Controlling Methane at ERD and ISCR Applications

Active measures to control the production of methane can offer multiple advantages in terms of cost, treatment efficiency and safety.

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In-situ chemical reduction (ISCR) and enhanced reductive dehalogenation (ERD) have demonstrated success in the remediation of numerous recalcitrant and toxic compounds, including chlorinated ethenes and hexavalent chromium. ISCR describes the combined effect of stimulated biological oxygen consumption (via fermentation of an organic carbon source), direct chemical reduction with zero-valent iron (ZVI) or other reduced metals. As described by Brown et al (2009)[1], the corresponding enhanced thermodynamic decomposition reactions that are realized at the lowered redox (Eh) conditions allow for more effective mineralization of many constituents of interest (COIs). A growing number of ERD substrates and other accelerated anaerobic bioremediation technologies (e.g., emulsified oils, non-emulsified oils, carbon-based hydrogen release compounds, vegetable matter + ZVI amendments, etc.) are available that facilitate the anaerobic biodegradation of halogenated compounds. Many readers will know from their own experiences that these amendments have been widely used with varying degrees of success in terms of overall remedial performance. One seemingly universal phenomenon has been the biological production of methane, especially during the early phases of remedial actions (look for it and you will find it). Active measures to control the production of methane can offer multiple advantages in terms of cost, treatment efficiency and safety.

What Is a Methanogen?

In the 1970s, Dr. Carl Woese and his colleagues at the University of Illinois at Urbana-Champaign studied prokaryotic relationships using DNA sequences, and they found that microbes that produce methane[2] – or methanogens – are Archaea. The identification of this new domain of microorganism was important for many reasons, but from our limited perspective, this vast difference in genetic com-

position means that methanogens are significantly different from typical heterotrophic bacteria and eukaryotes. In other words, Dehalococcoides ethenogenes are as different from methanogens as you are.

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 converting of CO_2 and H_2 to methane is greater than that of converting to acetate. 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 (See **Figure 1**). 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.[3]

What Is the Problem With Methane?

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. Table 1 presents a site example where hydrogen demand is calculated for a highly aerobic and oxidized 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 milligrams per liter (mg/L) of methane. The same is true of reducing all dissolved oxygen, nitrate, sulfate, and bio-available iron and manganese competing electron acceptors 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).

Potential Health and Safety Issues. Methane is considered to be a major greenhouse gas. It is explosive, with an LEL of 5 percent and an UEL 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 (as high as 1,000 mg/L have been reported), which can lead to accumulation in soil gas. Subsequent methane migration can pose serious concerns for utility corridors and vapor intrusion to indoor air.

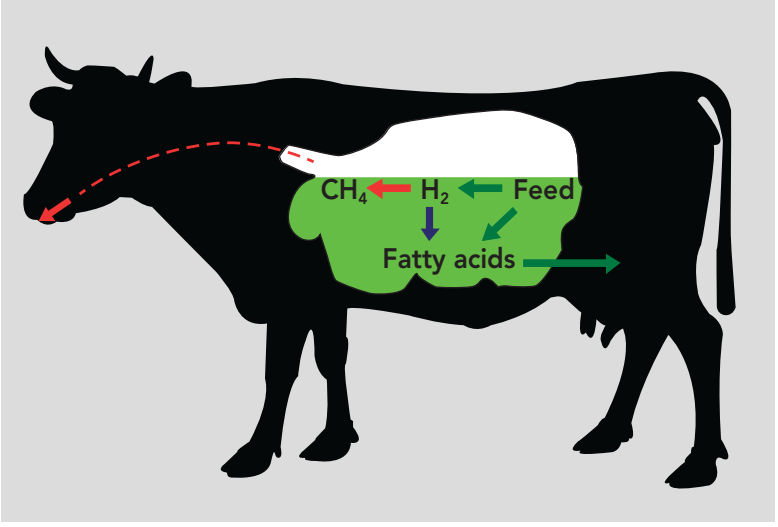

Figure 1: What happens to H_2 when methanogens are inhibited?[4]

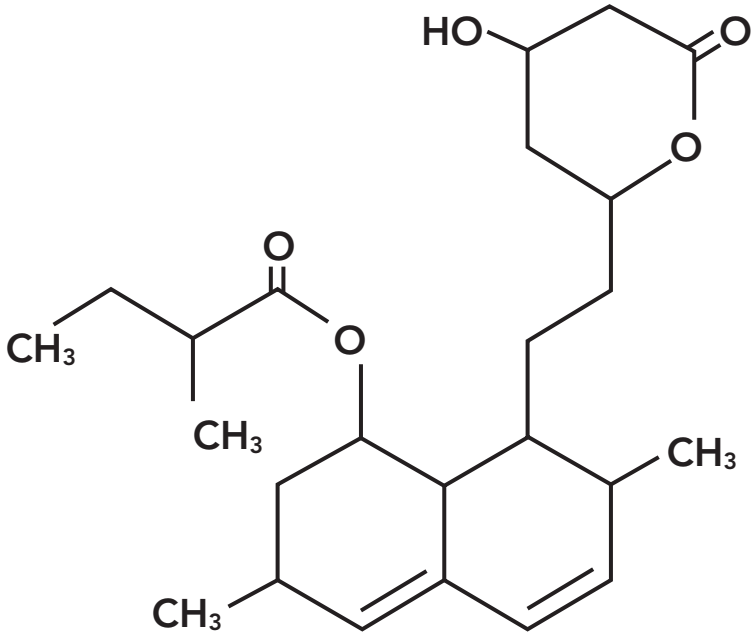

Figure 2: Chemical structure of Lovastatin

While this is perhaps more relevant in urban settings where methane can accumulate in basements, under slabs/foundations or migrate along utility corridors, excessive methane production has been observed in open spaces, and it can have unexpected, negative consequences.

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 to 28 mg/L (Indiana Department of Environmental Management, 2014). Notably, several ERD projects that were 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 communication – State of California; State of Minnesota). As a result, many remedial practitioners proactively design contingencies for conventional ERD/ISCR implementation in the event that dissolved methane exceeds a threshold level ranging from 1 mg/L to 10 mg/L. These contingencies often entail expensive and extensive systems for capturing and treating methane in soil gas/vapor captured via soil vapor extraction systems.

Why Is Excessive Methane Production Ubiquitous?

There are some recognized benefits to low levels of methanogenesis. For example, 1.) methanogens are known to play important roles in synergistic microbial ecology, 2.) their metabolic activity can help maintain anoxic conditions in treatment zones (through seasonal changes), and 3.) the activity of methane mono-oxygenases and other enzymes can stimulate co-metabolic activity of compounds such as TCE/DCE/VC in redox-recovery zones. Hence, limited production of methane is part of a healthy ERD/ISCR application. However, excessive methane production can be dangerous and represents a costly waste of amendment.

Complete reductive dechlorination of chlorinated ethenes relies on the utilization of H_2 produced by fermentative microbes. Competing against the complete dechlorinators, such as Dehalococcoides spp., Archaea can use this hydrogen to produce methane. The University of Colorado recently completed an assessment of microbial diversity in 146 soil samples taken 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.[4] In contrast, 574 samples collected around the United States found median Dehalococcoides spp. concentrations ranged between just 100 to 1,000 cells per milliliter, including sites in biostimulated conditions. Even at bioaugmented projects, the average Dehalococcoides spp. concentrations were just 10^5 cells per milliliter. With natural groundwater commonly containing microbial populations ranging between 10^5 to 10^7 cells per milliliter and biostimulated populations rising to more than 10^8 cells per milliliter, 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 the more dominant bacterial populations.

This constituted a 97 percent reduction and reduced soil concentrations well below the target Csat concentration of 1,200 mg/kg.

Can We Actively Manage Methanogens?

While various microbes have been cultured and used for the benefit of humans for millennia, it was within only the last 100 years that we discovered microbes also act to control each other. 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 arguably changed the course of human history and guided more research into how naturally-derived, complex molecules can be used to further improve our health and activity. 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.

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.[5] 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.

Statins That Inhibit Methanogenesis. Lovastatin ($C_{24}H_{36}O_5$; ***Figure 2***) 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.

Identification of a Methanogen-Inhibiting Yeast Strain. 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 non-beneficial methane production from cows.[6] In addition to its use as a nutritional supplement for humans and bovines, RYR is also used as a food coloring and food preservative.

Mechanism of Monacolin K Inhibition of Methanogens. Monacolin K can inhibit methanogenic Archaea because cell membrane production in Archaea shares a similar pathway with cholesterol biosynthesis.[7] More specifically, bacterial cell walls are predominantly composed 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 analogue 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), pseudomurein biosynthesis pathway is interrupted, and methanogens are restricted from growth and proliferation. And since Archaea methanogens are so different from bacteria, the inhibitory effect of RYR-derived monacolins is not observed in microbes that are typically associated with catabolism of organic contaminants (e.g., Pseudomonas spp.) or halo-respiration/biodegradation of chlorinated solvents (e.g., Dehalococcoides spp.).

Environmental Application and Test Results

While RYR has been used as a nutritional supplement and bio-manipulation strategy in animal husbandry for decades, environmental applications have only recently been deployed. Provectus Environmental Products Inc., of Freeport, Ill., has developed a food-grade product containing RYR designed for in-situ environmental applications. The product is being evaluated for effectiveness in inhibiting undesirable methanogenesis during the deployment of ERD/ISCR projects.

University Bench Testing Design. In collaboration with Western Michigan University, two anaerobic reactors were seeded with biomass that contained an active methanogenic population. The reactors were fed once per week to achieve chemical oxidant demand (COD) of 2,000 mg/L, and they were operated as anaerobic sequencing batch reactors at 22°C to 24°C. After one week of incubation, silty sand was added to each reactor resulting in a slurry having a solids concentration of 20 percent by weight. The reactors were allowed to operate for another week with the silty sand, to ensure that the sand did not affect methanogenic activity. During the first two weeks, both reactors were operated in an identical manner in order to establish baseline methanogenic conditions. During the third week, the test product was added to one reactor to achieve a concentration of 40 mg/L while maintaining the second reactor as an unamended Control (i.e., no product added). Because the 40 mg/L dose reduced methane production in the test reactor so rapidly and completely (see Table 2), it was decided to dose the Control reactor with 20 g/L of the product during the fourth week of operation.

Throughout the study, the volume of biogas produced was measured by periodically withdrawing a gas sample using a glass syringe inserted through a septum the top of each reactor. The methane content, as a percent of the overall biogas produced, was quantified by injecting into a gas chromatograph with a flame ionization detector (GC-FID). The reactors also had dedicated probes to measure pH and ORP. After each cycle (i.e., before feeding) a probe was inserted into the reactor to measure TDS, and a sample was collected to measure COD. The mixer was turned off during sampling and feeding to minimize the introduction of oxygen into the reactor contents.

University Bench Testing Results. Physical testing results are presented in **Table 2** below, including the volume of biogas produced, pH values, and the concentrations of COD, ORP, and TDS measured in the control and test reactors during the studies. The volume of biogas produced each feed cycle (i.e., each week) in the reactors ranged between 72 and 82 ml. Note that the volume of reactor gas produced was not affected by the introduction of silty sand during Week 2 of the Startup period. The reactors were fed a 2,000 mg/L COD solution each weekly cycle, which was rapidly consumed by the anaerobic culture. COD measurements after each feeding cycle ranged from 56 to 108 mg/L. Solution pH ranged between 6.1 and 6.4. ORP values were all close to -300 mV, which is typical of methanogenic conditions. The TDS in the reactors did not change appreciably over time, ranging from approximately 1,200 to 1,250 mg/L.

Prior to the addition of the test product, methane concentrations in the biogas varied from approximately 55 percent to 70 percent (Table 3), which indicated an active methanogenic culture. Following the addition of the test product to 40 mg/L in Reactor 2, the methane content of produced biogas was rapidly reduced from 62 percent to below detection (0.05 percent) within 11 days and remained below detection levels until the reactors were dismantled on day 17. Addition of the test product at 20 mg/L to Reactor 1 on day 7 reduced the methane content of biogas from 65 percent to below detection (0.05 percent) by day 17 (i.e., after 10 days). During the test period, the total volume of biogas produced per week in either reactor did not change appreciably (Table 1), only the methane concentration. Adding the product quickly shifted fermentative gas production from electron donor-consuming methanogenesis to primarily fermentation byproduct CO_2 (the bulk gas contained mostly CO_2).

Conclusions

Production of methane during the deployment of ERD/ISCR is a common occurrence that is receiving increased regulatory scrutiny in some situations. Natural methanogenic Archaea convert hydrogen, produced from injected carbon and the corrosion of ZVI, into methane. Studies have shown that initial conditions commonly favor Archaea over beneficial target microbes such as Dehalococcoides spp., based on superior baseline populations under variety of conditions. While low concentrations

of methane 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 bacterial populations.

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. The subgroup of monocolin statins derived from a strain of Monascus purpureus yeast have been identified as able to control undesirable methanogenesis. The tested product, Provect-CH4, is the first environmental remediation product to incorporate Monascus purpureus yeast with the goal of inhibiting methanogenesis. Recent university studies confirmed that in a closed, controlled system, the product effectively shut down methane production in an active methanogenic culture when added to create a solution containing at least 20 mg/L of the amendment. When deployed during ERD/ISCR, it may sharply reduce amendment waste associated with methanogenesis, as well as the health and safety risks associated with methane accumulation in places such as utility corridors and indoor air.

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Table 1. Hydrogen Demand for Complete Dechlorination of PCE/TCE in Hypothetical Source Area

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**

Table 2. A list of the biogas volume, pH values, and concentrations of COD, ORP, and TDS in the Control and the Test reactors throughout the studies.

Period	Gas Vol. (mL)	COD (mg/L)	pH	ORP (mV)	TDS (mg/L)
Reactor 1 ("Control" Reactor)					
Startup-Week +1	81	56	6.4	-302	1213
Startup-Week +2	72	91	6.3	-306	1241
Test-Week +1	75	61	6.2	-289	1258
Test-Week +2 (Add Provect-CH4 to 20 mg/L)	73	108	6.3	-296	1220
Reactor 2 (Initial Test Reactor)					
Startup-Week +1	79	72	6.2	-285	1244
Startup-Week +2	75	83	6.2	-298	1265
Test-Week +1 (Add Provect-CH4 to40 mg/L)	82	62	6.1	-306	1263
Test-Week +2	72	97	6.4	-287	1247

Table 3. Methane Concentrations (%) in Reactor Biogas during the 17 Day Test Period

Activity	Time (days)	Reactor 1 Methane (%)	Reactor 2 Methane (%)
Reactor 2 dosed with Provect-CH4 at 40 mg/L during Day 0	0	57	62 (+ Provect CH4)
	2	61	47
	4	68	32
	6	59	20
Reactor 1 dosed with Provect-CH4 at 20 mg/L during Day 7	7	65 (+ Provect CH4)	13
	9	51	6
	11	31	0
	13	22	0
	15	8	0
	17	0	0

Figure 4. Changes in Methane Concentrations over Time (Table 3 Data).