BIO-DECHLOR INOCULUM

Application Instructions

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BDI+ Quick Reference Table

The following table is a quick reference guidance providing only the most relevant information.
Please review the entire document carefully, plus the product Safety Data Sheet prior to any application.
Please contact REGENESIS Technical Support if you need any further assistance.

Viable application methods	Direct push. In wells.
Co-application	BDI+ is rarely applied as a stand-alone product. It is generally applied in same event with PlumeStop, 3-D Microemulsion, HRC, S-MicroZVI and/or CRS. Use same injection equipment and method used for injection of the other REGENESIS products.
Additional important items to source	Nitrogen gas cylinder with pressure regulator (to extract BDI+ from keg, and to form oxygen-free water). Sodium ascorbate (to form oxygen-free water as an alternative to nitrogen). Refrigeration system.
Storage	Keep product refrigerated at +2 / +4 °C. Consider providing a fridge onsite for long field activities; otherwise use container with coolers/ice-packs. **Do not** freeze.
Oxygen-free water	Oxygen-free water (<2 mg/L O_2) needed for both application methods. Sparge nitrogen gas or apply sodium ascorbate into tank with water.
Mixing & Application Method 1	Deliver BDI+ from keg into tank with oxygen-free water, using nitrogen gas to push the product. Mix quickly, but minimise air entering the water. Apply in the aquifer **AFTER** application of the other products.
Typical dilution factor – Method 1	1:40 for BDI+ (1 liter of BDI+ in 40 liters of water). 1:400 for BDI+ concentrate (0.1 liters of BDI+ Concentrate in 40 liters of water) (the exact dilution to be discussed with REGENESIS).
Mixing & Application Method 2	Mix the other REGENESIS products in oxygen-free water (follow relevant Application Instructions for details). Connect the BDI+ keg directly to the injection hoses, a suitable non-return valve **MUST** be used Slipstream the BDI+ in the injection line, to go in the aquifer directly together with the other products.
Typical dilution factor – Method 2	Use dilution factor recommended by REGENESIS.
Recommended injection pump	Diaphragm pump or piston pump. Use same pump utilized for applying the other REGENESIS products.
Recommended injection pressure	Low pressure injection. Typically, 1-5 bar (Note. This differs from the pressure required to dispense BDI+ from the keg, which is 10-15 psi (0.7-1 bar)). Adjust pressure using pressure regulator. Take note of pressure and flow rate for each step.
Direct push injection	Retractable screen tip recommended; pressure activated tip as an alternative. Typical injection steps every 30 cm.
In well application	Low pressure injection; **DO NOT** gravity feed. Use single or double packer. Flush well with oxygen-free water after application.
Other recommendations	Carefully refer to "User Manual for the Bio-Dechlor INOCULUM Plus Culture Delivery System" for all details on application. Always wash and flush equipment with oxygen-free water. Seal injection direct push points after injection. Do not operate P&T or other activities likely to disturb groundwater in surrounding area during and after injection.
Recommended monitoring	Refer to recommendations provided for the other products applied during the same application event.

General Information

This REGENESIS guide concerns the steps required for applying BDI Plus® into the target aquifer. For step by step instructions on dispensing BDI Plus from the delivered pressurised keg please refer to the 'User Manual for the Bio-Dechlor INOCULUM Plus Culture Delivery System', which is contained in the shipped cool box.

BDI Plus is an enriched natural microbial consortium containing species of Dehalococcoides. This microbial consortium has since been enriched to increase its ability to rapidly dechlorinate contaminants during in situ bioremediation processes. BDI Plus has been shown to stimulate the rapid and complete dechlorination of compounds such as tetrachloroethene (PCE), trichloroethene (TCE), dichloroethene (DCE), and vinyl chloride (VC). BDI Plus also contains microorganisms capable of degrading chloromethanes (carbon tetrachloride and chloroform) as well as chloroethanes like trichloroethane (TCA).

Treatment of chlorinated solvent contamination sometimes results in slow or incomplete degradation of the intermediate compounds. When faced with this circumstance, bioaugmentation with a microbial consortium such as BDI Plus offers a solution to accelerate or simply make possible the complete dechlorination of these otherwise recalcitrant compounds.

Material Packaging and Safety

BDI Plus is a mixture of living bacteria including members of the Dehalococcoides genus that are capable of anaerobically degrading chlorinated contaminants. The culture has been tested to ensure that it is free of the most common pathogenic bacteria, but like all living cultures it should be handled with due care to prevent contamination of work surfaces or field personnel.

During installation activities, REGENESIS recommends that field personnel use appropriate personal protection equipment (PPE) in accordance with your risk assessment. A Materials Safety Data Sheet (MSDS) is sent with each shipment and should be reviewed before proceeding with application activities.

Caution

- The BDI Plus container is pressurised to 10 to 15 psi with nitrogen before shipping.
- Wear suitable eye protection, gloves, respirator and protective clothing.
- Gas cylinders used to dispense culture **MUST** be equipped with a proper pressure regulator.
- During operation **DO NOT** exceed the containers maximum working pressure of 15 psi.

Unpacking

1. Carefully remove the container from shipping cooler and stand upright. **DO NOT** use the plastic sight tube as a handle.
2. Carefully check the container, connectors, valves and tubing for any damage or defects. If defects or damage is observed, do not use. Report any damage to REGENESIS. A backup set of quick connectors is provided in the packaging material.
3. Check and ensure that all valves are in the **CLOSED** position.
4. Refer to the 'User Manual for the Bio-Dechlor INOCULUM Plus Culture Delivery System', for details on how to connect the various tubes to the container.

Storage

BDI Plus is a robust formulation, capable of withstanding some exposure to oxygen and temperature change. The schedule of application requires adding the BDI Plus over a period of days, the keg(s) should be stored at a temperature of around 2-4 °C but freezing must be avoided. This can normally be achieved by storing the kegs on ice in the provided coolers. The keg should also be pressurised with nitrogen at
10 - 15 psi before storing to ensure a tight seal on the keg cap.

Oxygen Free Water

Where reference is made to 'oxygen free' water in this document it means water that has no more than 2mg/L of dissolved oxygen.

Specific Installation Procedures

BDI PLUS may be applied into the subsurface in two ways, either:

Method 1. Injection of BDI Plus alone, by diluting with 'oxygen free' water; or
Method 2. Co-injection with REGENESIS products ('slipstreaming').

Installation Method 1

BDI Plus may be applied into the same injection point (this can refer to either direct push or remediation well application) during the same injection event. We would recommend that this is done after application of other REGENESIS' products. For example, the injection point would have the required dose of 3-D Microemulsion® (3DMe®) applied to it, followed by the requisite amount of BDI Plus.

To make the injectable BDI Plus solution an appropriate amount of oxygen-free water should be prepared. To ensure that a sufficient quantity of 'oxygen free' water is available throughout the day, a large quantity of water can be prepared and the water to be injected may be transferred from this reservoir to the injection pump feed tank. The water can be prepared in two ways: a) Sparging with nitrogen; or b) Addition of sodium ascorbate.

Nitrogen Sparging

To ensure that the water has reached the desired state prior to mixing with BDI Plus nitrogen should be bubbled into a suitable vessel (e.g. a 200L or 55-gal drum) containing a given amount of water.

Nitrogen sparging is best accomplished by using a gas sparging device such as fish tank aerator. Adjust the nitrogen tank pressure regulator to 3-5 psi and immerse the aerator to the bottom of the vessel. By internal convection and oxygen stripping processes, the oxygen levels should diminish within an hour. Be careful to not consume too much gas and ensure you have enough nitrogen to empty the BDI Plus canister. Keeping an eye on tank pressure loss and dissolved oxygen level will indicate when one can trim down on the sparge pressure and conserve the nitrogen.

Sodium Ascorbate

As an alternative to nitrogen sparging, sodium ascorbate powder may be added to the vessel at a rate of 300mg/L and mixed gently. Monitor the dissolved oxygen level until it is no more than 2mg/L.

Once the reservoir of 'oxygen free' water has been prepared it can be transferred to the pump feed tank and mixed with BDI Plus. The tables provided below indicate the amount of water that a given amount of BDI Plus or BDI Plus Concentrate should be mixed with to make the injectable solution.

Volume of BDI PLUS (L)	Volume of dilution water (L)
5	200
1	40
0.5	20

Volume of BDI PLUS Concentrate (L)	Volume of dilution water (L)
0.5	200
0.1	40
0.05	20

Once the injectable solution has been prepared it may be applied to the subsurface in the same manner as any other Regenesis product, taking care to target the correct depth(s). Once all the BDI Plus has been applied we recommend that several litres of 'oxygen free' water is pumped to clear the BDI Plus from the injection hoses and injection rods or remediation well.

Installation Method 2

BDI Plus may be applied into the surface with other REGENESIS products such as 3DMe® or PlumeStop® by using these products as the carrier fluid ('slipstreaming'). To be able to do this a suitable connection on the injection hose must be made, fitted with a non-return valve, to enable BDI Plus to be applied into the injection hose directly from the canister. If in doubt, the applicator should use Method 1 above.

'Oxygen free' water, prepared using either of the steps above, should be added to the mixing tank, or the 'oxygen free' water can be prepared directly in the mixing tank. The requisite amount of REGENESIS product should then be added to the water to make up the injectable solution.

As the injectable solution is pumped to the injection point the requisite amount of BDI Plus should be dispensed from the canister directly into the injection line.