New Antimethanogenic Reagents (AMRs)

Session C5: Advances in Amendments, Wednesday April 11, 2018

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Battelle's 11th International Conference on Remediation
Of Chlorinated and Recalcitrant Compounds
April 8-12, 2018
Palm Springs, California

Presentation Outline

- What is the Problem with Methane?
- Antimethanogenic Reagents (AMRs)
- AMR-Based Environmental Biotechnologies
  - **Provect-CH4®** Methane Inhibitor
  - **ERD-CH4® Ole Ego** Liquid, Antimethanogenic ERD Reagent
  - **Provect-IR®** Solid, Antimethanogenic ISCR Reagent
  - **Provect-IRM®** Antimethanogenic ISCR/Heavy Metal Immobilization Reagent
  - **AquaGate-CH4®**Antimethanogenic Reactive Cap
  - **EZVI-CH4™** Antimethanogenic Reagent for DNAPL
- Case Study: Dry Cleaner in Urban Setting
- Summary and Conclusions

What is a Methanogen?

- Methanogens are microorganisms that produce methane
- Methanogens are Archaea (Woese and Fox, 1977) and hence, from a genetic perspective, *Dehalococcoides mccartii* 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)

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

Why Add Carbon/ZVI to Reduce ORP?

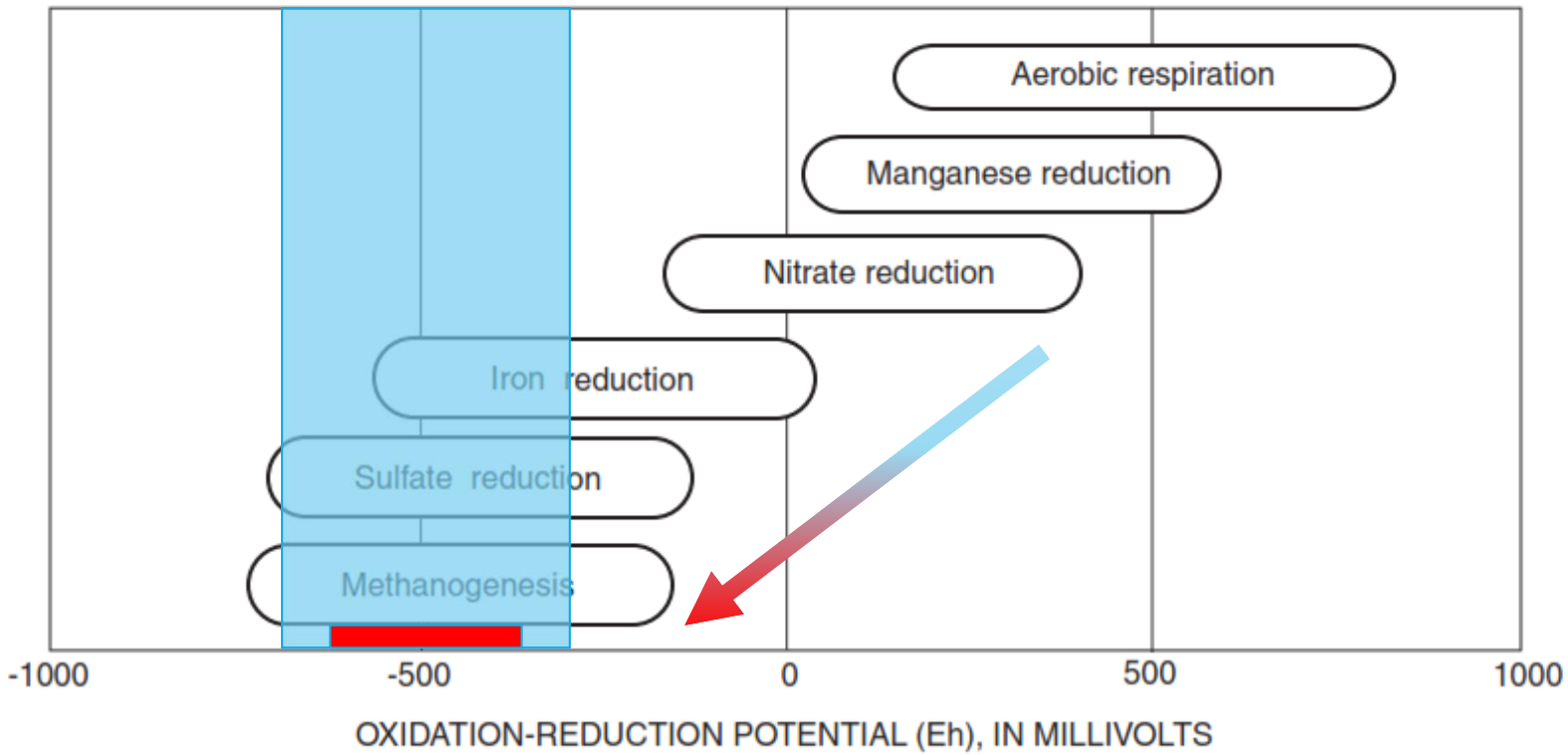
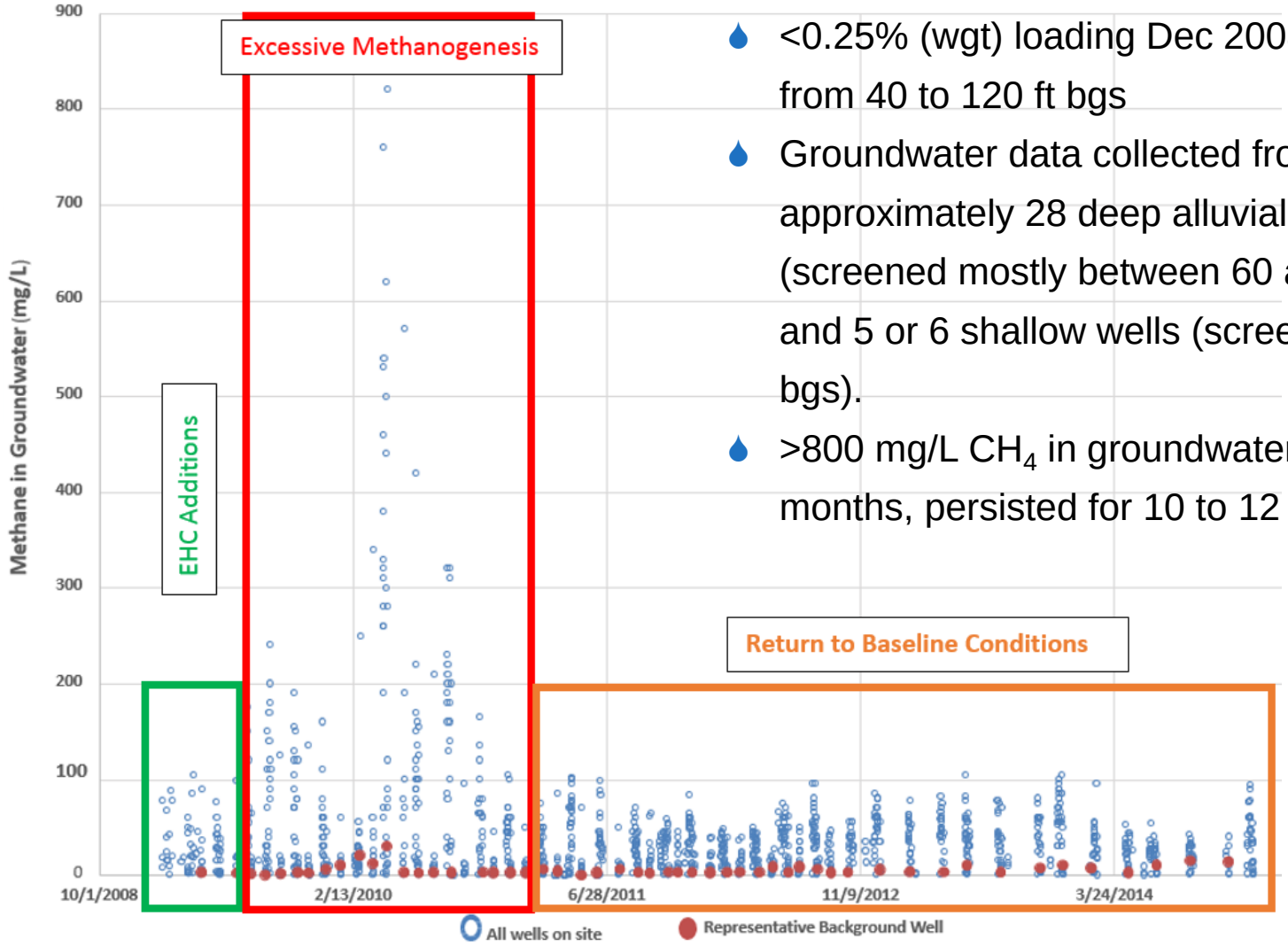


Figure 2. Oxidation-reduction potentials for selected microbial processes. (Modified from Stumm and Morgan, 1981.)

Example of Excessive Methanogenesis

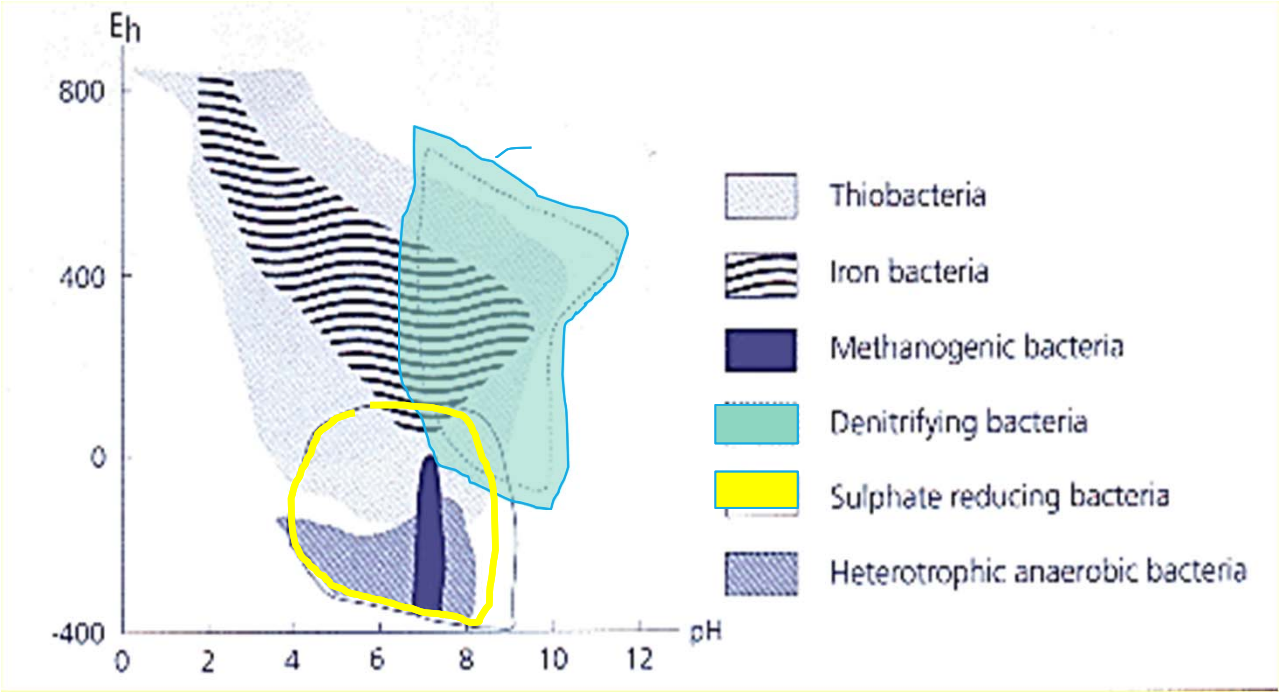


- <0.25% (wgt) loading Dec 2008-May, 2009 from 40 to 120 ft bgs
- Groundwater data collected from approximately 28 deep alluvial wells (screened mostly between 60 and 160 ft bgs) and 5 or 6 shallow wells (screened 25-35 ft bgs).
- >800 mg/L CH_4 in groundwater after 6 months, persisted for 10 to 12 months

Source: Peale et al, 2009; 2010

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



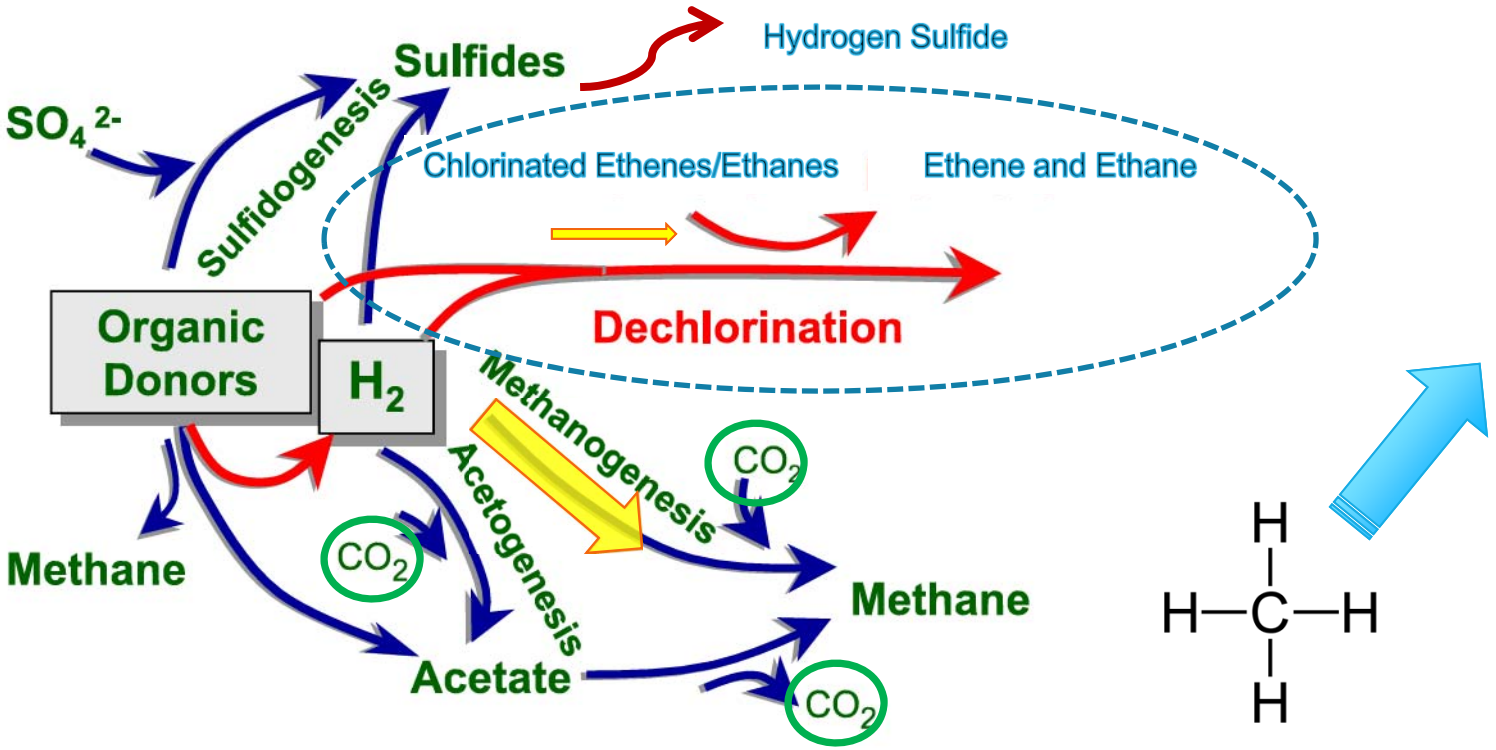
Zajic, 1969. Sigma Aldrich

Excessive CH_4 16 months post EVO

Source: US DOD, 2016; 2017

Hydrogen is the Currency

- **Where Does it Go? = Cost and Efficiency Issues**: Methanogens dominate anaerobic ecosystems and they can hinder dechlorination by competing for H_2 with dechlorinating bacteria (Yang and McCarty, 1998; yellow arrows modified by Provectus).



What is The Problem With Methane?

- **#1 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**

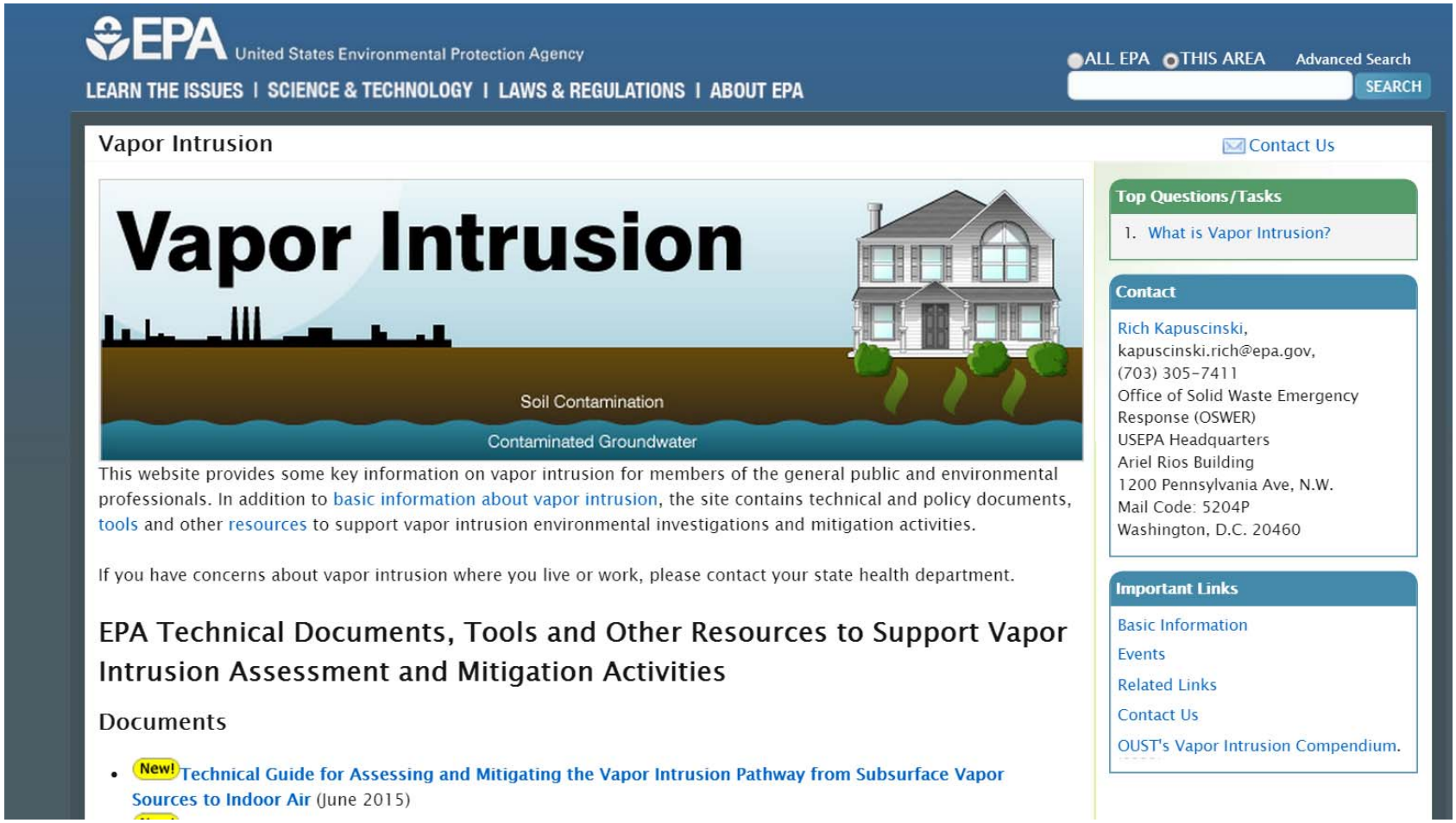
Even in a highly oxidized setting with relatively high total concentrations of PCE and TCE, generating just 20 mg/L of methane constitutes **greater than 33%** of the total amendment consumption based on moles of H_2.

What is The Problem With Methane?

- **#2 *Potential* Health and Safety Issues (*in Some Situations*):**
  - ✓ Methane is explosive, with an LEL of 5% and an UEL of 15%.

- 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 inactivation 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.

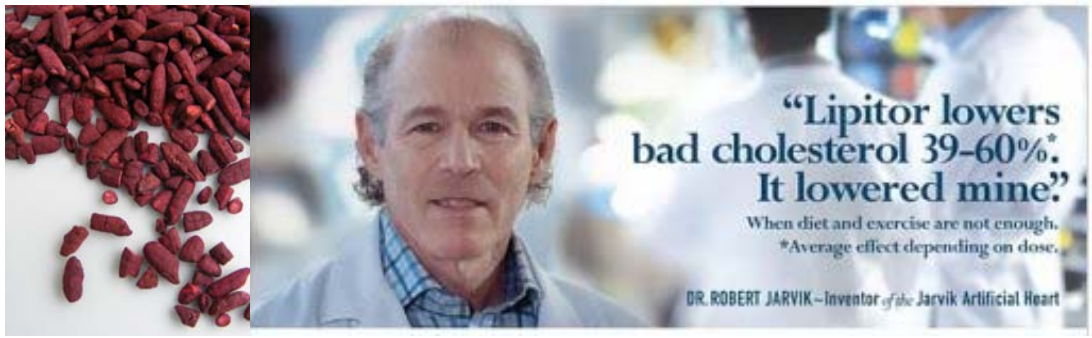
What Does the US EPA Say?



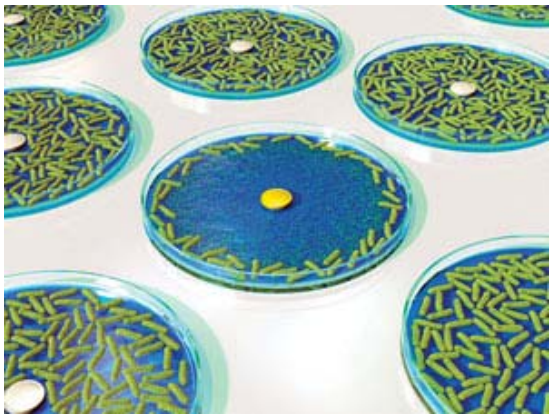
- **http://www.epa.gov/oswer/vaporintrusion/**

What is Red Yeast Rice (RYR) Extract?

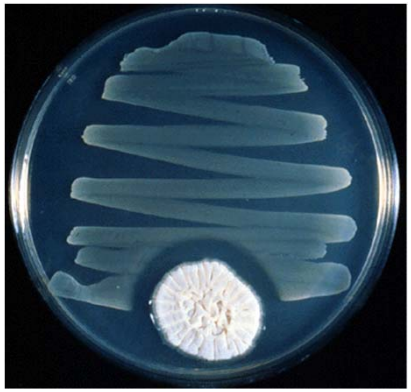
- RYR extract is a substance extracted from rice that has been fermented with a yeast called *Monascus purpureus*.
- RYR extract contains a number of natural statins - most importantly, Monacolin K - otherwise known as Lovastatin® / Lipitor® /etc.
- In addition to Monacolin K, RYR also contains 9 other 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.



Why Does RYR Produce Statins?

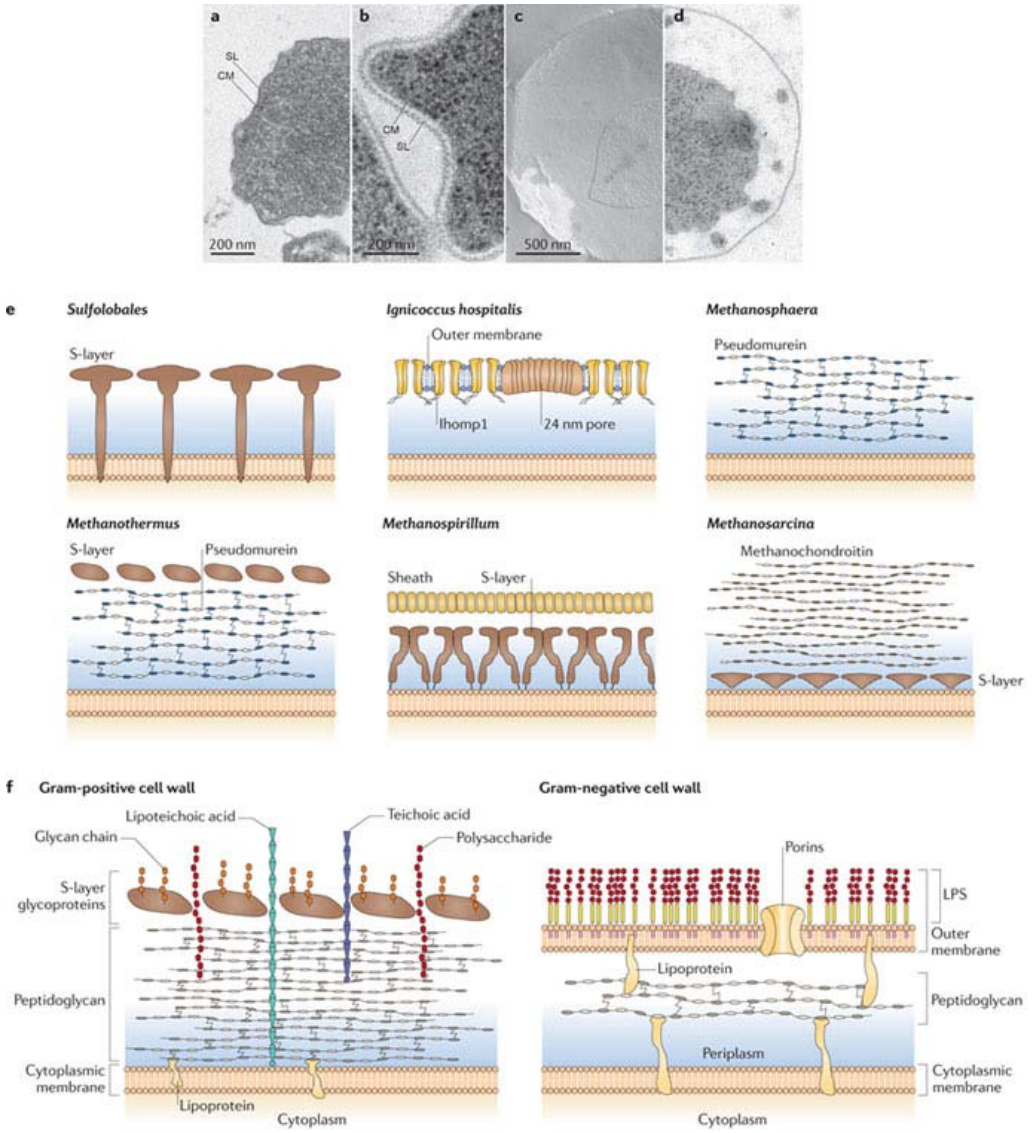


- Many microorganisms produce bioactive compounds that inhibit / regulate the growth and development of other organisms
- Example, antibiotics such as penicillin which is produced by mold of *Penicillium* genus

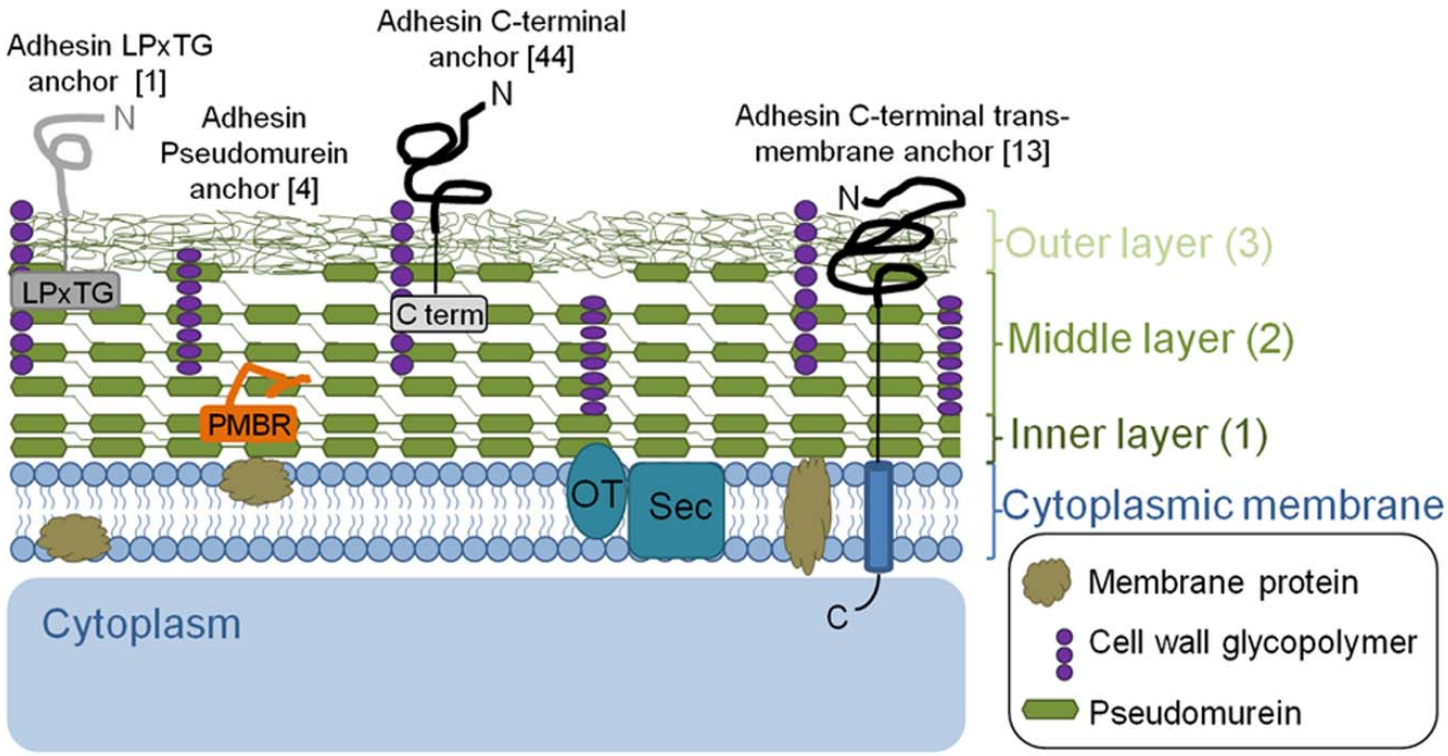

How Does RYR Control Methanogens?

- 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).



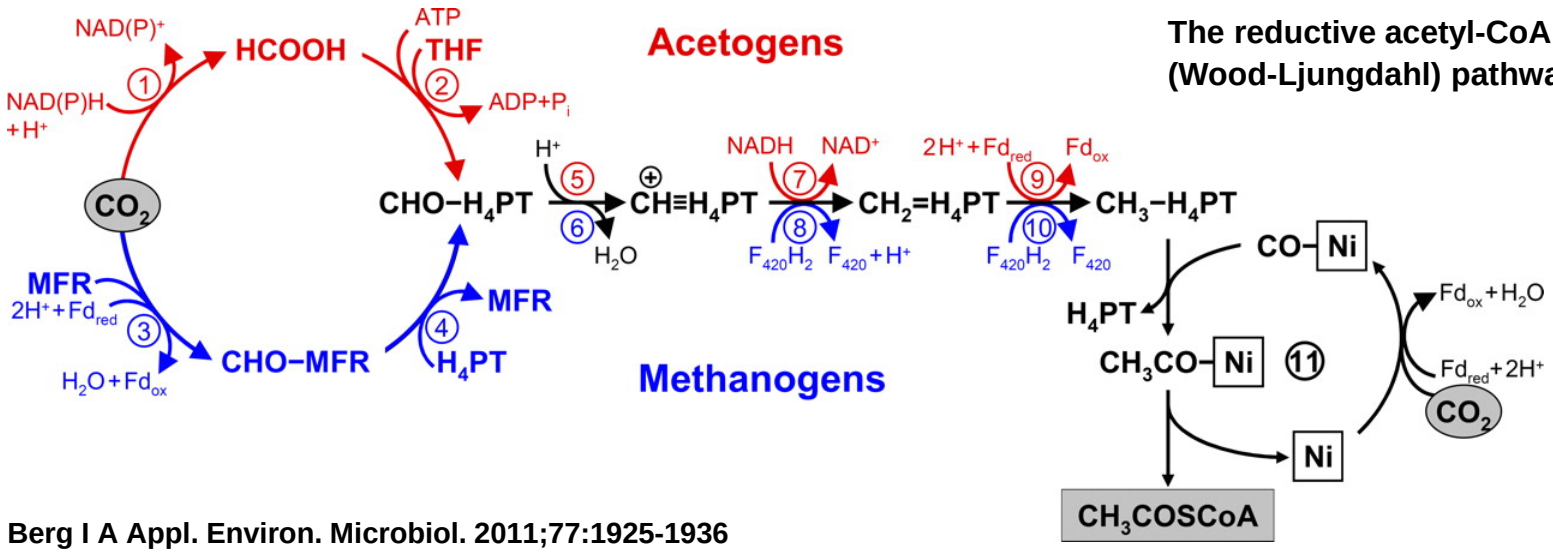
How Does Provect-CH4 Control Methane?

- 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?

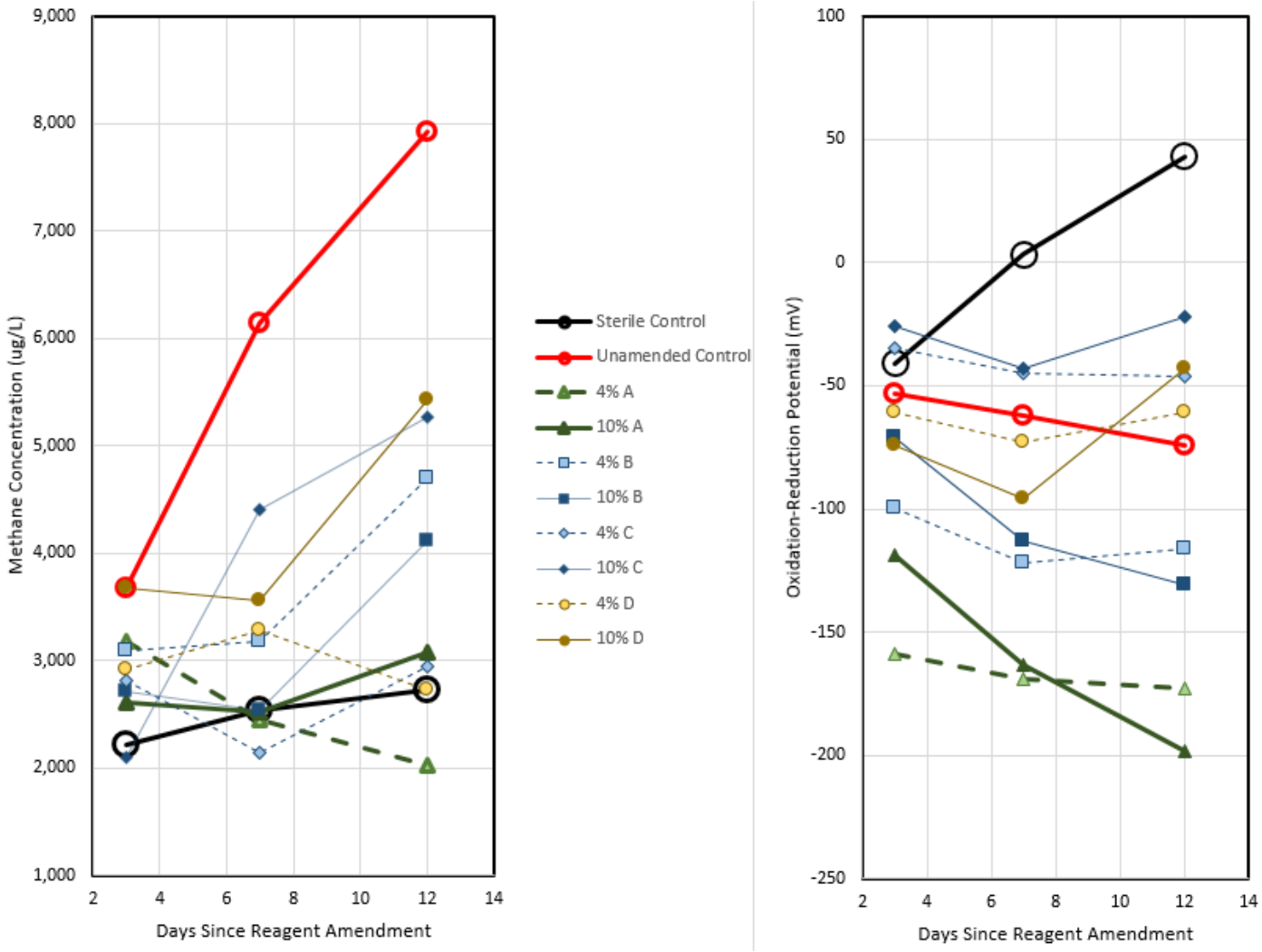
- Other compounds also interrupt F420 coenzyme synthesis and other systems unique to methanogens which restrict their growth and proliferation.
- Because Archaea are so different from other microbes, these inhibitory effects are selective to methanogens and are not observed in microbes that are typically associated with: i) catabolism of organic contaminants (such as *Pseudomonas spp*.) and/or, ii) halo-respiration/biodegradation of chlorinated solvents (such as *Dehalococcoides spp*).



The reductive acetyl-CoA (Wood-Ljungdahl) pathway

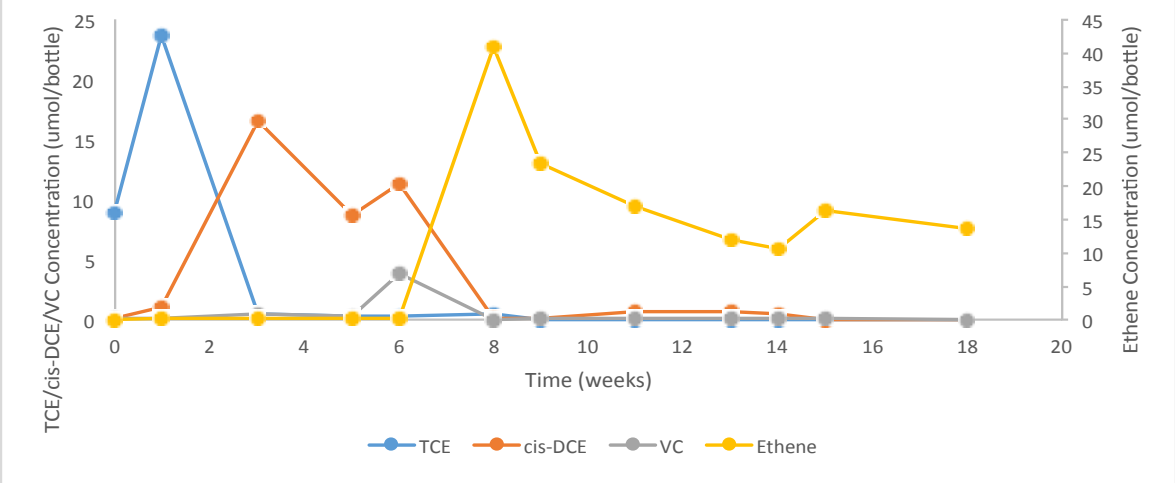
Berg I A Appl. Environ. Microbiol. 2011;77:1925-1936

Oil-Based AMRs: Initial Studies



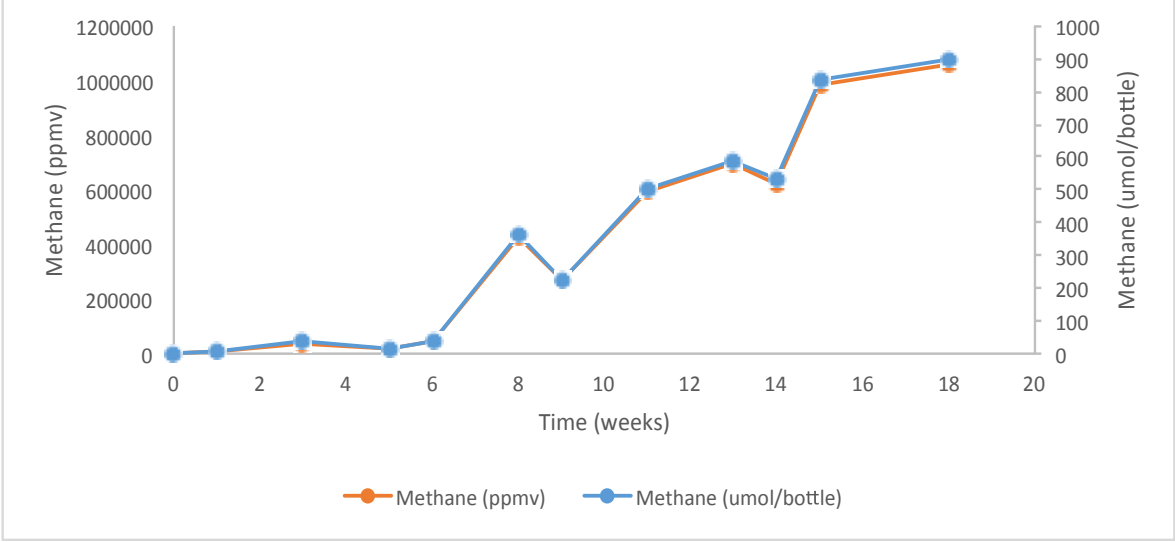
GO AMR Studies from Clemson

- 40 mM EVO to forward
- **No AMR**



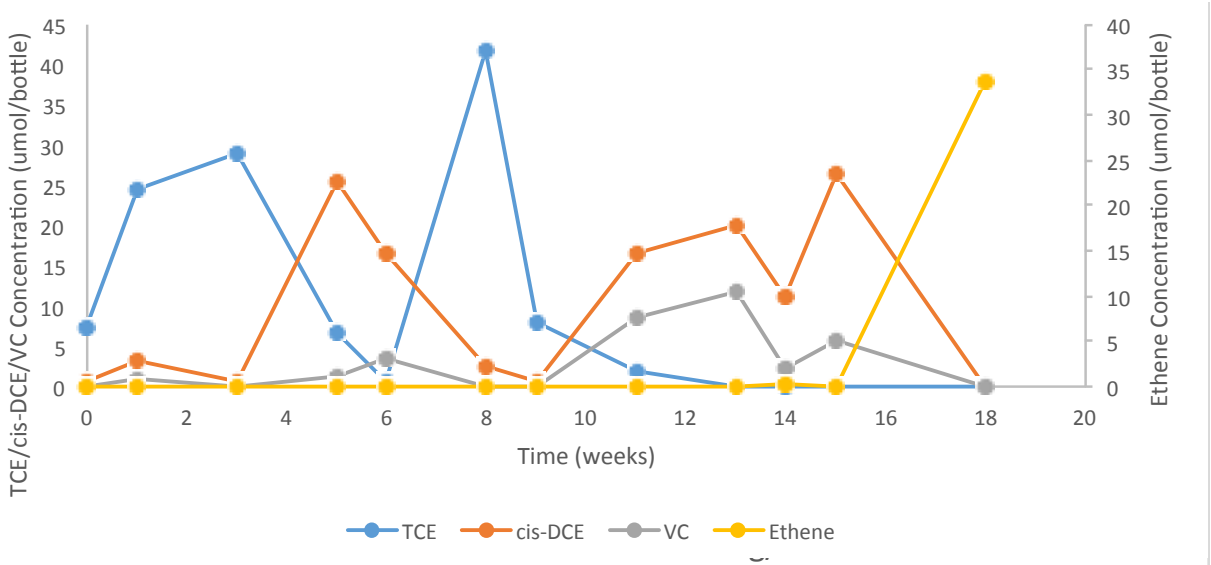
18 days

- CH4 @ 100,000 ppmV
- 900 umole



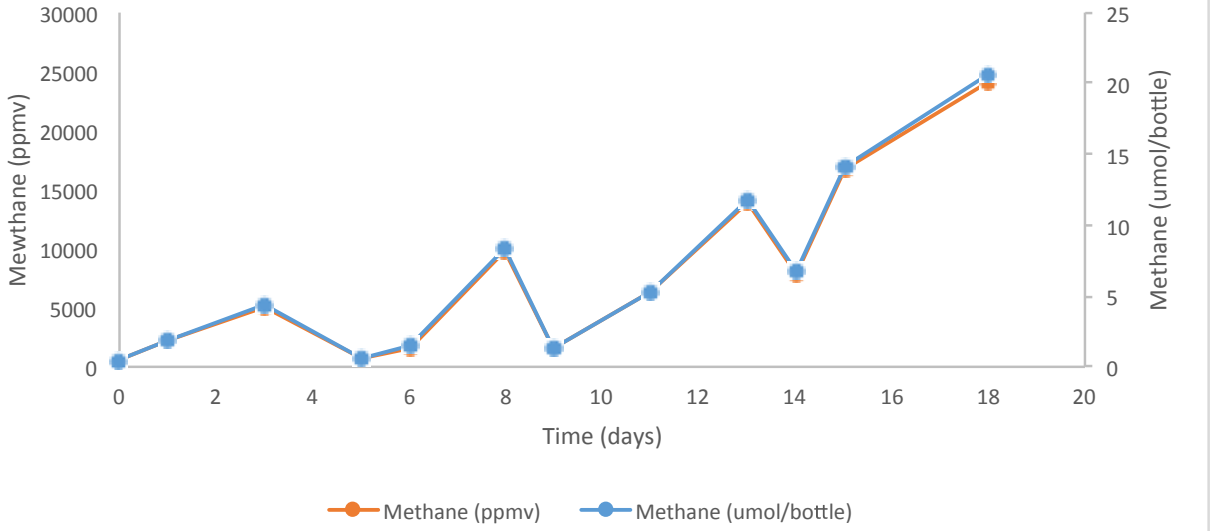
GO AMR Studies from Clemson

- 40 mM EVO to forward
- EGO AMR **@ 250 ppm**

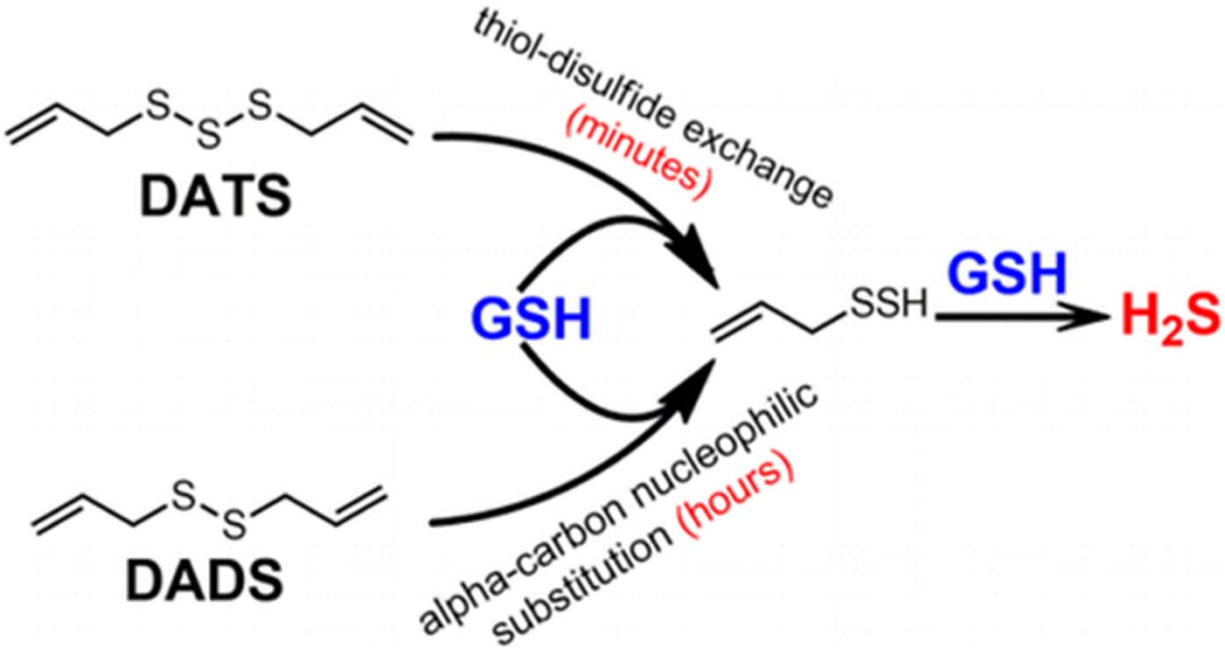


18 days

- CH4 @ <25,000 ppmV
- <20 umole
- 75 to >95% reduction in CH4



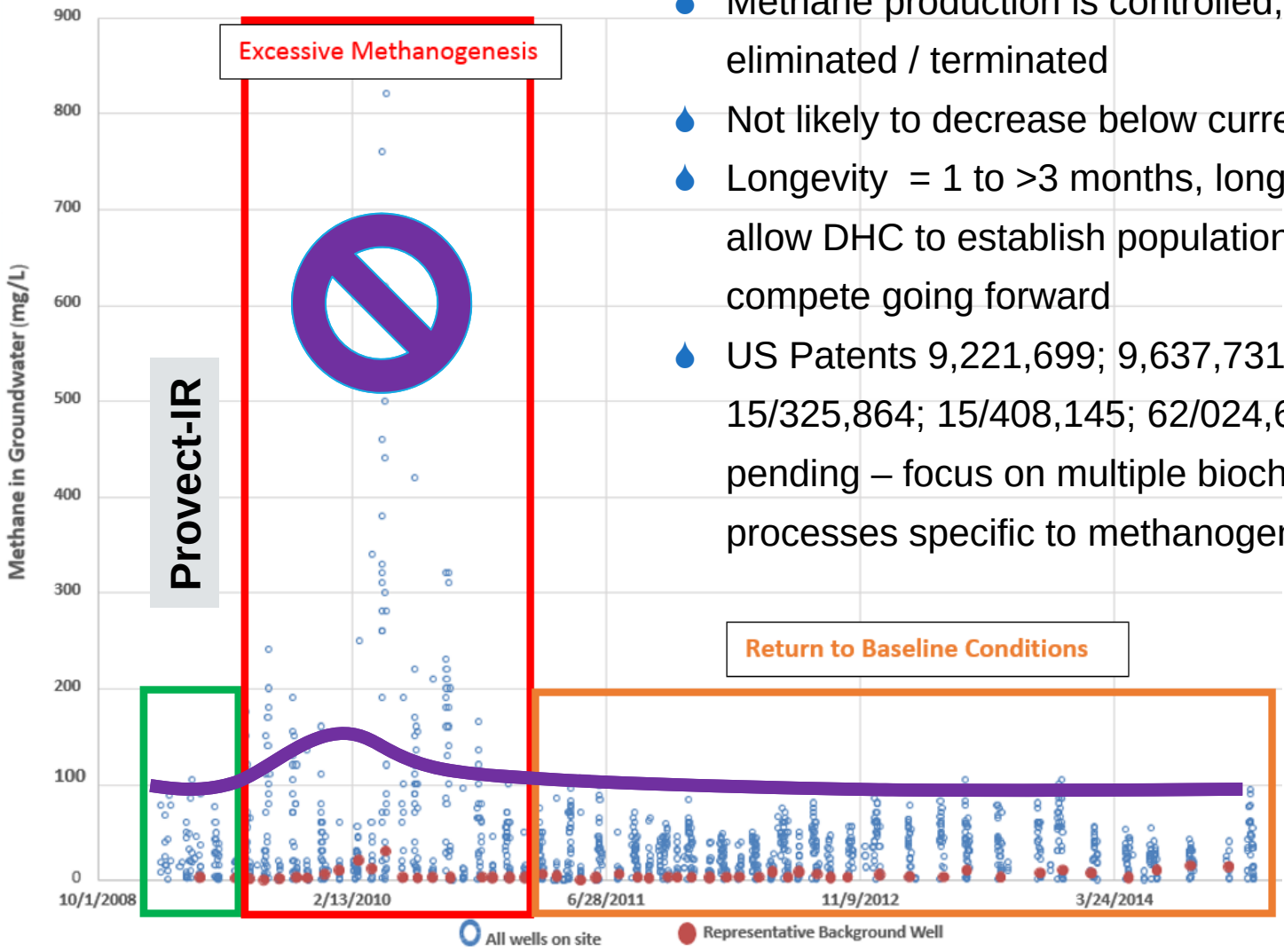
Synthetic Garlic Oil – Consist Quality



Diallyl Trisulfide Is a Fast H2S Donor, but Diallyl Disulfide Is a Slow One: The Reaction Pathways and Intermediates of Glutathione with Polysulfides

Dong Liang, Haixia Wu, Ming Wah Wong, and Dejian Huang
Organic Letters **2015** *17* (17), 4196-4199

Controlled Methanogenesis AMR



- Methane production is controlled, not eliminated / terminated
- Not likely to decrease below current conditions
- Longevity = 1 to >3 months, long enough to allow DHC to establish populations and better compete going forward
- US Patents 9,221,699; 9,637,731; 15/269,903; 15/325,864; 15/408,145; 62/024,640; patents pending – focus on multiple biochemical processes specific to methanogens

Provect-ERD CH4® Ole Ego™

- Liquid, AMR ERD substrate
- 60 to >90% FC
- Contains RYR and EGO

Photograph 1. Provect-ERD CH4™ 15:1 Water:Oil (Left), 85% Carbon + 4% AMR Self-Emulsifiable Oil Concentrate (Middle), and 85% Carbon Self-Emulsifiable Oil Concentrate, no AMR (Right).

Product Data

The materials are all combined at our own manufacturing facilities in the USA (and Europe) at proportions and formulations optimized for a given site. ERD-CH4™ is manufactured using 100% food grade ingredients that provide fast- and slow-release characteristics. Provect-CH4® antimethanogenic reagent (AMR) is typically added at three to five weight percent of the mass of the fermentable carbon. The common dosage of ERD-CH4 provides groundwater concentrations of 1,000 to 3,000 ppm TOC plus a minimum 150 ppm of AMR within the targeted treatment area.

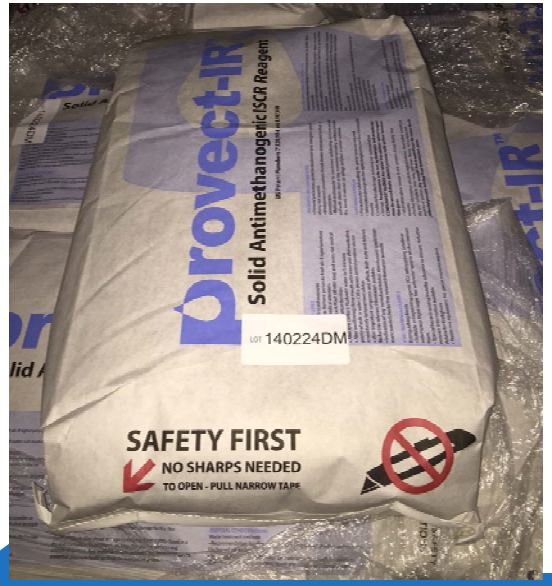
Color	Translucent Yellow
Density (lbs. / gal)	7.75 to 8.46 (varies based on AMR)
Physical State	Liquid
Odor	Earthy
Viscosity (Brookfield, 30 rpm @25°C)	50-100 cps
pH – 1% w/v in water	7.3

Oil Sample	Al	P	S	Zn	Fe	Mg	Ca	Na	K
Self-Emulsifiable Vegetable Oil	1.2 to 1.3	1,265 to 1,751	17.2 to 28.2	19.6 to 39.7	1,4 to 2.3	138 to 143	135 to 187	15.4 to 15.9	507 to 954

- **All units mg/kg oil**

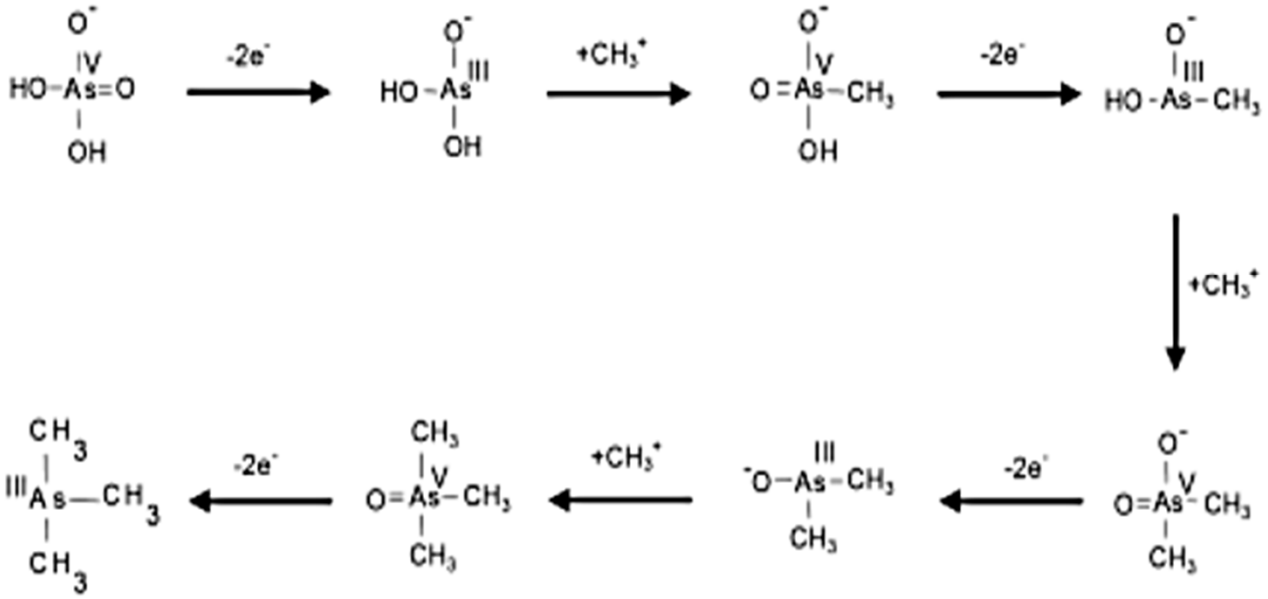
Provect-IR Solid, AMR ISCR Reagent

- Provect-CH4® AMR Technology
- Multiple, Complex, Hydrophilic, Timed-Release organic carbon source (plant materials, kelp, Ca propionate) @ 390 g H donor / lb product
- 15% to 85% (wgt) ZVI particles (from 3 to >495 micron)
- Integrated vitamins, minerals and nutrients (yeast extract) specially selected for anaerobes
- Chemical oxygen scavenger to maintain ZVI
- Package in 50 lb safety bags or 2,000 lb supersacs.



Methanogens (and others) Methylate Metals

- 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).

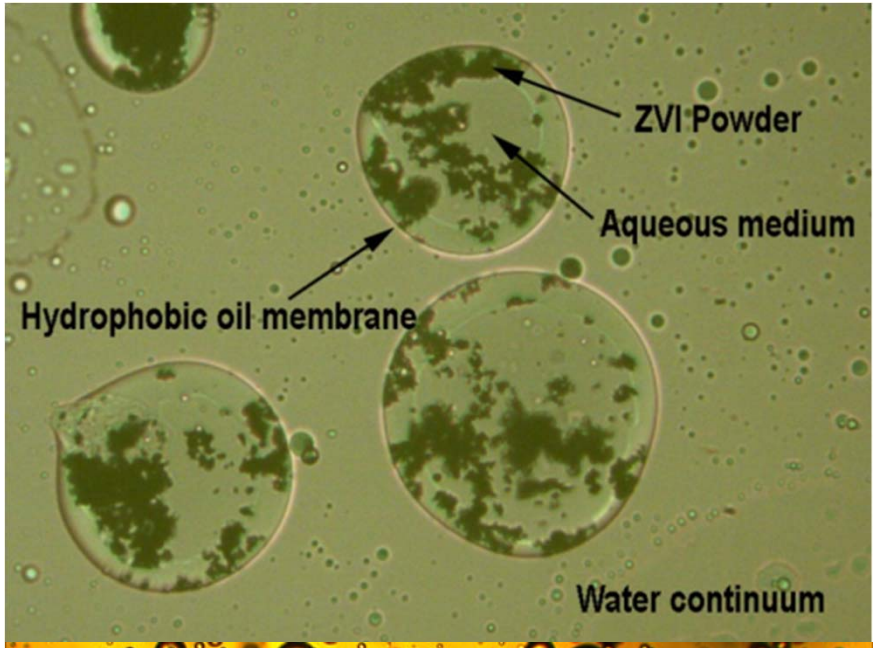


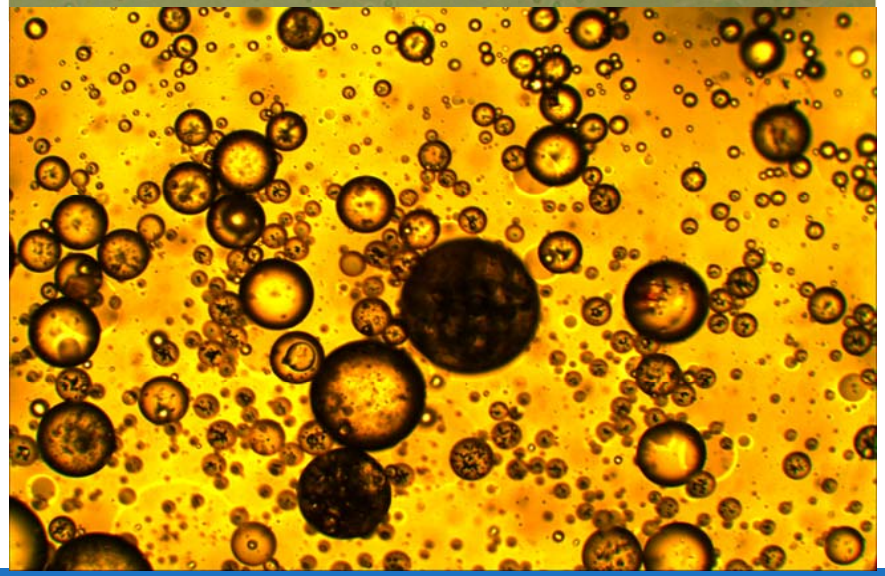
Challenger mechanisms for biosynthesis or Arsenate (Challenger, 1945)

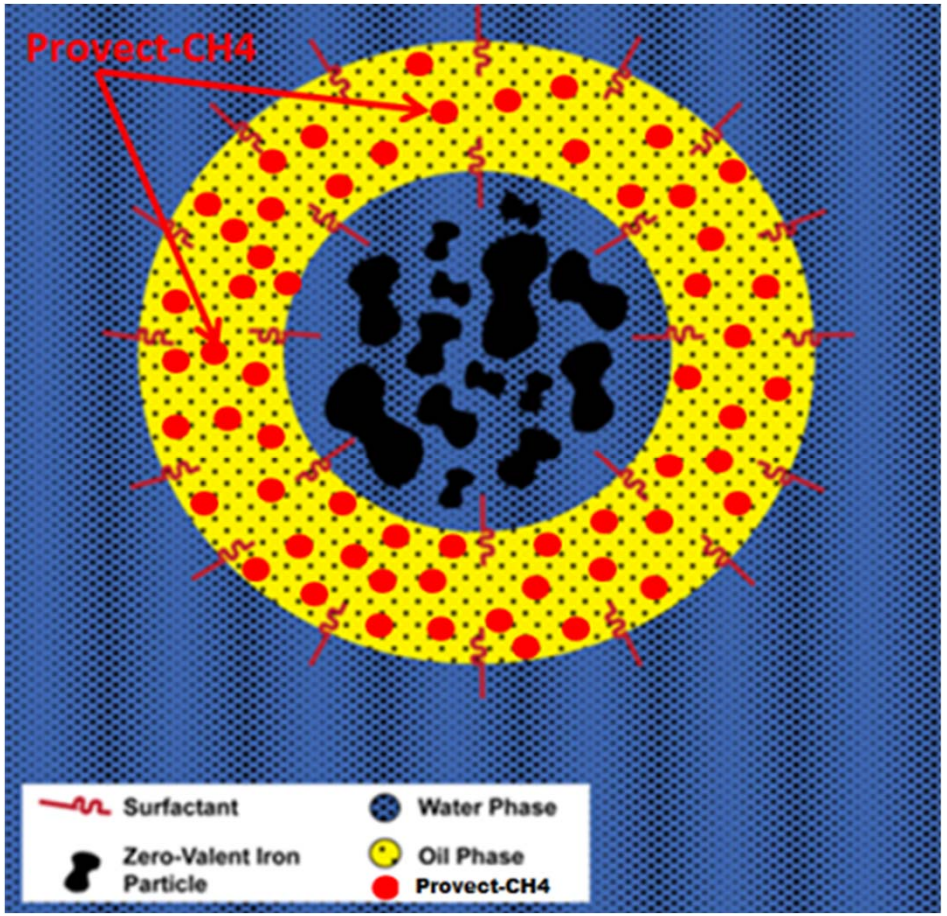
Provect-IRM Solid, Antimethanogenic ISCR Reagent for Heavy Metals

- **Formulated based on site requirements**
- Provect-CH4 AMR Technology
- Multiple, Complex, Hydrophilic, Timed-Release organic carbon source (plant materials, kelp, Ca propionate)
- Inorganic S source, if needed
- 15% to 85% (wgt) ZVI particles (from 3 to >495 micron)
- Integrated vitamins, minerals and nutrients (yeast extract) Chemical oxygen scavenger to maintain ZVI
- Package in 50 lb safety bags or 2,000 lb supersacs.

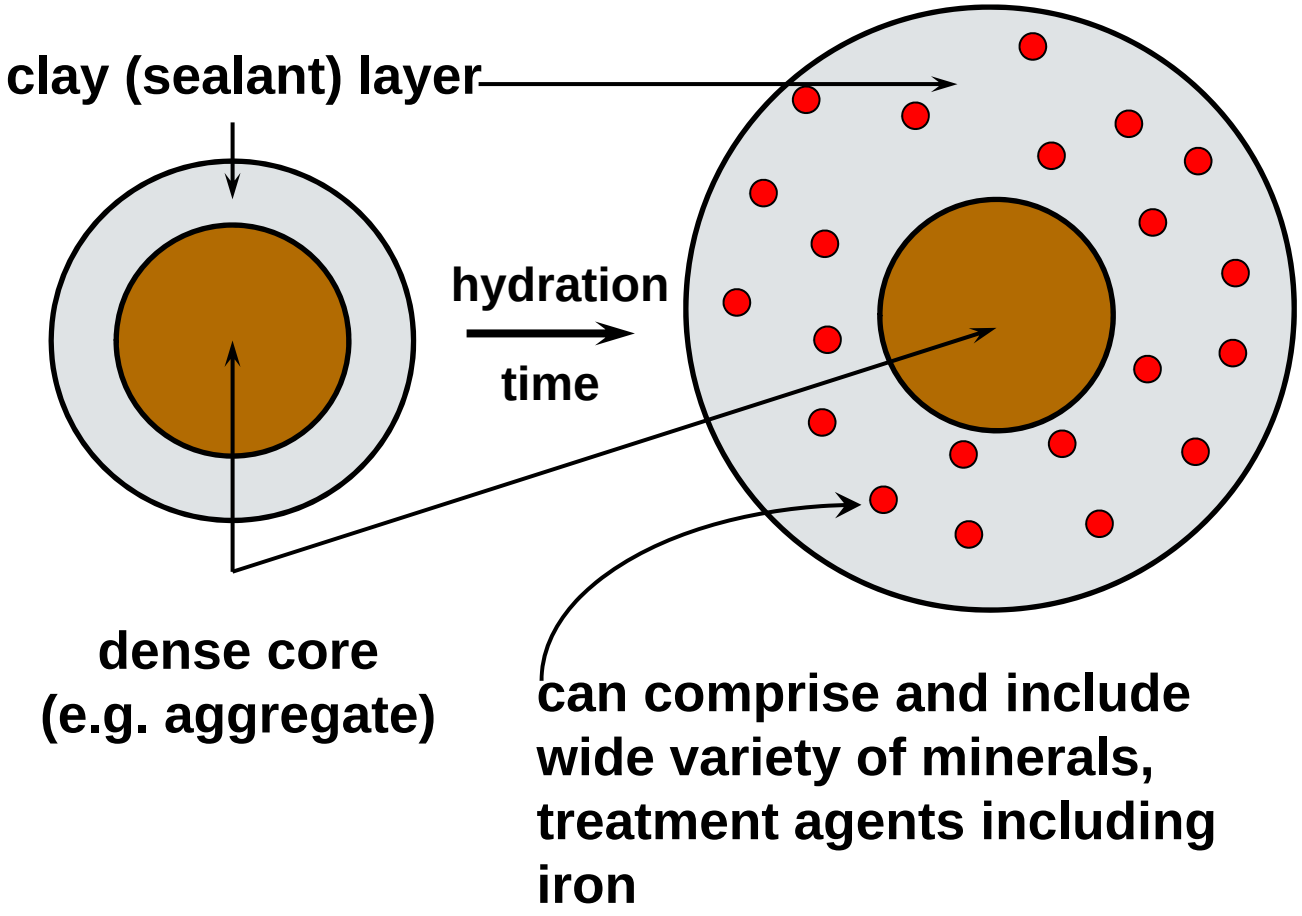
EZVI-CH4™ AMR DNAPL Technology







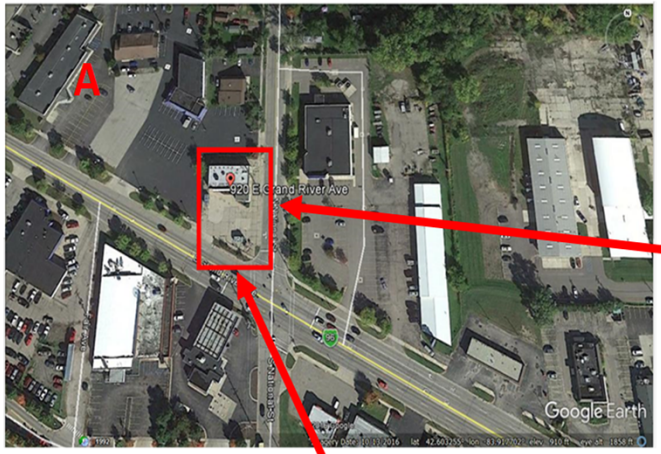
AquaGate®-CH4 AMR Capping

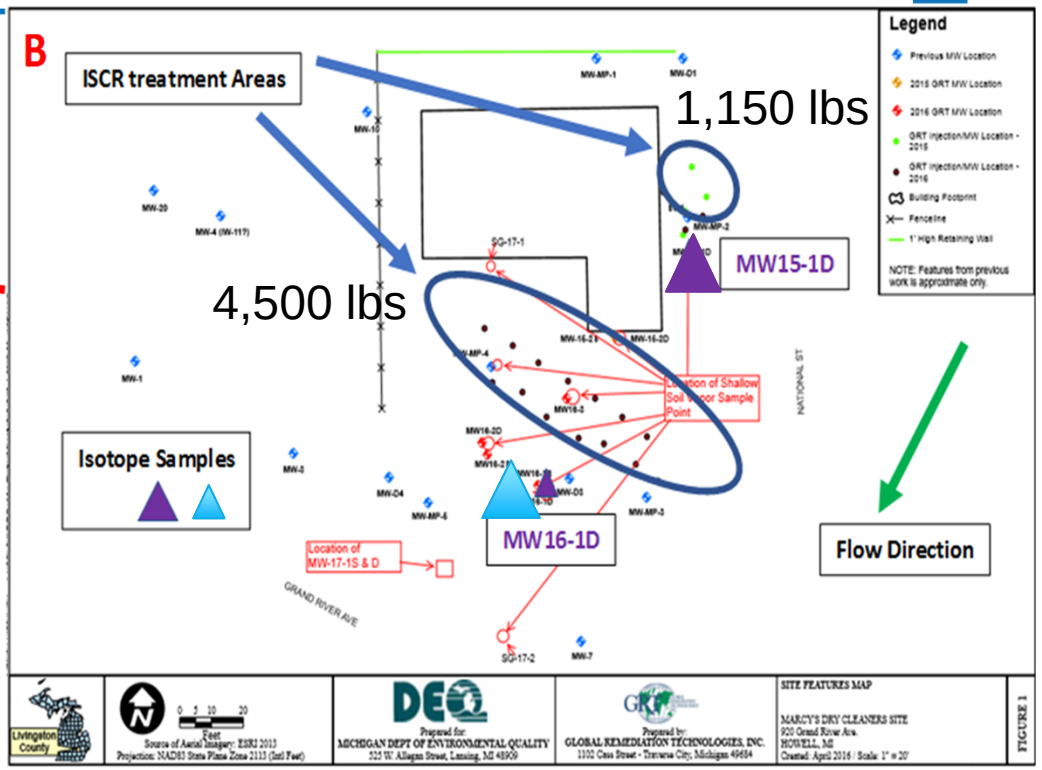


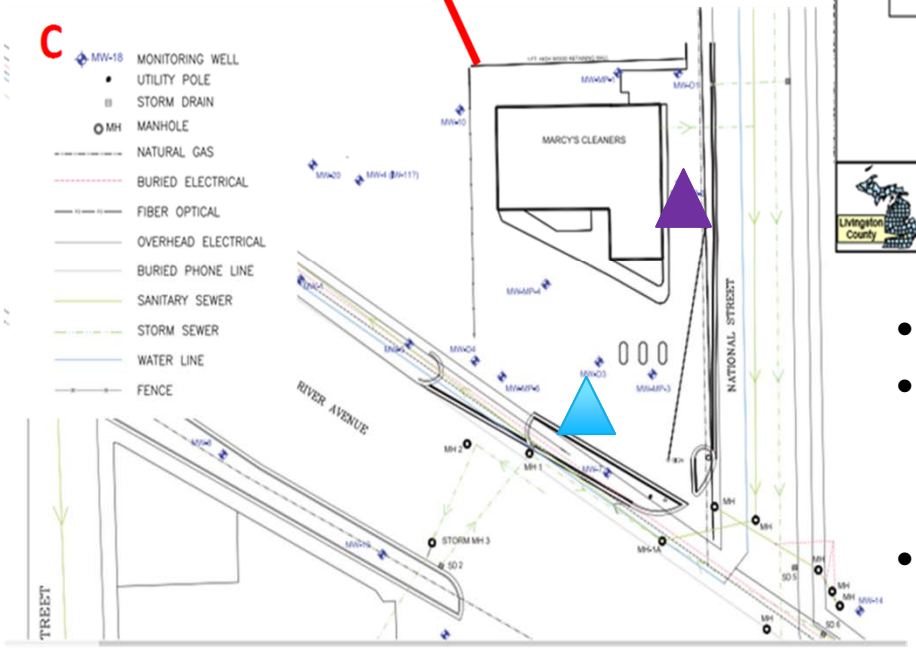
Case Study: Provect-IR®

- Active Dry Cleaning Facility, southern Michigan
- Shallow groundwater 5 ft bgs confined by a clay layer at 12 ft bgs.
- PCE (max. 35 ppm) and TCE (max. 14 ppm) along with an accumulation of anaerobic catabolites *cis* 1,2-DCE (max. 25 ppm) and some VC (max. 4 ppm).
- Source area up to 70 ppm total CVOCs
- Groundwater migrates through a sandy aquifer into a damaged storm sewer.
- A sanitary sewer feeder from the active dry cleaner exacerbating the PCE migration problem by allowing warm water with potential contaminants and surfactants to enter the groundwater.
- Consultant and Agency selected Provect-IR over conventional ERD and ISCR reagents known to induce methane production.

Phase II Implementation (March 2016)







- *>90% PCE removal, no DCE/VC stall*
- *ca*. 9 months post Provect-IR treatment CH4 from 5 to <10 mg/L was observed at two well locations ▲▲
- >200,000 ppmV in soil gas at MW-16S ▲

Origin of CH4 After 9 Months

$\Delta^{14}C$ and $\delta^{13}C$ Data Review

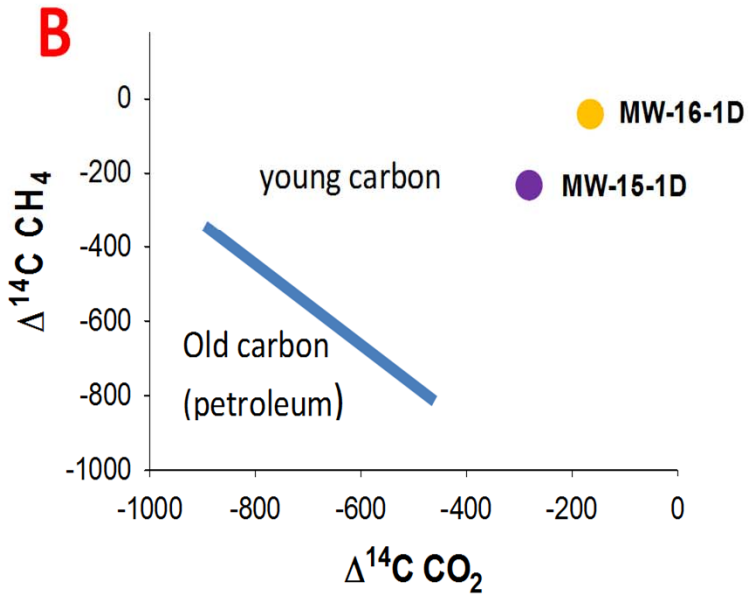
Stable Carbon and Radiocarbon Data Summary

A

Sample Identification	Type	F Modern	Fm Err	Age (years)	Age Err	$\delta^{13}C$	$\Delta^{14}C$
MW-16-1D-CO2, groundwater	CO2	0.8469	0.0020	1,340	20	-71.48	-159.96
MW-15-1D-CO2, groundwater	CO2	0.7261	0.0024	2,570	25	-16.65	-279.81
MW-16-1D-CH4, groundwater	CH4	0.9669	0.0019	270	15	-57.96	-40.95
MW-15-1D-CH4, groundwater	CH4	0.7677	0.0016	2,120	15	-60.61	-238.54

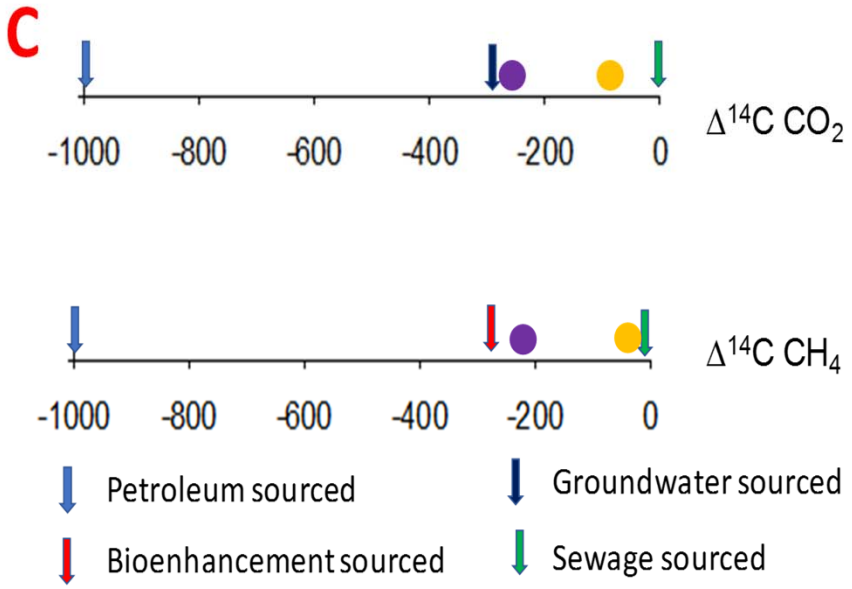
Radiocarbon Isotope CH_4 and CO_2 Data

B



Potential CO_2 and CH_4 $\Delta^{14}C$ Source Values

C



So, is Methanogenesis Important?

Some say…	Response
✓ We never see problems	mostly because we don't look
✓ My site is remote	ignores efficiency issue
✓ Our amendments don't make methane	then they are not working well
✓ It's bad to stop methanogens	it's beneficial to control them
✓ Our clients don't worry about it	then YOU better worry about it…
✓ We will just add more reagent	$$ not acceptable to our clients

You decide

- ✓ Look at your own data
- ✓ Evaluate your site conditions
- ✓ Co-metabolism (IRB, SRB) of CHCs is slow, and mostly stalls at DCE/VC
- ✓ Experienced clients understand the value added
- ✓ What do your regulators say …

Summary

- Natural statins in RYR, essential plant oils and other materials can be used to effectively and specifically control methanogenic activity
- The methane control technology has been integrated into various products designed for the environmental remediation industry
  - **Provect-CH4®** ERD Supplement / Methane Inhibitor
  - **ERD-CH4® Ole Ego™** Liquid, Antimethanogenic ERD Reagent
  - **Provect-IR®** Solid, Antimethanogenic ISCR Reagent
  - **Provect-IRM®** Antimethanogenic ISCR Reagent for Metals
  - **AquaGate®-CH4™** Antimethanogenic *In Situ* Sediment Capping Technology
  - **EZVI-CH4™** Antimethanogenic Source Area / DNAPL Treatment
- The main benefit is improved performance = “better gas mileage”
- Other potential benefits relate to safety, regulatory compliance, and sustainability

Provectus Environmental Products

- Complimentary Site Evaluation
- Complimentary review of quarterly field performance data for 1 year with every project
- Laboratory Treatability Studies
- Turn-Key, Pay-for-Performance Contracting Options
- Project Specific Guarantees and Warranties

- USA (Illinois, New York, Ohio, Pennsylvania, Louisiana)
- Brazil, China, Colombia, Israel, Italy, Spain and Taiwan