Private Webinar: Overview of Applied Environmental Biotechnologies

TBD LOCATION, TBD DATE

Jim Mueller – Freeport, IL

jim.mueller@provectusenv.com

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
- SBP Technologies, Inc. → RF Weston (1991 to 1997)
- Dames & Moore → URS (1997 to 2002)
- 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

<table>
<thead>
<tr>
<th>Application / Patent Number</th>
<th>Title</th>
<th>Filling Date</th>
<th>Issue Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>7,129,388 and 7,531,709</td>
<td>Method for Accelerated Dechlorination of Matter; Parts 1 and 2.</td>
<td>10/31/2006 and 05/12/2009</td>
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<tr>
<td>8,147,694</td>
<td>Method for the Treatment of Ground Water and Soils Using Mixtures of Seaweed and Kelp</td>
<td>04/03/2012</td>
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<tr>
<td>8,766,030</td>
<td>Utilization of Ferric Ammonium Citrate for In-Situ Remediation of chlorinated Solvents</td>
<td>01/30/2014</td>
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<tr>
<td>13/785,840 and 14/36,632</td>
<td>Method for Inhibition of methane production during anaerobic reductive dechlorination</td>
<td>03/05/2013 05/02/2014</td>
<td></td>
</tr>
<tr>
<td>CIP 14/268,637</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13/866,158</td>
<td>Use of encapsulated substrates to control the release rates of organic hydrogen donors</td>
<td>04/19/2013</td>
<td></td>
</tr>
<tr>
<td>13/891,934 and CIP 14/268,629</td>
<td>Chemical Oxidation and Biological Attenuation Process for the Treatment of Contaminated Media</td>
<td>05/10/2013</td>
<td></td>
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<tr>
<td>PCT/US14/36,642</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>62/024,640</td>
<td>Method and Composition for Inhibiting Methanogenesis During In Situ Sediment Treatment</td>
<td>07/15/2014</td>
<td></td>
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<tr>
<td>Filed</td>
<td>Inhibition of methane and hydrogen sulfide production in anaerobic digester animal farms, landfills, sediments and sewer systems</td>
<td>08/01/2014</td>
<td></td>
</tr>
<tr>
<td>Filed</td>
<td>Method and Composition for Inhibiting Heavy Metal Methylation During In Situ Remedial Actions</td>
<td>10/15/2014</td>
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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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Freeport, IL 61032
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Provectus, LATAM
São Paulo, Brazil
Bogota, Colombia
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
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 >10⁸ 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.

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 H₂.
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.
How Does RYR Control Methanogens?

- Bacteria cell walls contain peptidoglycan (murein).
- Methanogens cell walls contain pseudomurein.
- Pseudomurine 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).
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.
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.).

The reductive acetyl-CoA (Wood-Ljungdahl) pathway

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)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>qPCR Day 0</th>
<th>qPCR Day 25</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>9,840</td>
<td>18,400</td>
</tr>
<tr>
<td>P-IR 1.0</td>
<td>65,900</td>
<td>51,100</td>
</tr>
</tbody>
</table>

Does not impede DHC inoculants (SDC-9)
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 $\mu$g 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?

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 (R.Y.R) 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
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**

<table>
<thead>
<tr>
<th>Well Location</th>
<th>Pre-injection (ppm)</th>
<th>6 weeks Post-Injection (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CH4</td>
<td>PCE</td>
</tr>
<tr>
<td>MW-4</td>
<td>13.7</td>
<td>170</td>
</tr>
<tr>
<td>MW-207s</td>
<td>11.8</td>
<td>1,200</td>
</tr>
</tbody>
</table>

- 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**

<table>
<thead>
<tr>
<th>Well Location</th>
<th>CH4 PID (ppm)</th>
<th>CH4 FID (ppm)</th>
<th>CH4 TGA %</th>
<th>CO2 %</th>
<th>O2 %</th>
<th>Balance (N) Est. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>MW-4</td>
<td>ABC Only</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min</td>
<td>297</td>
<td>&gt;50,000</td>
<td>34.8</td>
<td>65.2</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>5 min</td>
<td>439</td>
<td>&gt;50,000</td>
<td>35.6</td>
<td>61.0</td>
<td>0.2</td>
<td>3.2</td>
</tr>
<tr>
<td>MW-207s</td>
<td>Provect CH4 added</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min</td>
<td>82</td>
<td>Out of range</td>
<td>0.5</td>
<td>1.0</td>
<td>12.7</td>
<td>85.8</td>
</tr>
<tr>
<td>5 min</td>
<td>41</td>
<td>1,599</td>
<td>0.4</td>
<td>0.7</td>
<td>20.2</td>
<td>78.7</td>
</tr>
</tbody>
</table>
CH4 - How to Monitor, Where to Monitor?
## Provect-CH4™ What Does it Cost?

### Treatment Zone Dimensions

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Width of targeted zone (perpendicular to gw flow)</td>
<td>266 ft</td>
</tr>
<tr>
<td>Length of targeted zone (parallel to gw flow)</td>
<td>266 ft</td>
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<tr>
<td>Depth to top of treatment zone</td>
<td>20 ft</td>
</tr>
<tr>
<td>Depth to bottom of treatment zone</td>
<td>45 ft</td>
</tr>
<tr>
<td>Treatment zone thickness</td>
<td>25 ft</td>
</tr>
<tr>
<td>Calculated Volume</td>
<td>1768900 ft³</td>
</tr>
</tbody>
</table>

### Methane Inhibitor Calculations

<table>
<thead>
<tr>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimated Porosity</td>
<td>30 %</td>
</tr>
<tr>
<td>Calculated impacted liquid</td>
<td>530,670 ft³</td>
</tr>
<tr>
<td>Targeted RYR Conc in GW</td>
<td>50 ppm</td>
</tr>
<tr>
<td>Methane Inhibitor for Project</td>
<td>1,656 lbs</td>
</tr>
<tr>
<td>ROUND TO 55 lb units (25 kg drums)</td>
<td>30</td>
</tr>
<tr>
<td>Unit Price as ERD Supplement (VOLUME DISCOUNTS APPLY)</td>
<td>$35/lb</td>
</tr>
<tr>
<td>TOTAL (excludes shipping)</td>
<td>$57,750</td>
</tr>
</tbody>
</table>

- 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
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
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- ISCO or ISCR?
- Services and Offerings
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

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
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
ABC-CH4™ What Does it Cost?

### Treatment Zone Dimensions

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- Can use 30% less ERD = 67,500 lbs x $1.50/lb = $101,250 (saves $33,750)
- 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
Introduction to Provectus Environmental Products, Inc.

Environmental Biotechnologies
- Provect-CH4™ Methane Inhibitor
- ABC-CH4™ Liquid, Antimethanogenic ERD Reagent
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ISCO or ISCR?

Services and Offerings
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

<table>
<thead>
<tr>
<th>Process</th>
<th>Amendments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enhanced Anaerobic Degradation / ERD</td>
<td>Molasses, emulsified vegetable oils, sodium lactate, polylactic acid, whey, simple H release compounds, ABC</td>
</tr>
<tr>
<td><em>In Situ</em> Chemical Reduction / ISCR</td>
<td>Provect-IR™, ABC+, EHC®, DARAMEND®</td>
</tr>
<tr>
<td>Antimethanogenic ISCR Reagents</td>
<td>Provect-IR®, Provect-IRM®, Aquablok®-CH4, and to some degree ABC-CH4™</td>
</tr>
</tbody>
</table>
Conventional ISCR Reagents (ca. 2003)

- Contaminant bound to soil surfaces
- Contaminant desorbs from binding site on soil and diffuses to ISCR particle surface water film
- Hydrated ISCR particles colonized by native soil bacteria
- Water film
- Native soil Archaea

Carbon Fermentation + ZVI Corrosion = ISCR Multiple Reaction Mechanisms

<table>
<thead>
<tr>
<th>Production of organic acids (VFAs):</th>
<th>electron donors for reduction of COIs, O\textsubscript{2}, NO\textsubscript{3}, SO\textsubscript{4}</th>
</tr>
</thead>
<tbody>
<tr>
<td>• By preventing basification, reduces precipitate formation on ZVI surfaces to increase rate of iron corrosion / H\textsubscript{2} generation / reactivity</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ZVI Reactions:</th>
<th>H\textsubscript{2} and Fe\textsuperscript{+2} and generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{Fe}^0) (\rightarrow) Fe\textsuperscript{2+} + 2e\textsuperscript{-}</td>
<td></td>
</tr>
<tr>
<td>2H\textsubscript{2}O (\rightarrow) 2H\textsuperscript{+} + 2OH\textsuperscript{-}</td>
<td></td>
</tr>
<tr>
<td>2H\textsuperscript{+} + 2e\textsuperscript{-} (\rightarrow) H\textsubscript{2}(gas)</td>
<td></td>
</tr>
<tr>
<td>R-Cl + H\textsuperscript{+} + 2e\textsuperscript{-} (\rightarrow) R-H + Cl\textsuperscript{-}</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ISCR = thermodynamic conditions for dechlorination:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Combined oxygen consumption from carbon fermentation and iron oxidation \rightarrow Strongly reduced environment (-250 to -500 mV)</td>
</tr>
<tr>
<td>• High electron/H\textsuperscript{+} pressure</td>
</tr>
</tbody>
</table>
Idealized Eh pH Ranges for Microbial Growth

<table>
<thead>
<tr>
<th>Microbe</th>
<th>Doubling Times</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehalococcoides spp.</td>
<td>24 to 48 hours</td>
</tr>
<tr>
<td>Methanogens with cytochromes</td>
<td>10 hours</td>
</tr>
<tr>
<td>Methanogens without cytochromes</td>
<td>1 hour</td>
</tr>
</tbody>
</table>
contaminant desorbs from binding site on soil and diffuses to ISCR particle surface water film

hydrated ISCR particles colonized by native soil bacteria

water film

native soil bacteria

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?

<table>
<thead>
<tr>
<th>Component / Feature</th>
<th>Provect-IR™</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Multiple, Complex, Mixed-Release Organic Hydrogen Donors,</strong> namely Kelp plus specially selected organic plant matter and readily fermentable propionate</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Integrated Methanogenic Inhibitor (patents pending)</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Chemical Oxygen Scavenger to maintain reduced ZVI during Mixing and Injection</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>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</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Calcium Propionate Primer for Enrichment of Indigenous Anaerobes</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Modified formulations for heavy metal immobilization</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td><strong>ZVI Component Average Particle Size (micron)</strong></td>
<td>15</td>
<td>100</td>
</tr>
<tr>
<td>current values assumed = not confirmed by vendors</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>% by weight ZVI</strong></td>
<td>10</td>
<td>40</td>
</tr>
<tr>
<td><strong>Square Feet of ZVI Surface per Pound of Product</strong> - Displayed values are based on estimated micron average size</td>
<td>25</td>
<td>15</td>
</tr>
<tr>
<td>19</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td><strong>Grams of Organic Hydrogen Donor/ Pound of Product</strong></td>
<td>390</td>
<td>272</td>
</tr>
<tr>
<td><strong>Price Ranges</strong> - based on information found in published documents (non-confidential, public, readily available)</td>
<td>$1.50 to $2.35/lb</td>
<td>&lt;$2.00 to $2.65/lb</td>
</tr>
</tbody>
</table>

*Price ranges may vary due to external factors such as market conditions and supplier variability.*
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$^{II-IV}$ 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.
## Contaminant Treatment Mechanisms in the ISCR zone

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

clay (sealant) layer

hydration

time

dense core (e.g. aggregate)

(not to scale)

can comprise and include wide variety of minerals, treatment agents, organics, seeds, etc.
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

delivery, or diffusion, of treatment reagents through sediment

extraction of treated or untreated pore waters from sediment

controlled flow induced through sediment

AquaBlok cap

permeable sand layer

substrate

contaminated sediment

extraction port

delivery port

piping systems

(not to scale)
AquaBlok-CH4™ Particle

can comprise and include wide variety of minerals, treatment agents including iron

dense core (e.g. aggregate)

clay (sealant) layer

hydration
time
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)

- Predominant direction of ground water flow

- *AquaBlok\(^+\)™ Reactive Cap*

- *Reactive Gates & AquaBlok\(^TM\) Cap*
Continuous PRB

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

Funnel and Gate
Presentation Outline

- Introduction to Provectus Environmental Products, Inc.
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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 (SO₄²⁻) 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**

- **US**
  - $1.80 / lb includes activator
- **THEM**
  - >$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 H$_2$S Formation
- Utilizes both iron and sulfate reducing bio processes

- When Chelated Fe$^{2+}$ used as activator EDTA Consumes the Oxidant
  - Short Lived Reaction
  - H$_2$S 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

### New Jersey Site

![Map of New Jersey Site(384,37),(913,306)

<table>
<thead>
<tr>
<th>Sampling Date</th>
<th>06/20/2013</th>
<th>10/02/2013</th>
<th>11/26/2013</th>
<th>02/28/2014</th>
<th>05/28/2014</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MW-2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sampling Date</strong></td>
<td>06/20/2013</td>
<td>10/02/2013</td>
<td>11/26/2013</td>
<td>02/28/2014</td>
<td>05/28/2014</td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>7.27</td>
<td>6.88</td>
<td>6.89</td>
<td>6.86</td>
<td>7.43</td>
</tr>
<tr>
<td><strong>ORP (mV)</strong></td>
<td>-14</td>
<td>+220</td>
<td>+86</td>
<td>+55</td>
<td>+292</td>
</tr>
<tr>
<td><strong>D.O. (mg/L)</strong></td>
<td>2.17</td>
<td>0.76</td>
<td>0.90</td>
<td>0.85</td>
<td>0.83</td>
</tr>
<tr>
<td><strong>Conductivity (mS/cm)</strong></td>
<td>0.97</td>
<td>3.44</td>
<td>1.52</td>
<td>2.38</td>
<td>1.55</td>
</tr>
<tr>
<td><strong>Temperature (°C)</strong></td>
<td>17.7</td>
<td>20.4</td>
<td>17.0</td>
<td>12.2</td>
<td>14.3</td>
</tr>
<tr>
<td><strong>Groundwater Elevation (ft)</strong></td>
<td>94.05</td>
<td>90.43</td>
<td>88.86</td>
<td>92.93</td>
<td>93.70</td>
</tr>
<tr>
<td><strong>Sulfate (mg/L)</strong></td>
<td>56.6</td>
<td>1,510</td>
<td>266</td>
<td>980</td>
<td>332</td>
</tr>
<tr>
<td><strong>Total Iron (mg/L)</strong></td>
<td>0.377</td>
<td>2.01</td>
<td>0.149</td>
<td>0.089</td>
<td>0.160</td>
</tr>
<tr>
<td><strong>Dissolved Iron (mg/L)</strong></td>
<td>0.249</td>
<td>1.83</td>
<td>0.0097</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Benzene (ppb)</strong></td>
<td>ND</td>
<td>7.20</td>
<td>43.4</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Toluene (ppb)</strong></td>
<td>ND</td>
<td>2.33</td>
<td>0.38</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Ethylbenzene (ppb)</strong></td>
<td>ND</td>
<td>4.08</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td><strong>Total Xylenes (ppb)</strong></td>
<td>ND</td>
<td>21.04</td>
<td>3.16</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>
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. Accordingly, various in-situ chemical oxidation (ISCO) technologies using oxidizing agents such as hydrogen peroxide (Fenton's chemistry), permanganate, ozone or activated persulfate, 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 or surfactant-assisted oxidation 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. 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

<table>
<thead>
<tr>
<th>COIs</th>
<th>ISCO</th>
<th>ISCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Provect-OX</td>
<td>ZVI</td>
</tr>
<tr>
<td>Chlorinated Ethenes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Chlorinated Ethanes Chlorinated Methanes</td>
<td>Yes</td>
<td>Not 1,2-DCA, CA, DCM, CM</td>
</tr>
<tr>
<td>OC Pesticides</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Organic Explosives</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>TPH GRO and DRO</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Mixed CVOCs and TPH</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Metals (Cr, Pb, Cu, As, Ni)</td>
<td>Emerging</td>
<td>Yes</td>
</tr>
<tr>
<td>Mixed CVOCs and Metals</td>
<td>Emerging</td>
<td>Yes</td>
</tr>
<tr>
<td>Mixed TPH and Metals</td>
<td>Sequential Treatment</td>
<td></td>
</tr>
</tbody>
</table>

## Contaminant Concentration

### Media

<table>
<thead>
<tr>
<th>Media</th>
<th>COI Concentrations</th>
<th>ISCO</th>
<th>ISCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil</td>
<td>Up to 10 mg/kg</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>&gt; 10 mg/kg</td>
<td>Yes</td>
<td>Maybe</td>
</tr>
<tr>
<td></td>
<td>NAPL</td>
<td>Yes</td>
<td>No*</td>
</tr>
<tr>
<td>Groundwater</td>
<td>ppb Level &lt;1,000 ppb</td>
<td>No**</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>ppm Level 1 ppm to 100 ppm</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>High ppm Level, &gt;100 ppm</td>
<td>Yes</td>
<td>No*</td>
</tr>
</tbody>
</table>

### Remedial Application Strategy

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

* Based on time and cost

---

## Site Logistics / Costs

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

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).
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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Phone (815) 650-2230
info@provectusenv.com
Chloromethane Issues?

Reactions with halogens
Methane reacts with halogens, given appropriate conditions:

\[ X_2 + UV \rightarrow 2 X^\cdot \]
\[ X^\cdot + CH_4 \rightarrow HX + CH_3^\cdot \]
\[ CH_3^\cdot + X_2 \rightarrow CH_3X + X^\cdot \]

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\(_3\)Cl) and a new chlorine atom.\(^{[1]}\) Similar reactions can produce dichloromethane (CH\(_2\)Cl\(_2\)), 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)