Managing Excessive Methanogenesis During ERD/ISCR Remedial Action

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Excessive production of methane has been observed at some remediation sites following the addition of organic hydrogen donors such as (emulsified) oils/lecithin, sugars, and conventional carbon + zero-valent iron (ZVI) amendments. This is due to the fact that methanogens are commonly the most ubiquitous indigenous microbes in anoxic aquifer settings, and, under enriched environmental conditions, methanogens replicate every one to two hours (whereas Dehalococcoides *spp., e.g., double in 24–48 hr). Hence, methanogens often bloom and dominate the microbial ecosystem following the addition of remedial amendments, thereby liberating large amounts of methane gas. There are at least three important consequences of this response:*

i. *By utilizing hydrogen, the methanogens compete with dechlorinating microbes, thus making inefficient use of the remedial amendment (just 20 ppm methane in groundwater represents an approximate 30 percent "waste" of added fermentable substrate (i.e., hydrogen donor)—this is a common and tangible detriment);*
ii. *Methanogens can methylate heavy metals and their rapid growth consumes alkalinity, while generating acidity, thereby facilitating multiple potential mechanisms for creating secondary contaminant issues (i.e., arsenic plumes); and*
iii. *Elevated methane concentrations can exceed current and pending regulations of <10 to <28 ppm methane in groundwater and/or 0.5 percent by volume methane in soil gas (e.g., 10 percent of the lower explosive limit) and/or indoor air (methane is flammable between 5 percent and 15 percent by volume) and this will induce migration of contaminant vapors potentially causing indoor air issues.*

Considering the recent guidelines for indoor air published by the US Environmental Protection Agency, it is increasingly important to prevent excessive methanogenesis associated with remedial actions. From a regulatory perspective, public safety issues are paramount; from a property re-use or real estate (brownfield) developers' perspective, project delays are costly and can jeopardize an entire program. The use of antimethanogenic compounds as inhibitors of protein biosynthesis and the activity of enzyme systems unique to Archaea (i.e., methanogens) during in situ *remedial action can improve contaminant removal while offering safer, more efficacious treatment, simply by impeding the methanogenic bacteria's ability to proliferate and out compete desired bacterial communities (e.g.,* Dehalococcoides *spp.).*

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INTRODUCTION

As described by Brown et al. (2009)—and many others—there are, in general, two reductive processes used to remove chlorinated volatile organic solvents (CVOCs) and other halogenated compounds from contaminated environments: (i) biologically mediated reductive dechlorination/enhanced reductive dechlorination (ERD) and (ii) *in situ* chemical reduction (ISCR). While both ultimately involve the transfer of electrons to the chlorinated solvent, resulting in dechlorination, the pathways and the mechanisms are quite different. Under certain conditions, heavy metals such as arsenic, cadmium, chromium, copper, lead, and zinc can also be managed via *in situ* immobilization, resulting from various adsorption or precipitation reactions that can occur under ERD or ISCR conditions.

ERD involves a distinct metabolic process whereby halo-respiring bacteria use the CVOC as an electron acceptor. The electron donor is typically an added carbon substrate or molecular hydrogen (produced from the fermentation of a carbon substrate). The dechlorination reaction is a sequential hydrogenolysis process wherein chlorines are replaced by a hydrogen ion (H^+). Both the hydrogen ion addition and the chlorine removal require an electron. Therefore, the reduction involves two sequential electron transfers that are mediated by halo-respiring bacteria. Exhibit 1 depicts the complete reductive dechlorination pathway for tetrachloroethylene (PCE) that was first described by Vogel and McCarty (1985), and has been subsequently well studied. The degradation process is sequential dechlorination from PCE → trichloroethylene (TCE) → dichoroethylene (DCE; there are three different isomers of DCE – *cis*, *trans*, and 1,1-; however, *cis*-1,2-DCE is the dominant product) → vinyl chloride (VC) → ethene. The terminal end product can be carbon dioxide or methane.

ISCR can be described as the combined effect of stimulated biological oxygen consumption (via fermentation of an organic carbon source) plus direct chemical reduction with zero-valent iron (ZVI) or other reduced metals. Under ISCR conditions, significantly lower redox potential (e.g., Eh ← 400 to –750 millivolts [mV]) is frequently observed, which enables more effective mineralization of CVOCs (Dolfing et al., 2008; Shi et al., 2011). The corresponding catabolic reaction products are representative of those observed via iron-mediated reductive pathways, in that the primary reaction products from the reduction of chlorinated ethenes are acetylenes, not ethenes. As summarized in Exhibit 2 (based on Gillham & O'Hannesin, 1994), this can occur via a β-elimination reaction in which chlorines on adjacent carbon atoms are removed, forming a C–C bond. Abiotic reduction of the CVOCs can also go through the hydrogenolysis pathway, but this typically accounts for only 10 percent of the reduction of the parent compound. However,

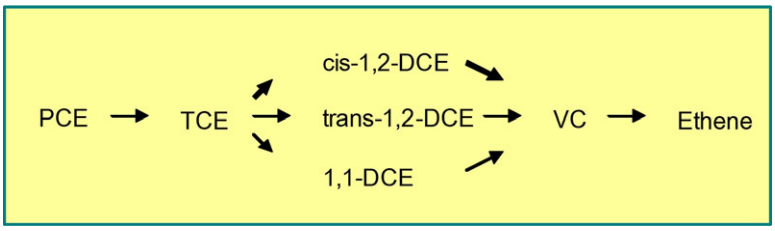

Exhibit 1. Sequential reductive dechlorination of PCE/TCE

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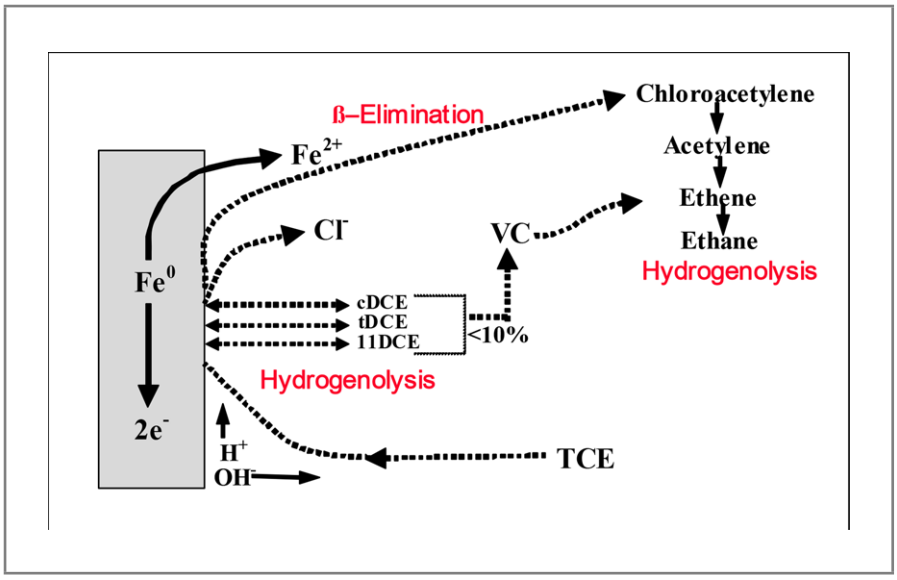

Exhibit 2. Abiotic reduction of TCE by ZVI

hydrogenolysis reactions may be used to further reduce the chloroacetylenes that are formed via the β-elimination pathway. Whether hydrolyzed or further reduced, chloroacetylenes are short-lived intermediates in groundwater environments.

Recent studies have shown that ZVI alone can also affect contaminant reduction by several novel pathways that are not observed with dual valent iron (DVI) or other metals and minerals (Chen et al., 2014). These pathways include (i) dechlorination by intramolecular nucleophilic substitution presumably catalyzed by hydroxyl groups associated with oxides on actively corroding ZVI; and (ii) epoxide ring opening by electron transfer from reduced iron. Hence, ISCR conditions as defined previously have an expanded potential to treat a range of halogenated compounds and, ideally, without the stoichiometric production of catabolites and potential accumulation of dead-end intermediates.

An ever-growing number of ERD substrates and other accelerated anaerobic bioremediation technologies (e.g., emulsified oils/lecithins, nonemulsified oils/lecithins, carbon-based hydrogen release compounds, vegetable matter + ZVI amendments, oils + ZVI reagents) are available to facilitate the anaerobic biodegradation or ISCR of halogenated compounds. Many remediation professionals know from their own experiences that these amendments have been used with varying degrees of success in terms of overall remedial performance. Inherent to the biological fermentation process is the production of methane. As discussed next, this can be significant, especially during the early phases of remedial actions. A renewed focus on efficiency and safety governed by compliance with new regulatory guidelines encourage changes in the standard practice of applied bioremediation.

WHAT IS A METHANOGEN?

In the 1970s, Dr. Carl Woese and his colleagues at the University of Illinois–Urbana studied prokaryotic relationships using DNA sequences and they found that microbes that

produce methane—or methanogens—are Archaea (Woese & Fox, 1977). The identification of this new domain of microorganism was very important for many reasons, but from our perspective herein, this vast difference in genetic composition means that methanogens are significantly different from typical heterotrophic bacteria and eukaryotes. In other words, *Dehalococcoides ethenogenes* are as different from methanogens as humans are, and technologies can therefore interact with them quite specifically.

Methanogens are often the dominant hydrogenotrophs (i.e., consumers of hydrogen) in many environments because they have a lower utilization threshold for H_2 than do acetogens, and because the energy yield from the conversion of CO_2 and H_2 to methane is greater than that for conversion to acetate (Bates et al., 2011). If a given environmental setting is biogeochemically reducing, it is predictable that indigenous methanogens are the most numerous, fastest growing microbes present. However, when methanogens are inhibited, acetogens, such as *Clostridium* and many other microbes with a broad range of catabolic abilities, will thrive and produce acetyl-CoAQ/acetate and other volatile fatty acids (VFAs) from H_2 and CO_2 via the Wood–Ljungdahl pathway. In an anaerobic environmental remediation setting, halorespiring and other bacteria, such as *Desulfobacter* spp. and *Desulfuromonas* spp., will also utilize the available hydrogen for dechlorination of targeted COIs, and the VFAs will be fermented to ultimately yield CO_2 (Schauder et al., 1986).

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PROBLEMS ASSOCIATES WITH EXCESSIVE METHANE PRODUCTION DURING ERD AND ISCR REMEDIAL ACTIONS

There are important consequences to excessive methanogenesis during a remedial action, which requires an increased awareness on the part of remedial practitioners:

- cost and efficiency issues;
- generation of secondary plumes of arsenic (and other heavy metals);
- potential health and safety issues; and
- new and emerging regulatory issues.

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. Exhibit 3 (Mueller et al., 2014a) presents a site example where hydrogen demand is calculated for a highly aerobic source area measuring approximately 1,850 cubic yards. Hydrogen demand for complete dechlorination of all PCE and TCE mass to ethene within this source area example, including both adsorbed and dissolved contaminants, is less than the amendment consumed to generate 20 mg/L of methane. The same is true of reducing all competing electron acceptors (e.g., dissolved oxygen, nitrate, sulfate, and bio-available iron and manganese) within the hypothetical treatment zone. So, even though this example site is highly oxidized with relatively high total concentrations of PCE and TCE, generating just 20 mg/L of methane constitutes greater than 33 percent of the total amendment consumption based on moles of hydrogen equivalent (H_2). Some ERD and ISCR sites have initially produced >800 mg/L methane, which resulted in effervescence and sample

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Exhibit 3. Hydrogen demand for complete dechlorination of PCE/TCE in hypothetical source area (Courtesy Troy Fowler, IET Inc.—per Mueller et al. [2014a])

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	632
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

off-gassing (Peale et al.,2010), something we now recognize as undesirable and potentially problematic.

Generation of Secondary Plumes of Arsenic (and Other Heavy Metals)

Arsenic plumes have often been associated with reduced environments, such as those associated with landfills (US Geological Survey [USGS], 2004) and other carbon-rich environments (Brown et al., 2010), which can be induced by standard ERD practices. When an arsenic plume results from an ERD application, it is often attributed to the fact that arsenate (AsV) is reduced to the more soluble arsenite (AsIII). Under ISCR conditions, arsenic plumes should be less extensive given the presence of ZVI (and potentially sulfides), which aids in the formation of stable arsenic complexes, such as arsenopyrite (Blowes et al., 2000; Craw et al., 2003; Manning et al., 2002). Nonetheless, secondary arsenic plumes have also been observed under ISCR conditions (Mueller et al., 2014b), which was confusing until recent studies helped bring attention to a part of the equation that is often overlooked involving methylation of arsenic and other heavy metals.

It is known that methanogens (and of course other microorganisms) can methylate almost all Groups IV, V, and VI heavy metals with the possible exception of lead (Magnun

et al., 2015; Thomas et al., 2011). As such, a wide variety of methylated metalloids and metals can be found in the environment. These methylmetal(loids) are usually volatile, and with few exceptions, they are more toxic than their inorganic counterparts due to increased water solubility and lipophilicity (Michalke et al., 2006). It is long understood that microorganisms are primarily responsible for the biosynthesis of organo-metals (Challenger, 1945), and that the activity of methanogens is a main source of their production. Hence, if the number and activity of methanogens are limited under antimethanogenic ERD or ISCR conditions, then the targeted metal contaminants are more likely to be included in the desired stabilization reactions. Moreover, the overall toxicity of the site is not increased via the generation of methylmetal(loids) (e.g., biomethylation of arsenate) as an undesired consequence of the remedial treatment process.

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Potential Health and Safety Issues

Methane is the second most prevalent greenhouse gas emitted in the United States, accounting for about 10 percent of all U.S. greenhouse gas emissions from human activities in 2013 (US EPA at www3.epa.gov/climatechange/ghgemissions/gases/ch4.html). Methane's lifetime in the atmosphere is much shorter than CO_2, but methane is more efficient at trapping radiation than CO_2. Pound for pound, the comparative impact of methane on climate change is more than 25 to 36 times greater than CO_2, over a 100-year period.

Methane is explosive, with a lower explosive limit (LEL) of 5 percent and an upper explosive limit of 15 percent. As a result of the microbial fermentation process, methane will be produced in most situations following the addition of any conventional ERD or ISCR amendment. Excessive and extended production of methane can result in elevated groundwater concentrations (methane concentrations exceeding 800 mg/L have been reported following the addition of EHC®, a conventional ISCR Reagent—see Peale et al. [2010]), which can lead to methane accumulation in soil gas. Subsequent methane migration can pose serious concerns for utility corridors and vapor intrusion to indoor air. While this is perhaps more relevant in urban settings where methane can accumulate in basements, under slabs/foundations, and/or migrate along utility corridors, excessive methane production has been observed in open spaces (nonurban) and it can have unexpected, negative consequences.

New and Emerging Regulatory Issues

Recognizing the issues mentioned previously, Federal (USEPA, 2015) regulations and state-specific guidelines for methane in indoor air have been promulgated, with others pending for groundwater and soil gas. For example, Exhibit 4 summarizes current guidelines for methane in groundwater specifically for anaerobic (e.g., ERD or ISCR) bioremediation applications in Indiana (Indiana Department of Environmental Management [IDEM], 2015). In brief, a methane mitigation plan should accompany all anaerobic bioremediation work plans and soil gas monitoring should be undertaken during remedial action, especially if induced methanogenic conditions are below a structure where oxygen is not as easily replenished. If sub-slab methane concentrations exceed 10 percent of the LEL, then mitigation should be undertaken. Likewise, if anaerobic

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Exhibit 4. Summary of IDEM's suggested screening and action levels for methane production at anaerobic bioremediation sites (IDEM, 2015)

Samples Medium	Methane Concentration	Recommended Action
Groundwater	>10 mg/L	Monitor soil gas
	>28 mg/L	Mitigate
Soil gas outside building	>10% LEL	Check for receptors
Soil gas inside building (subslab)	<10% LEL	Monitor and report
	>10% LEL	Mitigate
Indoor air	>10% LEL	Evacuate/mitigate

conditions are induced in the vicinity of subsurface confined spaces, a mitigation contingency plan in addition to monitoring should be proposed. Notably, according to discussions with project managers several ERD projects, which were intended to use liquid carbon (emulsified oils/lecithins) sources, have failed to receive regulatory approval from various state agencies due to issues associated with excessive production of methane during previous technology applications.

HOW CAN WE ACTIVELY MANAGE METHANOGENS DURING REMEDIAL ACTIONS?

Clearly, it is increasingly important for remediation practitioners to consider methane production during bioremediation projects. If methanogenesis is excessive with consequences noted previously, then active mitigation measures such as vapor extraction or venting are required. In some cases where there are known receptors or other issues, proactive measures would be desirable in order to prevent excessive methanogenesis from causing potential problems. Here, newly developed technologies, as described by Scalzi and Karachalios (2015), can represent active measures to control the production of methane, which can offer multiple advantages in terms of cost, regulatory compliance, treatment efficiency, and safety.

Methanogens Are Everywhere

Under idealized conditions such as those induced during ISCR applications, methanogens can double their cell numbers in less than one hour, whereas *Dehalococcoides* spp. typically require 24 to 48 hr. Hence, methanogens, due to their much faster rate of replication, often dominate the subsurface microbial ecosystem associated with ISCR remedial approaches. The University of Colorado recently completed an assessment of microbial diversity in 146 soil samples collected from a range of ecosystem types around the world. The study concluded that an average of 2 percent of all soil microbes are Archaea,

with some samples exceeding 15 percent of the total estimated soil populations based on 16S rRNA gene sequencing (Bates et al., 2011). In contrast, 574 samples collected around the United States contained median *Dehalococcoides* spp. concentrations ranged between just 100 to 1,000 cells/mL, including sites in biostimulated conditions. Even at bioaugmented projects, the average *Dehalococcoides* spp. concentrations were only 10^5 cells/mL. With natural groundwater commonly containing microbial populations ranging between 10^5 and 10^7 cells/mL and biostimulated populations rising to over 10^8 cells/mL, Archaea populations can be orders of magnitude greater than target *Dehalococcoides* spp. microbes. Under both bioaugmented and biostimulated conditions, the vastly inferior *Dehalococcoides* spp. population typically struggles to compete against methanogenic Archaea for available hydrogen and nutrients, regardless of the electron donor/fermentative carbon source used. By inhibiting the growth and proliferation of methane producing Archaea, chlororespiring bacteria can become a more dominant component of the bacterial populations.

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Methanogens Are Useful

There are recognized benefits to low levels of methanogenesis, hence our desire to control or impede their activity during remedial action but not to eliminate them. For example, (i) methanogens are known to play important roles in synergistic microbial ecology, (ii) their metabolic activity can help maintain anoxic conditions in treatment zones (through seasonal changes), and (iii) the activity of methane mono-oxygenases and other enzymes can stimulate co-metabolic bacterial activity for compounds, such as TCE/DCE/VC in redox-recovery zones. Hence, limited production of methane is part of a healthy ERD/ISCR application. Complete reductive dechlorination of chlorinated ethenes relies on the utilization of hydrogen gas produced by fermentative microbes. Archaea (methanogens) use this hydrogen to produce methane, which is in direct competition with the targeted, complete dechlorinators, such as *Dehalococcoides* spp. Therefore, as discussed previously, excessive methane production represents a costly waste of remedial amendments, and it can yield undesired and unsafe consequences.

Microorganisms Can Control Each Other

Microbes are known to synthesize bioactive compounds that allow them to compete more effectively for food, nutrients, and space. For example, in 1928, Sir Alexander Fleming discovered that *Penicillium notatum* mold produced a substance that inhibited the growth of *staphylococci*. Many years of research ultimately discovered that the *Penicillium* mold produced a complex organic molecule that interfered with the cross-linking of some types of bacterial cell wall components, notably in Gram-positive bacteria. This discovery of the first commercial antibiotic arguably changed the course of human history, and guided additional research into how naturally derived, complex molecules can be further utilized to improve our health and quality of life.

Penicillium spp. continued to be investigated for other potential benefits and, in 1971, a class of complex organic molecules named "statins" were discovered as a way to inhibit cholesterol production. Cholesterol, first identified in 1769, is a large organic molecule essential to the structural integrity and fluidity of animal cell membranes. Plants also make small amounts of cholesterol-type compounds, named phytosterols. Cholesterol came to

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the forefront of the heart disease discussion in 1961, when data from the five-year Framingham Heart Study were published and suggested that men under the age of 50 and with elevated blood cholesterol levels were at greater risk of heart disease. This finding triggered a race to find a molecule that lowered blood serum cholesterol levels. Statins can be defined as "a class of lipid-lowering drugs that reduce serum cholesterol levels by inhibiting a key enzyme involved in the biosynthesis of cholesterol." The most likely target was 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, a key enzyme involved in the human cholesterol biosynthesis pathway (Alberts et al.,1980).

Statin Warfare

With the mechanism for the human production of cholesterol identified, Dr. Akira Endo's work with *Penicillium citrimum* enabled the discovery of a large complex molecule named mevastatin, the first compound that effectively inhibited the pathway known as the mevalonate (or HMG-CoA reductase) pathway (Endo, 2010). It turns out that the mevalonate/HMG-CoA pathway is key to both higher eukaryotes, as well as, many bacteria for the production of proteins, hormones, protein anchors, and steroid synthesis. Much as penicillin works to interfere with the structural integrity of bacterial cell walls to offer a competitive advantage, *Penicillium*-excreted mevastatin stops the mevalonate pathway to interfere with the growth of competitive bacteria. Mevastatin never ended up being marketed due to harmful side effects for humans. However, this discovery prompted other researchers to look for other variants.

Identification of a Methanogen-Inhibiting Yeast Strain

Lovastatin ($C_{24}H_{36}O_5$) is a fungal metabolite isolated from cultures of *Aspergillus terreus* and other organisms. Lovastatin is widely known as a potent HMG-CoA pathway inhibitor and has been used for decades to lower cholesterol in human blood. Lovastatin was the first statin approved by the United States Food and Drug Administration in 1987 as a hypercholesterolemic drug.

Continued research into the complex organic molecules produced by various fungi found a statin-producing yeast strain of interest, named *Monascus purpureus*. When cultured with rice as the growth substrate, the yeast successfully produces Monacolin K (Lovastatin) along with a host of other monacolins. Known for its distinctive color, the end product is commonly marketed as nutritional red yeast rice (RYR) extract known to provide a supplemental source of mono-unsaturated fatty acids, vitamins, and other nutrients. Red yeast extract has also been used in the cattle industry for decades in efforts to manage rumen microbiology and to control nonbeneficial methane production in cows (Henderson et al., 2010). In addition to its use as a nutritional supplement for humans and bovines, RYR is also used as a food coloring and food preservative.

Monacolin K inhibits methanogenic Archaea because cell membrane production in Archaea shares a similar pathway with cholesterol biosynthesis (Miller & Wolin, 2001).

Mechanism of Monacolin K Inhibition of Methanogens

Monacolin K inhibits methanogenic Archaea because cell membrane production in Archaea shares a similar pathway with cholesterol biosynthesis (Miller & Wolin, 2001). More specifically, bacterial cell walls are predominantly comprised of murein (peptidoglycan). Archaea, however, do not produce murein, rather, their cell walls are

composed of various sulfated-heteropolysaccharides, proteins, and glycoproteins/lipids along with pseudomurein—a structural analog of murein. Murein is biosynthesized via activity similar to that of HMG-CoA reductase, which yields cholesterol in humans. In the presence of a red yeast–derived monacolins (e.g., Monacolin K), the pseudomurein biosynthesis pathway is interrupted, and methanogens are restricted from growth and proliferation. Because Archaea methanogens are so uniquely different than bacteria, the inhibitory effect of RYR-derived monacolins is not observed in microbes that are typically associated with (1) catabolism of organic contaminants (e.g., *Pseudomonas* spp.) and/or (2) halo-respiration/biodegradation of chlorinated solvents (e.g., *Dehalococcoides* spp.).

Laboratory proof-of-concept studies documented the ability of the methane control technologies to yield expected results under controlled conditions.

FIELD APPLICATION OF ANTIMETHANOGENIC REAGENTS DURING REMEDIAL ACTIONS

Humans have been consuming statins since 1978, and RYR has been used as a nutritional supplement and bio-manipulation strategy in animal husbandry for decades. But its use during environmental remediation applications has only recently been deployed (Mueller et al., 2015; Scalzi & Karachalios, 2015). Provectus Environmental Products, Inc., of Freeport, Illinois, provides remedial amendments containing RYR designed for *in situ* environmental applications including (i) Provect-CH4® methanogen control technology for supplemental use with conventional ERD/ISCR amendments sold by myriad others, (ii) Provect-IR® antimethanogenic ISCR reagent, (iii) Provect-IRM® antimethanogenic ISCR reagent for heavy metal immobilization, (iv) EZVI-CH4™ antimethanogenic ISCR reagent for DNAPL treatment, and (v) AquaGate®-CH4 antimethanogenic reagent for *in situ* sediment capping. Laboratory proof-of-concept studies documented the ability of the methane control technologies to yield expected results under controlled conditions (Mueller et al., 2014a). Results from field-scale applications summarized next further validate the beneficial use of methane control during *in situ* remedial actions.

Case Study—Active Dry Cleaning Facility in an Urban Setting

Site background

Shallow groundwater at a site in the Midwest United States was located approximately 1.5 m below ground surface (mbgs) and was confined by a clay aquitard at about 4 mbgs. Operations at an active dry cleaning facility since 1968 resulted in releases of PCE from a variety of sources, including a former aboveground storage tank and a former underground storage tank both of which were previously removed. The shallow groundwater in a suspected source area was impacted by PCE (maximum 35,000 ppb) and TCE (maximum 14,000 ppb) along with an accumulation of anaerobic catabolites *cis*-1,2-DCE (maximum 25,000 ppb) and lesser amounts of VC (maximum 3,800 ppb). Contaminated groundwater migrated through a sandy aquifer into a damaged storm sewer. Moreover, an active sanitary sewer feeder from the active dry cleaner operations was thought to be exacerbating the PCE migration problem by allowing warm water with potential contaminants and surfactants to enter the groundwater.

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Remedy selection

A remedial response action was proposed to reduce the concentration of PCE entering the storm sewer and subsequently impacting surface water. Given the shallow groundwater in an urban setting, the design engineers and the state regulators had special interest in assuring that the remedial action would not stimulate excessive methanogenesis, which could create indoor air/vapor intrusion issues (methane could induce contaminant migration) and other potential safety issues associated with high levels of methane itself. A rigorous, critical analysis of various commercially available remedial amendments (e.g. EHC®, EZVI, Ferox-Plus™, and Provect-IR®) considered cost, longevity, injection capabilities, and predicted performance—including methane production. Provect-IR® was identified as the best alternative for this site based on its ability to (i) minimize the potential issues associated with elevated methane in groundwater, soil gas, and indoor air, and (ii) promote more efficient use of the hydrogen donor so less amendment was required.

Field application

For initial proof-of-concept field testing, 454 kg of antimethanogenic ISCR reagent was injected into the targeted aquifer zone as an aqueous slurry containing about 23 percent solids via four injection points, spaced approximately 2 to 3 m apart. The pilot test area measured approximately 6 m long × 6 m wide × 2 m deep (from approximately 1–3 mbgs) surrounding an existing monitoring well, MW-MP-2 (screened from 1–3 mbgs with water at approximately 1.5 mbgs), located within a known source area (Exhibit 5). A total of 3 L of DHC inoculum (SDC-9® source CB&I—Oak Ridge, TN) containing >5 × 10E10 DHC/L was distributed evenly throughout each injection interval along with the Provect-IR amendment.

Performance monitoring

To validate performance in terms of CVOCs removal, without the accumulation of catabolic intermediates or the generation of excessive methane, data were obtained from seven monitoring points (Exhibit 6) using a variety of field measurements and laboratory analyses, as described next. Groundwater samples were acquired from four well locations, MW-MP-2, MW-15-1S, MW-15-1D, and MW-15-2D, at Day = 0, immediately after the injection event (Day = 1), and 30, 60, and 90 days after injections were completed. Groundwater samples were collected utilizing low-flow sampling techniques. The samples were properly preserved, packaged, and delivered to an off-site environmental laboratory and analyzed for the following:

- pH—excessive fermentation can acidify aquifer groundwater and impede microbiological activity;
- Turbidity—excessive turbidity may compromise data and indicate methane bubbles (above the saturation level);
- Groundwater elevation—influence during injection can provide an estimate of injection radius of influence (ROI) or subsurface amendment distribution;
- DO/ORP—rapid reductions in redox should be observed in the treatment zone within the ROI;

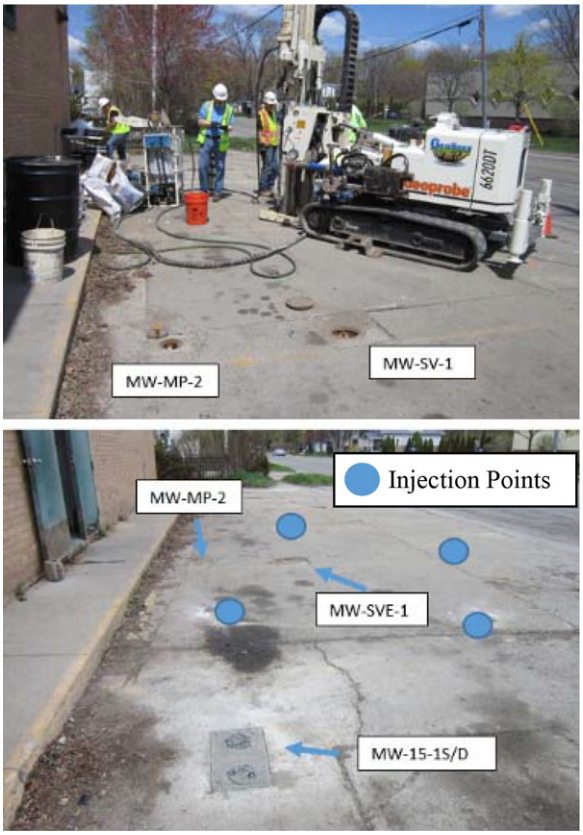

Exhibit 5. Layout of Provect-IR® injections and location of monitoring wells

Exhibit 6. Summary of sampling points for field-scale pilot test

Monitoring Well Name	Proximity to Treatment Zone	Screen Interval (mbgs)	Parameters
MW-MP-2	Within treatment zone	1.2 to 2.7	Air and groundwater
SVE-1	Within treatment zone	0.5 to 1.2	Air only
MW-15-1S	1.5 m downgradient	0.9 to 1.8	Air and groundwater
MW-15-1D	1.5 m downgradient	1.8 to 3.3	Groundwater only
MW-15-2S	6 m downgradient	1.2 to 2.1 (dry)	Air only
MW-15-2D	6 m downgradient	1.8 to 3.3	Groundwater only
SV-1/Sewer lines	Outside treatment zone		Hand held air only

- Dissolved gasses (CH_4, CO_2, ethane, and ethene)—documents effectiveness;
- VOCs—documents effectiveness and ROI along with mass loading;
- Total and dissolved metals, including iron, calcium, magnesium, manganese, and Michigan 10 metals;
- Anion scan (chloride, sulfate, nitrate, and nitrite); and
- Total organic carbon (TOC)—indicates the presence of ISCR reagents and microbiological activity.

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Soil and air samples were collected from four wells: MW-MP-2 and SVE-1 both located within the treated area, MW-15-1S (shallow) located approximately 1.5 m downgradient of the treated area, and MW-15-2S (shallow) located approximately 6 m downgradient of the pilot test area. In addition, air samples were obtained from the nearby storm sewer grate and well SV-1 located inside the dry cleaning facility. Air samples were analyzed for the following parameters:

- Well head gases using a portable detector (CH_4/LEL, CO, H_2S, VOCs, CO_2,)—health and safety issues; and
- Laboratory samples (for CH_4, ethane, and ethene)—documents effectiveness, helps determine presence of ISCR reagents.

Results within treatment zone (source area)

Monitoring well MW-MP-2 was located directly within the pilot test area, which had the highest level of CVOCs (being a suspected source area). Following the application of Provect-IR, there were modest increases in dissolved iron and TOC which can indicate the presence of the Provect-IR reagent (data not shown), and this was accompanied by a rapid (within 30 days) decrease in ORP from a baseline of +14 mV to –358 mV and reducing conditions (about –50 mV) were sustained for the entire 90-day monitoring period. The injection event was accompanied by a notable increase in CVOCs, which is a common phenomenon attributable to perturbations and desorption associated with the energy of physically adding reagents to an aquifer matrix (Mueller et al., 2010). However, the concentration of PCE and TCE in groundwater were decreased by 72 percent (from 35 ppm at Day 1 to 9.9 ppm at Day 90) and 50 percent (from 14 ppm at Day 1 to 7.1 ppm at Day 90), respectively, without the stoichiometric accumulation of *cis*-DCE or VC. Total CVOCs in groundwater were reduced by 47 percent over the 90-day period (from 76.7 ppm at Day 1 to 40.6 ppm at Day 90). In the absence of any residual CVOC source, continued incubation would be expected to yield further reductions in contaminant mass.

Given its proximity to the Provect-IR injections, soil gas data from MW-MP-2 were expected to provide the most likely opportunity to measure the effect of the active methane control features of the Provect-IR amendment.

Given its proximity to the Provect-IR injections, soil gas data from MW-MP-2 were expected to provide the most likely opportunity to measure the effect of the active methane control features of the Provect-IR amendment. There were no increases in groundwater methane concentrations during any sampling event, with dissolved methane concentrations ranging from 1.1 mg/L at Time = 0 to a maximum of 1.4 mg/L 60 days after Provect-IR additions (data not shown). Vapor readings from this well showed a very slight increase in methane from a baseline reading of 100 ppmv to 300 ppmv 30 days after the injection event, with subsequent monitoring showing no methane present (<20 ppmv). Soil gases contained measurable and variable concentrations of PCE, TCE, *cis*-1,2-DCE, *trans*-1,2-DCE, 1-1-DCE, and VC. But given its proximity to the source area, changes over time were difficult to interpret. Soil gas data from SVE-1 showed similar responses (data not shown).

Results 1.5 m downgradient of Provect-IR treatment zone

Monitoring wells MW-15-1S (shallow) and MW-15-1D (deep) were located about 1.5 m downgradient from the amendment injection area. As these monitoring locations were

proximal to the injection points, the injection event was again accompanied by a slight increase in groundwater CVOCs concentrations immediately following the addition of reagent to the subsurface (data not shown). Subsequently, however, the concentrations of PCE and TCE in groundwater for MW-15-1S decreased by >99 percent (from 39 ppb at Day 1 to <1 ppb at Day 90 and from 29 ppb at Day 1 to <1 ppb at Day 90, respectively), which was accompanied by a 96 percent reduction of *cis*-DCE (reduced from 140 ppb at Day 1 to 5 ppb at Day 90) and 94 percent reduction in VC (reduced from 360 ppb at Day 1 to 20 ppb at Day 90). Total CVOC reduction of 95 percent was observed over the 90-day period (from 603 ppb at Day 1 to 28 ppb at Day 90). In the absence of a residual CVOC source, continued incubation would predictably yield further reductions. Similarly, MW-15-1D exhibited significant reductions in CVOCs, with the concentration of PCE and TCE in groundwater being reduced by >99 percent (from 3,500 ppb at Day 1 to <1 ppb at Day 90 and from 1,600 ppb at Day 1 to <1 ppb at Day 90, respectively). There were no discernible changes in *cis*-DCE or VC, which indicated CVOC destruction without the accumulation of catabolites, that are representative of ISCR conditions. Total CVOCs in groundwater were reduced by 63 percent over the 90-day period (from 8,720 ppb at Day 1 to 3,252 ppb at Day 90).

Immediately following the application of Provect-IR, there was a rapid (within 30 days) decrease in ORP, with the deeper well showing more extensive and sustained reductions from a baseline of –47 mV to –345 mV after 30 days, and reducing conditions (about –48 mV) being sustained for the entire 90-day monitoring period. There were no discernible increases in dissolved iron or TOC, which would directly indicate the presence of the Provect-IR reagent. For MW-15-1S, the dissolved methane concentrations ranged from 0.74 mg/L at Time = 0 to 1.6 mg/L 60 days after Provect-IR additions. For MW-15-1D, the dissolved methane concentrations ranged from 1.3 mg/L at Time = 0 to a maximum of 1.9 mg/L 90 days after Provect-IR additions.

Immediately following the application of Provect-IR, there was a rapid (within 30 days) decrease in ORP, with the deeper well showing more extensive and sustained reductions from a baseline of –64 mV to –134 mV after 30 days, and reducing conditions (about –84 mV) being sustained for the entire 90-day monitoring period.

Results 6 m downgradient from Provect-IR treatment zone

Monitoring wells MW-15-2S (shallow) and MW-15-2D (deep) were located about 6 m downgradient from the pilot test area. Groundwater samples were not consistently recoverable from the shallow well (dry), hence data are not presented.

Being more distant from the injection points, the injection event would be expected to yield less noticeable effects on CVOC concentrations in MW-15-2D immediately following the application of Provect-IR. However, the concentrations of PCE and TCE in groundwater decreased by 95 percent (reduced from 19 ppb at Day 0 to 1 ppb at Day 90) and by 87 percent (reduced from 7.5 ppb at Day 0 to 1 ppb at Day 90), respectively. This was accompanied by a 64 percent reduction in *cis*-DCE (reduced from 120 ppb at Day 0 to 43 ppb at Day 90) and 40 percent reduction in VC (reduced from 52 ppb at Day 0 to 31 ppb at Day 90). Total CVOC reduction of 62 percent was observed over the 90-day period (reduced from 199 ppb at Day 0 to 76 ppb at Day 90).

Immediately following the application of Provect-IR, there was a rapid (within 30 days) decrease in ORP, with the deeper well showing more extensive and sustained reductions from a baseline of –64 mV to –134 mV after 30 days, and reducing conditions (about –84 mV) being sustained for the entire 90-day monitoring period. There was a slight increase in dissolved iron but no change in TOC, which would directly indicate the

presence of the Provect-IR reagent. There were no notable increases in groundwater methane concentrations during any sampling event. The dissolved methane concentrations ranged from 1.7 mg/L at Time = 0 to a high of 2.2 mg/L 60 days after Provect-IR additions. Soil gases collected from SV-1 showed a very slight increase in methane from a baseline reading of <20 ppmv to a high of 94 ppmv 30 days after the injection event, with subsequent monitoring showing no methane present (Day 60 and Day 90 < 20 ppmv). Soil gases contained measurable and variable concentrations of PCE, TCE, *cis*-1,2-DCE, *trans*-1,2-DCE, 1,1-DCE, and VC.

Molar analysis

Over the 90-day period, total CVOC reductions of 47 percent, 79 percent, and 62 percent were observed within the Provect-IR treatment zone (source area), 1.5 m downgradient, and 6 m downgradient thereof, respectively. When analyzed from a total molar perspective, the significance of these reductions becomes clear. In the source area (with approximately 70 ppm total CVOCs) that was directly treated with Provect-IR, the prime contaminants PCE and TCE were reduced from 51 percent molar mass to 30 percent molar mass and there was not an accumulation of DCE or VC as dead-end catabolites (MW-MP-2 per Exhibit 7a). Proximal to the treated source area, the parent compounds were completely removed and ethene/ethane represented a majority of the molar mass (MW-15-1S/D per Exhibits 7b and 7c). Further downgradient from the treated area, these responses were slower to develop, which would be expected given the time and distance (MW-15-2D per Exhibit 7d) and early profiles can be used as comparative purposes to account for changes associated with extraneous processes.

Discussion

Given the shallow water table and the presence of the site building, the production of methane was a concern along with potential off-gasing of CVOCs. To minimize these concerns, the size of the pilot test cell was minimized and located outside the building footprint, and Provect-IR antimethanogenic ISCR reagent was chosen. Nevertheless, the project identified the following contingencies:

Given the shallow water table and the presence of the site building, the production of methane was a concern along with potential off-gasing of CVOCs.

- Daylighting: If daylighting occurred, further injections would be terminated until the issue was resolved (possible lower injection rate, more injection points with less product per point, longer period between injections). Any product that surfaced was properly managed.
  Results: Some surfacing issues at the shallower injection intervals were encountered in areas proximal to a former excavation. The issue was managed by increasing the depth of the injection intervals noting that the amendment would rise upward into the targeted interval.
- Indoor Air: Excessively elevated levels of VOCs or methane would result in additional vapor sampling in the subsurface (including vapor points located inside the drycleaners and outside the treatment zone) and in the breathing zone. The building and/or subsurface would be ventilated as necessary.
  Results: No issues encountered until the end of the study at one proximal well location, MW-15-1S. Follow-up monitoring to confirm soil gas data and potentially delineate the source of VOCs (BTEX and related nonchlorinated hydrocarbons), CVOCs (some previously undetected),

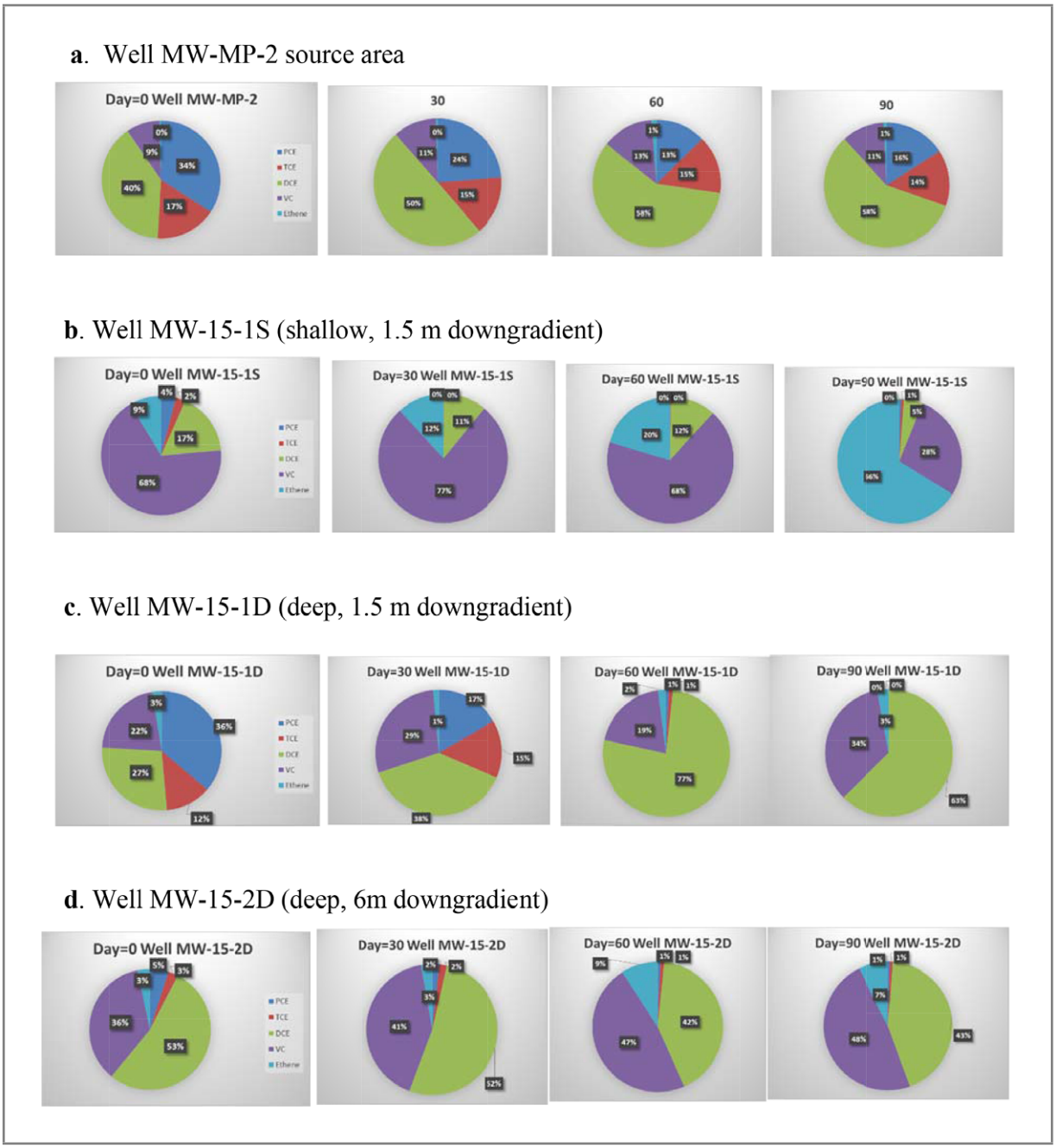

Exhibit 7. Molar analysis

CH_4, and other gas constituents was recommended (release from dry cleaners, vehicle emissions, etc.).

CONCLUSIONS

Ubiquitous, natural methanogenic Archaea convert hydrogen, produced from injected carbon and the corrosion of ZVI, into methane. Production of methane—sometimes in great excess—during the deployment of conventional ERD/ISCR reagents is a common

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occurrence that is receiving increased attention and regulatory scrutiny. While low level activity of methanogens may be beneficial in maintaining anoxic conditions and stimulating co-metabolism, higher concentrations indicate that the electron donor capacity of injected amendments is wasted because it was not utilized by acetogens or other microbes for dehalorespiration. By restricting the growth and proliferation of methane-producing Archaea, chlororespiring bacteria can develop more dominant populations, enabling them to better compete for the supplied electron donor materials during remedial actions. This results in increased remediation efficiency and cost effectiveness (i.e., less substrate required = lower amendment costs; decreased implementation time due to less substrate = lower implementation costs). Controlled methanogenesis can also reduce potential health and safety risks associated with induced contaminant migration and/or methane accumulation in places such as utility corridors and indoor air.

The discovery of statins, a group of biologically active compounds that interfere in the production of cholesterol in humans, has been shown to have evolutionary roots in inhibiting the growth of certain competitive microbes. Statins derived from a strain of *M. purpureus* yeast have been identified as able to control undesirable methanogenesis through the interruption of enzymatic processes unique to Archaea under various environmental settings. This capability is now available to the remediation industry (Scalzi & Karachalios, 2015) within several unique products that incorporate *M. purpureus* yeast with the goal of inhibiting methanogenesis across the full range of ERD/ISCR remedial implementations. Recent laboratory and field studies clearly document the benefits to project cost, health and safety, and regulatory compliance.

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