Provectus ENVIRONMENTAL PRODUCTS

OVERVIEW

Provect-CH4TM is a food-grade, natural source of Monacolin K (otherwise known as Lovastatin) and other statin compounds with a demonstrated ability to prevent excessive methane (CH₄) 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 / Lecithins
- 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-CH4TM is the only reagent designed <u>to actively control the production of methane</u> in a safe, reliable and predictable manner (<u>US Patent No. 9,221,669 B2</u>).

MATERIAL PACKAGING, HANDLING AND STORAGE

Provect-CH4[™] is packaged as a dry powder in 55-lb plastic bags housed in sturdy cardboard drums. Typical shipments consist of individual drums delivered via overnight courier; larger orders consist of multiple drums placed on 4x4 wooden pallets. Each pallet is neatly wrapped in water-resistant plastic, but direct exposure to rain should be avoided.

GENERAL HEALTH AND SAFETY GUIDELINES

Provect-CH4[™] is non-hazardous and safe to handle. The use of standard personal protective equipment is always recommended, including safety glasses, steel-toe boots, gloves, hearing protection (in the proximity of loud machinery) and hard hat. Dust mask may be desired when working with the material under certain conditions. The SDS is posted on our web site at the following link: <u>Click Here!</u>



CONCEPTUAL REMEDIAL DESIGNS

There are at least two scenarios where Provect-CH4[™] methane inhibitors can be used: i) "preemptively", as part of newly implemented ERD or ISCR treatment, or ii) "curatively", in an attempt to ameliorate a situation where excessive methanogenesis has been established due to previous use of conventional amendments. For preemptive applications, 75 to 100 ppm Provect-CH4 in groundwater is targeted using total aquifer porosity; for curative applications, a concentration of 125 to 150 ppm Provect-CH4[™] in groundwater is targeted. For example, treatment of an area measuring 45 ft long x 90 ft wide x 60 ft deep (targeted vertical depth interval from 75 to 135 ft bgs would require 171 lbs of Provect-CH4[™] to yield of 75 ppm in groundwater in order to prevent a bloom of methanogens immediately resulting from the addition of an ERD amendment (Table 1).

Table 1: Provect-CH4[™] mass requirements and injection details for Source Area Treatment.

15

36450

75

170.7

%

ft3

lbs

3

ppm

Treatment Zone Dimensions		
Width of targeted zone (perpendicular to gw flow)	45	ft
Length of targeted zone (parallel to gw flow)	90	ft
Depth to top of treatment zone	75	ft
Depth to bottom of treatment zone	135	ft
Treatment zone thickness	60	ft
Calculated Volume	243000	ft3
Methane Inhibitor Calculations		

Estimated Porosity

Calculated impacted liquid Targeted Provect-CH4 Concentration in GW

Methane Inhibitor for Project

ROUND TO 55 lb units

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<u>Performance Monitoring – Soil Gas Analysis</u>: An effective soil gas monitoring network should be established to facilitate vadose-zone soil gas sampling from the capillary fringe, deep vadose-zone soil, and shallow vadose-zone of treated area along with background locations. Soil gas composition can be recorded using a FROG GC/MS or LandTec GEM5000 Landfill Gas (LFG) Meter or equivalent with an infrared detector calibrated to 15% methane.

<u>Performance Monitoring – Groundwater Analysis</u>: An effective groundwater monitoring network should be established to facilitate the collection of groundwater samples from transects throughout the treatment Areas, ideally with duplicate sample points located within the treatment zone. Routine monitoring of field parameters could include groundwater level, pH, DO/redox, and temperature. Laboratory analyses could include: i) chemical COI analyses (US EPA standard methods), ii) microbiological analysis of dehalogenators, Archaea and total microbes using DNA micro-arrays for functional groups and PCR with DGGE analysis for target populations (Microbial Insights, TN),and/or iii) Microbiological analyses via quantitative polymerase chain reaction (qPCR) DNA analyses to enumerate *Dehalococcoides* (DHC) and methanogens (MGN) along with the number of microorganisms expressing the functional genes *bcv*A Vinyl Chloride Reductase (BAV1), *tce*A TCE Reductase, and *vcr*A Vinyl Chloride Reductase.

GENERAL GUIDELINES FOR SCREENED WELL SYSTEMS

Provect-CH4[™] is commonly used as a supplement to EVO and other liquid ERD amendments that are added to an aquifer via screened wells. Here, it is important to note that Provect-CH4[™] is mostly cold-water soluble, but small amounts of suspended solids (insoluble rice starch) will settle out of the mixture over time (3 to 5 hours), especially without frequent mixing. Provect-CH4[™] can be mixed directly with the EVO and applied <u>the same day</u> as a single amendment (requires frequent mixing) at about 2.5% weight basis, or less. Alternatively, Provect-CH4[™] can be applied to the aqueous chase water at a concentration ranging from 2% solids (see example **Photograph B**)

Water Volume (USG)	Water Weight (lb)	Provect CH4 (lbs)	% Solids / Slurry	Slurry Viscosity
100	832	26 lb (1/2 drum)	2% (very thin-soluble see photo A)	tbd
50	416	26 lb (1/2 drum)	6% (thin-soluble)	tbd
50	416	55 lb (1 drum)	10% (slurry like see Photo B)	tbd



Provect-CH4[™]

Handling and Application Guidelines



Photograph A (left) = 2% Provect-CH4 in solution with mixing. **Photograph B** (right) = aqueous suspension of Provect-CH4 with ca. 10% solids (some solids will settle out of suspension over time)

If the density of the mixture or the total volume of amount of slurry added per point is problematic, the dimensions, mass requirements, mixing and other application details can be modified in the field based on site specific conditions (for example, the density of the slurry can be changed to modify the total injection volume or the injection layout/number of injection points could be altered depending on recommendations from the contractor).

GENERAL GUIDELINES FOR DIRECT PUSH INJECTION OF AQUEOUS SLURRY

<u>Mixing Equipment</u>. Reagent slurry has been prepared in various ways, ranging from in-line automated mixing systems, to manual mixing using a hand-held drill with a mixing attachment, to more creative processes. Particularly for larger projects, experienced drillers will have some form of mechanical mixing system on site that includes a tank with a paddle-type mixer at the bottom. The slurry is then transferred to a feed tank connected to an injection pump so that slurry can be prepared continuously while injections are being performed (see example, ChemGrout mixing system (<u>CG-500</u> <u>High Pressure Series</u>). Slurry mixes quickly in these systems (<1 minute), and injections can proceed without interruption.





Provect-CH4[™]

Handling and Application Guidelines

<u>Pumps</u>. Experienced drillers will have a variety of pumping equipment on site. For injecting slurries, an injection pump capable of generating at least 300 psi of pressure at a flow rate of >5 gpm is desired. Obviously, the pump needs to be able to handle solids, such as piston pumps, grout pumps, and progressing cavity pumps - with a preference towards the piston and grout pumps. Slurry is typically injected at pressures of 100 to 200 psi; however, higher pressures are sometimes required to initiate the injection. It is recommended to have a higher pressure pump available on site that can generate over 500 psi and ca. >10 gpm, as deeper installations often require higher injection pressures.

<u>Tooling</u>. Experienced drillers will have sufficient rod length on site to allow 3 to 5 injection points to be capped overnight to allow pressure to dissipate. This can help prevent backflow and surfacing of slurry as the injection rods are retracted. Likewise, experienced drillers will have on hand a variety of injection tips, some that direct the slurry horizontally (see for example GeoProbe's pressure activated tip at geoprobe.com).

In a "top-down" injection approach, the rods are initially advanced to the top of the targeted depth interval, and a specified volume of slurry is injected while recording flow rate, injection pressure, and slurry

volume delivered. The injection rods are then further advanced a distance ranging 2 to 4 feet and the process is repeated to help ensure even distribution of slurry over the targeted depth interval. At the end of each injection point, a small volume of water (15 USG) is often used to clear the rods and the injection tip of any slurry.

OPTIONAL USE OF INOCULANTS FOR ENHANCED TREATMENT

The aquifer is highly aerobic, as indicated by elevated levels of DO. As such, aquifer conditions are not generally conducive to ERD/ISCR. If desired, microbiological assays can be conducted to enumerate DHC type microbes. Low number of DHC-type organisms at some sites, along with the absence of PCE Catabolites, often indicates that the naturally occurring microbial population may be catabolically limited and hence the remedial process might benefit from the addition of inoculants with known abilities to rapidly biodegrade DCE and related compounds. 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 $1 \times 10 \times 10$ cfu/L of live bacteria including high numbers of *Dehalococcoides* species with known abilities to biodegrade DCE. The target density of DHC cells in the treated aquifer area should be about $1 \times 10 \times 10$ cfu/L.



OPTIONAL USE OF BUFFERING AGENTS

For ERD and ISCR to be most effective, aquifer pH should be near neutral or between 6 and 8. If aquifer pH is acidic, possibly due in part to the previous use of non-buffered, carbon-only amendments, pH could be raised by using supplemental ZVI and/or one of several alkaline buffering agents such as CaCO₃-based solid materials (*e.g.*, pulverized limestone or dolomite powders) or liquid buffers such as solutions of Ca(OH)₂, Mg(OH)₂, or NaHCO₃. If pH is basic, acidifying compounds such ammonium sulfate can be employed.

For conceptual purposes, the use of lime at 0.5% soil mass can be assumed, but to best evaluate the type and amount of buffered required a site-specific laboratory buffering test could be conducted which would involve a titration of a slurry of site soil and groundwater with selected reagents. A potential procedure for testing of solid CaCO₃ involves buffer dosing in 0.25% (to soil mass) increments from 1% to 2% in 1:4 soil to water slurries and an equilibration time of 1 day. The solid buffer testing is performed in separate batches for each dose. For testing liquid buffers [e.g. saturated solutions of Ca(OH)₂ or Mg(OH)₂], a standard titration test would be performed, whereby increments of a liquid buffer is added periodically to a soil-water slurry.

CONTACT US FOR A COMPLIMENTARY SITE EVALUATION

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