetrachloride (CCl_4), depending upon reaction conditions and the chlorine to methane ratio.

- Not a likely issue with ERD/ISCR
- Can be an issue with ISCO
- Is an issue with WWT (UV OX)