

Provect-ERD[™] contains proprietary fermentable carbon sources plus water-soluble, dual-valent iron (DVI) and where appropriate - antimethanogenic reagent (AMR) technology to yield a liquid *in situ* chemical reduction (ISCR) amendment to enhance the removal of chlorinated volatile organic compounds (CVOCs) from soil and groundwater. As outlined herein, this approach offers several benefits:



- Can be applied via screened wells
- Ease of use (can self-perform)
- Increased reliability and performance beyond enhanced reductive dechlorination (ERD) alone
- In situ formation of mackinawite and other iron sulfides
- Extended longevity (remedial action persists for years)
- Reduced risk of regulatory exceedances for methane
- Avoidance of possible health and safety issues (vapor intrusion, induced plume migration)
- Custom formulations available

PROVECT-ERD[™] TECHNOLOGY BACKGROUND

Provectus' enhanced reductive dechlorination (ERD) technology represents a significant advancement in environmental biotechnology by combining the proven biochemistry of ERD with the power of the Provect-CH4[®] methanogen inhibitors to yield a truly unique liquid, antimethanogenic ERD reagent.

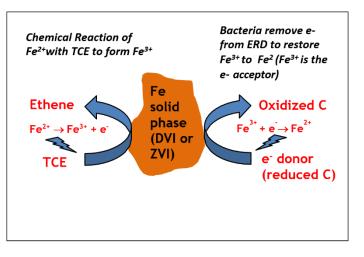
<u>Fermentable Carbon Source</u>: The amendment is manufactured and shipped as a site-specific mixture that typically contains 60 - 70% fermentable carbon (FC) and depending on formulation the following:

- Optional Provect-CH4[®] AMR (two types typically at 4% to 8% weight of FC)
- Glycerin as fast-release H donors
- Soluble lactic acid as mid-release H donors
- Ethyl lactate as a green solvent and H donor
- Dissolved fatty acids as long-term release H donors
- Dipotassium phosphate for micronutrients and pH buffering

<u>Antimethanogenic Reagents (AMR) to Control of Methanogenesis:</u> Provect-CH4[®] is a food-grade, natural source of Monacolin K (i.e., Lovastatin) and other statin compounds and/or essential plant oils with a demonstrated ability to prevent excessive methane (CH₄) production by inhibiting the growth and proliferation of methanogenic Archaea in environmental remediation applications. Provect-CH4[®] can be used as a supplement to conventional ERD and ISCR amendments to control methanogenesis thereby rendering them safer, more effective, and more cost efficient.



Dual Valent Iron: Provect-ERD[™] is supplemented with a source of a soluble, reduced iron (*i.e.*, present as ferrous [Fe²⁺] iron). The DVI supports direct chemical dechlorination via alpha-elimination pathways and supports the in situ formation of reactive ferrous minerals (e.g., magnetite) and - in the presence of a sulfur source - reactive iron sulfides (e.g., mackinawite) to yield abiotic reductive dechlorination. These abiotic pathways often result complete in dechlorination (Weber et al., 2006) and can persist for many years (if an electron donor is



available) being catalyzed by indigenous iron-reducing bacteria. Notably, biotransformation of Fe²⁺ does not require direct contact with the iron solid phase as a variety of naturally occurring biological molecules, such as humic acids, can facilitate electron shuttle dynamics.

ADVANTAGES OF USING PROVECT-CH4® METHANE CONTROL TECHNOLOGY

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 create and 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 represents a costly waste of the amendment since the hydrogen released as methane was not utilized by the targeted microbes, such as *Dehalococcoides* spp., *Dehalobacter* spp., or other related bacteria. In addition, excessive methanogenesis can pose significant safety issues (methane is explosive), it will induce vapor migration and it can lead to exceedances of new and emerging regulatory guidelines. Moreover, uncontrolled methanogenesis can be interpreted (by some) to represent an avoidable contribution to greenhouse gas emissions, hence its active control can have a positive impact on one's overall sustainability index.

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PROVECT-ERD[™] + DVI PRIMARY FEATURES

Because Provect-ERD^{$^{\text{M}}$} provides both fermentable carbon and supplemental DVI, the Fe⁺² ions donate electrons and are oxidized to Fe⁺³ for an extended period (years). Where needed (*i.e.*, in the presence of low sulfate), an additional source of sulfur is included in the product formulation. General physical parameters are as follows:

- Viscosity = 10 to 15 cP at 20 C
- Specific Gravity = 1.00 to 1.2
- Density 7.75 to 8.5 lbs/USG
- Hydrogen Yield= 0.2 g to 0.4 H₂/g ERD-CH4
- Fermentable carbon @ 60 to 70% weight basis
- AMR 4% to 8% of the FC content
- Soluble organic Fe content 5 to 20% weight basis

Provect-ERD[™] is the only ERD Reagent that includes Provect-CH4[®] AMR technology in an engineered, pre-mixture formulation to rapidly improve remedial performance while simultaneously 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 and <u>at least 15 to >30% less ERD</u> amendment can be applied.

♦ <u>Safer</u>: 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.

• <u>Green and Sustainable Technology:</u> Formulated with byproducts from "green" energy processes, so it is better for the environment.

• <u>Patented Technologies</u>: Technology end users and their clients are fully protected from all Patent and other legal issues.

• Ease of Use:

- o No need to emulsify the product with specialized tooling and equipment
- No laborious material transfers and dilutions
- Lower injection pressures
- Formulated for each site-specific application
- o Avoids cost and need for secondary treatment to manage excessive methane

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- <u>Carbon Longevity (> 2 years)</u>: Contains C14 to C18 fatty acids that have been shown in the field to last for over two years.
- <u>Natural Co-Solvent</u>: May include ethyl lactate which is a "green" co-solvent. This helps dissolve fatty acids, and it also aids desorption of bound contaminants to accelerate treatment.
- <u>Cost Competitive</u>: Standard formulations containing 60% fermentable carbon + 4% (FC weight basis) AMR methane inhibitor + 10% weight basis DVI is the most cost-efficient way of procuring the combined technologies.

OPTIONAL INOCULANTS AND BUFFERING AGENTS

If aquifer conditions are not optimal for ERD/ISCR, then the indigenous microbial population may be catabolically limited and any ERD remedial process will benefit from the addition of inoculants with known abilities to rapidly biodegrade DCE and VC. Once favorable redox conditions (ca. ORP < -100 mV, DO <1 mg/L, pH between 6.5 and 7.5) have been attained DHC cultures can be added to enhance complete mineralization and minimize DCE stalls. The DHC inoculant should contain at least 1x10E10 cfu/L of live bacteria including high numbers of *Dehalococcoides* species with known abilities to biodegrade DCE and VC. The target density of DHC cells in the treated aquifer area should be >1x10E6 cfu/L.

For ERD and ISCR to be most effective, aquifer pH should be near neutral or between 6 and 8. If the aquifer pH is acidic, an alkaline buffering agent such as $CaCO_3$ -based solid materials (*e.g.*, pulverized limestone or dolomite powders) or liquid buffer such as solutions of $Ca(OH)_2$, Mg(OH)₂, or NaHCO₃ can be applied.