Provect-CH4® Methane Inhibitor

TECHNOLOGY DESCRIPTION

Provect-CH4 is a food-grade, natural source of Monacolin K (otherwise known as Lovastatin) that is used to prevent methane (CH_4) production by inhibiting the growth and proliferation of methanogenic Archaea. In environmental remediation applications, it can be used as an additive to conventional enhanced reductive dehalogenation (ERD) and *in situ* chemical reduction (ISCR) amendments rendering them safer and more effective. These include:

- Oils
- Emulsified Oils
- Sugars (lactate, dextrose, glucose)
- Other carbon sources (*e.g.,* molasses, whey)
- Plant based carbon (*e.g.,* cellulose and hemi-cellulose)
- Carbon + ZVI amendments (conventional ISCR reagents)

With widely varying degrees of success, other approaches such as managing pH and using slower-release, cellulose based carbon sources (lignolytic bacteria are not commonly thought to produce methane) have attempted to manage methane production during remedial applications. However, Provect-CH4 is the only reagent designed **to actively control the production of methane** in a safe, reliable and predictable manner (US Patent No. 9,221,669 B2). In addition to the safety issues, associated with elevated methane in groundwater, soil gas, and indoor air, this effect also promotes more efficient use of the hydrogen donor.

WHAT IS THE PROBLEM WITH METHANE?

There are recognized benefits to methanogens and of limited methanogenesis. 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 activity of 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.

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** (below) 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 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% of the total amendment consumption based on moles of H2.

Table 1. H 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**

Potential Health and Safety Issues: Methane is considered to be a major greenhouse gas. It is explosive, with an LEL of 5% and an UEL of 15%. 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 in groundwater concentrations (as high as 1,000 ppm have been reported) which can lead to accumulation in soil gas subsequently impacting 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 also been observed in more rural settings and other open spaces.

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 CH_4/L (Indiana Department of Environmental Management, 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 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 methane exceeds a threshold level ranging from 1 to 10 ppm groundwater. These contingencies often entail expensive and extensive systems for treating methane in soil gas/vapor captured via SVE systems.

PROVECT-CH4: MODE OF ACTION – HOW DOES IT WORK?

What is a Methanogen? In the 1970s, Dr. Carl Woese (1928 to 2012) 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 and Fox, 1977). The identification of this new Domain of microorganism was very important for many reasons, but from our limited 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 you are.

What is a Statin? A Statin 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". 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, potent statin used for decades to lower cholesterol in human blood by inhibiting 3-hydroxyl-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which is a key enzyme in the cholesterol biosynthesis pathway (Alberts *et al.,* 1980). It was the first statin approved by the United States Food and Drug Administration in 1987 as a hypercholesterolemic drug.

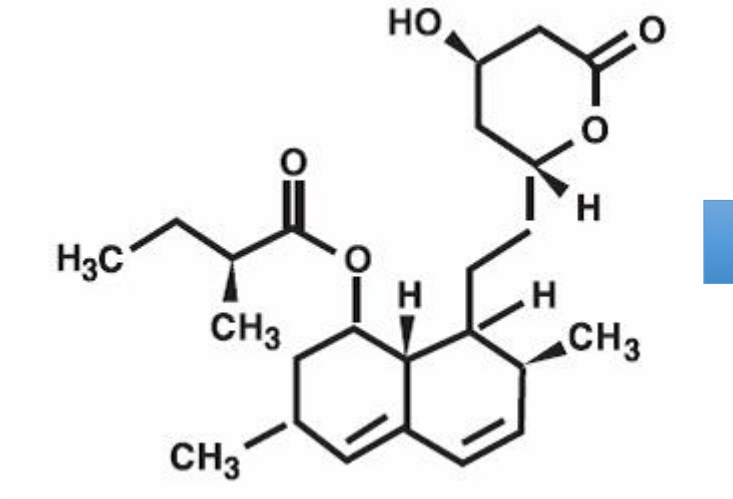


Figure 2. Chemical structure of Lovastatin

What is Red Yeast (Rice) Extract? The red yeast rice (RYR) extract that is component of Provect-CH4 is a substance extracted from rice that has been fermented with a type of yeast called *Monascus purpureus*. Red yeast extract has been used in the cattle industry for decades in efforts to manage rumen microbiology and to control methane production on cows (Henderson *et al*., 2010). It is also used as a food coloring, food additive/preservative, and is widely consumed by humans. The RYR extract contains a number of monacolins - most importantly, Monacolin K, otherwise known as Lovastatin or Mevinolin. Monacolin K is the only naturally occurring statin compound. In addition to Monacolin K, RYR extract also contains other statins, mono-unsaturated fatty acids, vitamins and other nutrients that will effectively stimulate anaerobic bacteria in the subsurface.

So – How Does Provect-CH4 **Inhibit a Methanogen?** Monacolin K can inhibit methanogenic archaea because cell membrane production in archaea shares a similar pathway with cholesterol biosynthesis (Miller and 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 analogue of murein - which is biosynthesized via activity similar to that of HMG-CoA reductase which yields cholesterol in humans. In the presence of a statin, HMG-CoA reductase is inhibited, pseudomurein biosynthesis pathway is interrupted, and methanogens are restricted from growth and proliferation. And since methanogens are so uniquely different than bacteria, the inhibitory effect of Provect-CH4 is not observed in microbes that are typically associated with: i) catabolism of organic contaminants (such as pseudomonas species) and/or, ii) halo-respiration/biodegradation of chlorinated solvents (such as *Dehalococcoides* species).

SAFER, MORE EFFICIENT ERD / ISCR TREATMENT

In situ chemical reduction (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), 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 number of enhanced reductive dehalogenation (ERD) substrates and other accelerated anaerobic bioremediation technologies exist (*e.g.*, emulsified oils, non-emulsified oils, carbon-based hydrogen release compounds, vegetable matter + ZVI amendments) that facilitate biodegradation of related compounds. Provect-IR antimethanogenic ISCR substrate uniquely combines RYR extract with of a variety of specially selected reagents in order to induce genuine ISCR conditions and faciliate the destruction of targeted COIs in a safer, more efficacious manner. Provect-IR has been used full–scale to ameliorate aquifers impacted by chlorinated solvents, pesticides, heavy metals and other COIs (Provectus Environmental Products, Inc. – http://www.provectusenvironmental.com/).

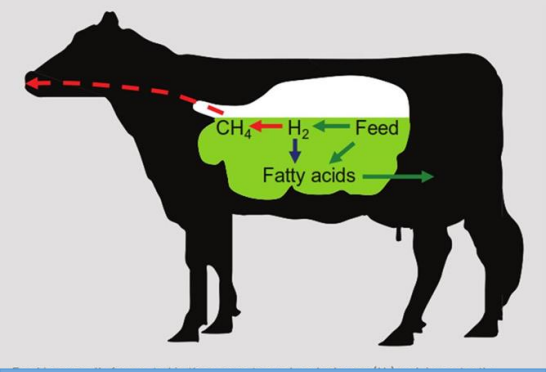


Figure 1. What happens to H2 when methanogens are inhibited? (*By Henderson et al, 2011)*

Methanogens are often the dominant hydrogenotrophs (*i.e.*, consumers of hydrogen) in many environments because they have a lower 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. 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 VFAs from H_2 and CO_2 via the Wood-Ljungdahl pathway (See **Figure 1**). By inhibiting the growth and proliferation of methane producing Archaea, chlororespiring bacteria can become the more dominant bacterial populations. In an anaerobic environmental remediation setting, halorespiring and other bacteria such as Desulfobacter spp. and Desulfuromonas spp. will more effectively utilize the available hydrogen for dechlorination of targeted COIs, and the VFAs will be fermented to ultimately yield CO_2 (Schauder *et al.,* 1986).

PROOF OF CONCEPT

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 COD of 2,000 mg/L, and they were operated as anaerobic sequencing batch reactors at 22°C- 24°C. After one week of incubation, silty sand was added to each reactor resulting in a slurry having a solids concentration of 20% 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, Provect-CH4 was added to one reactor to achieve a concentration of 40 mg/L while maintaining the second reactor as an un-amended Control (*i.e.*, no Provect-CH4 added). Because the 40 mg/L dose of Provect-CH4 reduced methane production in the Test reactor so rapidly and completely (see below), it was decided to dose the "Control" reactor with 20 mg/L of Provect-CH4 during the fourth week of operation.

Figure 2. Close up showing biogas being collected with a syringe to monitor

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 (**Figure 2**). The methane content of the biogas samples 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.

RESULTS:

Table 1 lists 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 72-82 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 COD measurements after each feeding cycle ranged from 56 to 108 mg/L. The reactors were fed 2,000 mg COD/L each cycle, which was apparently rapidly consumed by the anaerobic culture. Values of pH ranged between 6.1 and 6.4. Values of ORP were all close to -300 mV, which is typical of methanogenic conditions. The TDS in the reactors did not change over time, ranging from approximately 1,200 to 1,250 mg/L.

Table 1. 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.

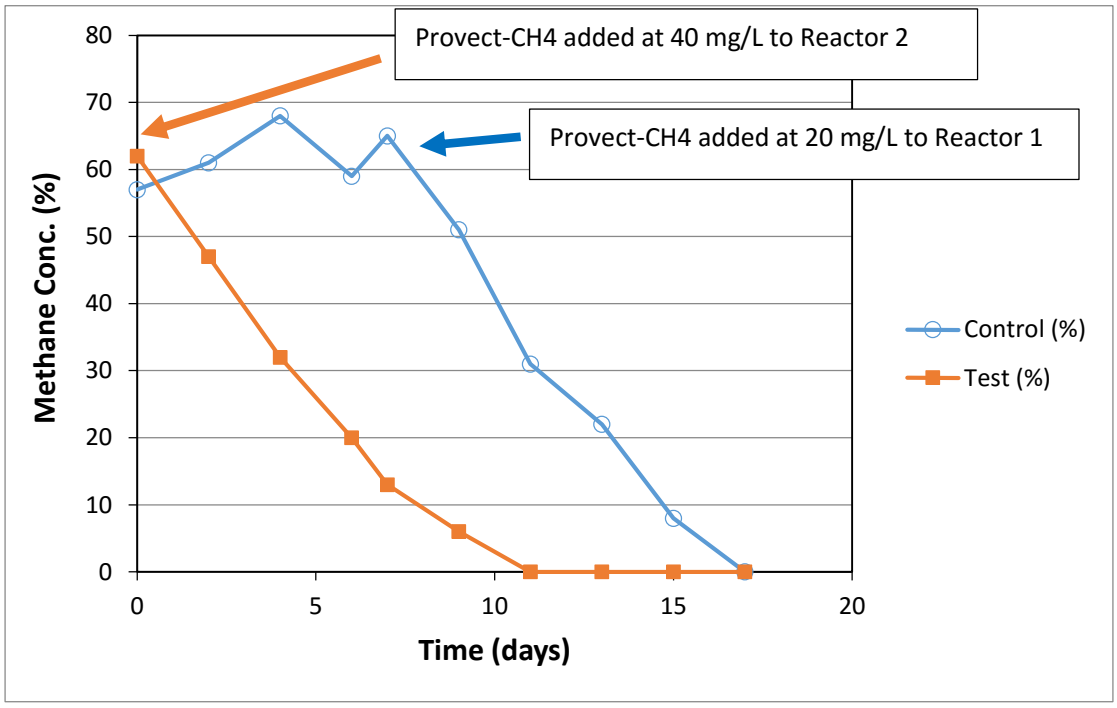
Period	Gas Vol. (mL)	COD (mg/L)	pH	ORP (mV)	TDS (mg/L)
Reactor 1					
Startup-Week 1	81	56	6.4	-302	1213
Startup-Week 2	72	91	6.3	-306	1241
Test-Week 3	75	61	6.2	-289	1258
Test-Week 4 (Provect-CH4 at 20 mg/L)	73	108	6.3	-296	1220
Reactor 2					
Startup-Week 1	79	72	6.2	-285	1244
Startup-Week 2	75	83	6.2	-298	1265
Test-Week 3 (Provect-CH4 at 40 mg/L)	82	62	6.1	-306	1263
Test-Week 4	72	97	6.4	-287	1247

Prior to the addition of Provect-CH4, methane concentrations varied from approximately 55% to 70% (**Table 2**), which indicated an active methanogenic culture. Following the addition of Provect-CH4 at 40 mg/L to Reactor 2 the methane content of biogas was rapidly reduced from 62% to below detection (0.05%) after 11 days (**Figure 3)**. With only a single addition of Provect-CH4 to the closed system the methane concentration remained below detection levels until day 17, when the reactors were dismantled. Addition of Provect-CH4 at 20 mg/L to Reactor 1 on Day 7 reduced the methane content of biogas from 65% to below detection (0.05%) by day 17 (*i.e.,* after 10 days). During the Test period, the total volume of biogas produced in either reactor did not change appreciably (Table 1), only the methane concentration of the biogas (the bulk gas contained mostly CO_2).

Table 2. Methane Concentrations (%) in Reactor Biogas during the 17 Day Test Period (*i.e.*, after dosing with methane inhibitor).

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

Figure 3. Changes in Methane Concentrations over Time (Table 2 Data).



CONCLUSIONS:

These studies demonstrated that in a closed, controlled system Provect-CH4 effectively shut down methane production in an active methanogenic culture when added at least 20 ppm. In an aquifer setting, a targeted dose of 50 ppm is generally recommended: site specific treatability studies can be conducted to help validate potential effectiveness and optimize the design specifications.

PRIMARY FEATURES:

Provect-CH4 is the only ERD/ ISCR supplement that will rapidly improve remedial performance while simultaneously preventing or significantly minimizing the production of methane. The benefits are notable:

- More Efficient = More Cost Effective: Production of methane is a direct indication that the hydrogen generated from the organic carbon amendments was used by methanogens and the amendment has been wasted because it was not utilized by acetogens or dehalorespiration. By inhibiting the growth and proliferation of methane producing Archaea, chlororespiring bacteria can become the more dominant bacterial populations.

- Safer: Methane is explosive with an LEL of 5% and an UEL of 15%. Production of methane will result from the addition of any conventional ERD or ISCR amendment: excessive and extended production of methane can result in elevated in groundwater concentrations (as high as 1,000 ppm have been reported) which can lead to accumulation in soil gas subsequently impacting indoor air. State specific regulations for methane in groundwater have been promulgated, with others pending for soil gas and indoor air.

- Ease of Use: Green and sustainable. All components integrated in a single package. Logistics with no surprises.

- Patented Technologies: Technology end users and their clients are fully protected from all Patent and other legal issues.

EXAMPLE APPLICATION GUIDELINES (50 ppm Provect-CH4 in groundwater):

Treatment Zone Dimensions

Width of targeted zone (perpendicular to gw flow)		150	ft
Length of targeted zone (parallel to gw flow)		125	ft
Depth to top of treatment zone		10	ft
Depth to bottom of treatment zone		20	ft
Treatment zone thickness		10	ft
Calculated Volume		187500	ft3

Methane Inhibitor Calculations

Estimated Porosity		35	%
Calculated impacted liquid		65625	ft3
Methane Inhibitor for Project	ROUND TO NEAREST 25 lb	204.8	lbs

* PLEASE CONTACT US (info@provectusenv.com) FOR PRICING INFORMATION AND DELIVERY QUOTES.

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