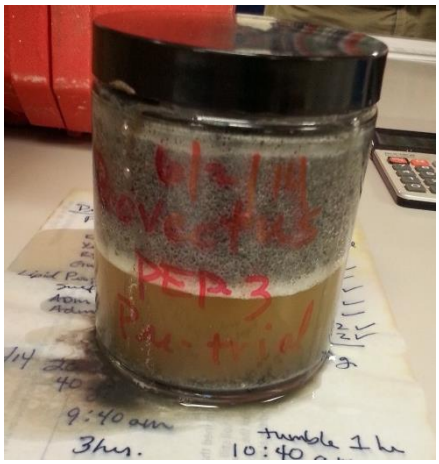
Provect-IR™
Antimethanogenic ISCR Reagent



TECHNOLOGY DESCRIPTION

Provect-IR is a unique mixture of reagents combined into a single product that optimizes the *in situ* reductive dechlorination of chemicals present in soil, sediment, and groundwater. It acts by promoting synergistic interactions between:

- Natural antimethanogenic compounds
- Hydrophilic, nutrient rich organic carbon sources
- Zero-Valent Iron (ZVI)
- Chemical oxygen scavengers
- Vitamin and mineral sources

This distinctive, patented combination of natural and food-grade chemicals promotes ISCR conditions for fast and effective destruction of targeted constituents of interest (COIs) such as chlorinated solvents, organochlorine pesticides, and other halogenated compounds (Brown *et al.,* 2009; Dolfing *et al.,* 2008; US Patent Office Scalzi *et al* 2012). Notably, Provect-IR is the only ISCR reagent to simultaneously inhibit the production of methane during the requisite carbon fermentation processes (US Patent Office Scalzi *et al*, 2013, 2014). This promotes more efficient use of the hydrogen donor while avoiding negative issues associated with elevated methane (CH4) in groundwater, soil gas, and indoor air.

Current regulations for methane in groundwater vary from *ca*. 10 to 28 mg CH4/L (Indiana Department of Environmental Management, 2014). More State regulations are pending, with several ERD projects which intended to use liquid carbon (emulsified oils) sources failing 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). Many remedial practitioners have subsequently been required to establish contingencies for conventional ERD/ISCR implementation in the event that methane exceeds a threshold level ranging from 1 ppm to 10 ppm groundwater. These contingencies often entail expensive and extensive systems for capturing and treating methane in soil gas/vapor captured via SVE systems.

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 is a widely known, potent statin used for decades to lower cholesterol in human blood by inhibiting 3-hydro-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.

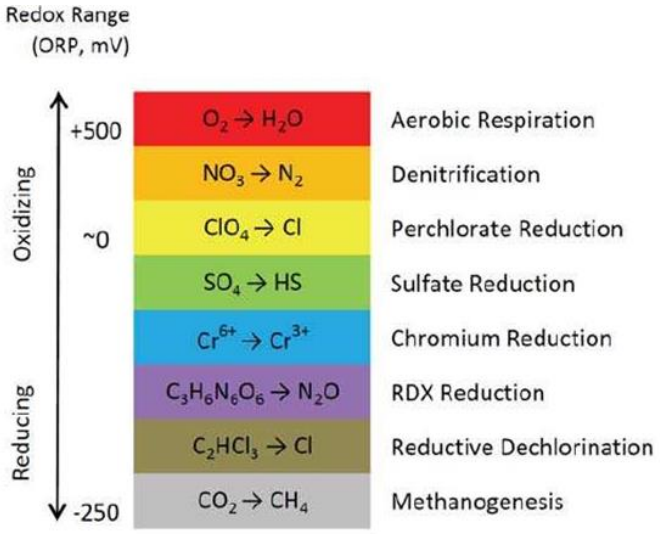
What is Red Yeast (Rice) Extract? The red yeast rice (RYR) extract that is component of Provect-IR is a substance extracted from rice that has been fermented with a type of yeast called *Monascus purpureus*. Red yeast extract is used as a food coloring, food additive/preservative, and is widely consumed 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 mono-unsaturated fatty acids and other vitamins that will effectively stimulate anaerobic bacteria in the subsurface.

So - How Does a Statin Inhibit a Methanogen? Interestingly, Monacolin K is a potent inhibitor of methanogenic archara because cell membrane production in archaea shares a similar pathway with cholesterol biosynthesis (Miller and Wolin, 2001). And since methanogens are so uniquely different than bacteria, the inhibitory effect 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). RYR has been used in the cattle industry for decades in efforts to manage rumen microbiology and control methane production in cows.

ATTENUATION PROCESSES – SAFER, MORE EFFICIENT ISCR TREATMENT

In situ chemical reduction (ISCR) as defined by Dolfing *et al* (2008) 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. The corresponding enhanced thermodynamic decomposition reactions that are realized at the lowered redox (Eh) conditions allow for more effective mineralization of many 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 purportedly offer similar responses. However, the Provect-IR antimethanogenic ISCR substrate is unique in its ability to yield Eh values most conducive to reductive dechlorination while simultaneously preventing methane production - which is a waste of the H being generated and potentially a safety issue under field conditions.



Provect-IR 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. As outlined below, it can be used to manage environments impacted by chlorinated solvents, pesticides, heavy metals and other COIs.

Specially Selected Organic Hydrogen Donors: A variety of hydrophilic, nutrient rich organic carbon sources are incorporated in Provect-IR that assist in promoting the ISCR process. The Provect-IR bioremediation amendments consist of slow, medium and long-term release carbon sources. Such a formulation is desirable because it provides both a rapidly utilized electron donor (calcium propionate), slow-release long-term electron donors (kelp meal and yeast extract) and long-term release carbon sources (other cellulose and hemi-cellulose carbon such as soy meal). More specifically,

- Calcium propionate and other readily biodegradable carbon sources: Following the addition of simple carbon sources such as lactate, formate, ethanol or glucose to an aquifer setting these compounds are often converted rapidly to hydrogen and acetate. Although this is the desired response, the process is sometimes too rapid, and this can result in aquifer acidification (due to rapid VFA production) and the liberation of too much hydrogen (which allows methanogens and sulfate reducers to compete effectively with dehalogenators, which tend to grow more slowly). Hence, calcium propionate is used as a readily biodegradable carbon source.
- Yeast extract: This supplement provides a variety of organic hydrogen donors that have slower release profiles (*i.e.*, they are not fermented as rapidly as proprionate). Yeast extract also contains biological components that are very useful to anaerobes, but are not available through other carbon-only media. In particular, yeast extract provides an abundant source of priming ATPase along with trace nutrients and vitamin B complexes.
- Kelp meal/Cellulose based carbon: These hydrogen sources are composed of a hydrophilic, solid and complex carbon that ferment more slowly and inherently generate less methane. The hydrophilic organic component of the kelp meal, for example, is composed of cellulose and hemicellulose and it may be treated during the manufacturing process so that some of the components more easily undergo hydrolysis to glucose while maintaining an overall longevity of 3 to 5+ years.

Chemical Oxygen Scavengers: The presence of chemical oxygen scavengers such as sodium sulfite helps minimize performance lag phases that are often observed following the injection of remedial amendments. This is due, in part, to the presence of oxygen that is introduced as a result of the field mixing and blending operations. It takes a cerain amount of time and reagent consumption to remove that introduced oxygen and allow the ISCR reactions to proceed. Provect-IR is unique it that manages this impact chemically, which is a more effective, reliable manner thus allowing the ISCR process to be more effective.

Zero-Valent Iron: The presence of ZVI in Provect-IR is critical to ISCR reactions. The ZVI is added as a reduced material that is oxidized during the reductive dechlorination reactions which use ZVI as the reducing agent. The *beta*-elimination reaction mainly produces (chloro)acetylene, ethane/ethane and chloride ions, without the accumulation of potentially problematic catabolites typical of microbiologically mediated sequential reductive dehalogenation processes (*e.g.,* DCE “stall”). As the ZVI reacts, hydroxyl ions are released and pH increases which is useful in neutralizing the acidity generated during the fermentation of carbon, where acids are generated. Oxidized iron species are also prodcued, where are useful in *alpha*-elimination reactions and iron cycling. One limitation to ZVI reactions is that they are surface mediated which means that direct contact is required for direct COI destruction.

RYR Extract: Provect-IR is the only ISCR amendment that will rapidly induce ISCR conditions while simultaneously preventing or significantly minimizing the production of methane. The benefits are notable:

- 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.

- 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.

PRIMARY FEATURES:

- Effective: No accumulation of dead-end catabolic intermediates as a function of substrate addition (as is common with [emulsified] oils and sources of carbon only).
  - Does not rely on physical sorption/sequestration as a major "removal" mechanism (as is common with oils).
  - Inherently buffered for pH control – will not acidify an aquifer and liberate heavy metals as potential secondary COIs.
- Efficient: Significantly lower costs as a result more efficient amendment utilization and avoidance of contingencies for methane management. No need for additional buffers.
- Safe: Fewer health and safety concerns as compared with use of traditional ERD or ISCR reagents; Avoid issues associated with new and emerging methane regulations.
- Ease of Use: Green and sustainable. All components integrated in a single package. Logistics with no surprises.
- Longevity: Engineered profile of carbon sources for multi-year longevity estimated at 3 to 7 years based on site-specific hydrogeology. Reagent will stay in place and remain active which prevents rebound.
- Improved Performance: More efficient use of hydrogen donors (does not get wasted as methane).
- Adaptable Formulations for Heavy Metals: Will not mobilize arsenic or other heavy metals yielding secondary contaminants (as is common with [emulsified] oils and sources of carbon only). Can be formulated to manage environments that are co-impacted by various inorganic contaminants (*e.g.*, As, [Hg], Ni, Pb, Zn) while simultaneously mineralizing the organic compounds.
- Patented Technologies: Technology end users and their clients are fully protected from all Patent and other legal issues.

PHYSICAL PROPERTIES:

Particle Size: ranges from ca. <5 to 100 micron (can be manufactured to specifications).

Dry Density: ranges from 0.4 to 0.5 g/cm3

29% Aqueous Slurry Density: ranges from 0.9 to 1.0 g/cm3

29% Aqueous Slurry Viscosity: ranges from 500 to 1,500 cP

SLURRY PREPARATION GUIDELINES:

Percent Solids Content	Mass of Provect-IR	Volume of Water (US gallons)
10%	25 lb	27
20%	25 lb	12
30%	25 lb	7

LITERATURE CITED:

Alberts, A., J. Chen, G. Kuron, V. Hunt, J. Huff, C. Hoffman, J. Rothrock, M. Lopez, H. Joshua, an E. Harris; 1980. Mevinolin: a Highly Potent Competitive Inhibitor of Hydroxymethylglutaryl-coenzyme A Reductase and a Cholesterol-Lowering Agent. Proceedings of the National Acadaemy of Sciences of the United States of America 77:3957-3961.

Brown, R., J. Mueller, A. Seech, J. Henderson and J. Wilson. 2009. Interactions between Biological and Abiotic Pathways in the Reduction of Chlorinated Solvents. Remediation Journal Winter 2009, pages 9-20.

Dolfing, J., M. Van Eekert, A. Seech, J. Vogan and J. Mueller. 2008. *In Situ* Chemical Reduction (ISCR) Technologies – Significance of Low Eh Reactions. International Journal Soil & Sediment Contamination 17 (1) : 63-74.

Miller, T.L. and M.J. Wolin. 2001. Inhibition of growth of Methane-Producing Bacteria of the Rumen Forestomach by HydroxymethylglutarylSCoA Reductase Inhibitors. J. Dairy Sci. 84:1445-1448.

Scalzi, M. and McGill, J. 2012. Method for the Treatment of Groundwater and Soils using Mixtures of Seaweed Kelp. US PTO No. 8,147,694 B2 (April 3, 2012).

Scalzi, M. and A. Karachalios. 2013 and 2014. Inhibition of Methane Production during Anaerobic Reductive Dechlorination. US PTO 13/ 785,840 and CIP 14/268,637

Woese, C.R. and G.E. Fox (1977). "Phylogenetic structure of the prokaryotic domain: the primary kingdoms". *Proceedings of the National Academy of Sciences of the United States of America* **74** (11): 5088–5090.

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