

Bio-Dechlor INOCULUM PLUS (BDI PLUS®)

Installation Instructions:

General Guidelines

Bio-Dechlor INOCULUM PLUS (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).

Recent trends in engineered bioremediation indicate that the 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.

REGENESIS® believes that the best approach to install BDI PLUS into the subsurface is by direct-push methods. This allows for the BDI PLUS solution to be applied directly into the aquifer material and provides greater coverage/treatment over the life of the project. As a minimum, the following equipment will be needed to perform this type of installation:

- Direct-push drilling unit
- Grout pump (e.g. Geoprobe GS 2000)
- Appropriate hose assembly including a fitting that links a hose from the grout pump to the direct-push rods (provided by REGENESIS with shipment)
- One or more 55+ gallon water drums, fitted with an appropriate lid that has at least one bung hole (number of drums depends on size of application)
- Rotary transfer pump (or equivalent) with appropriate amount of hose to connect from 55-gal drum to hopper of grout pump (similar to Grainger No. 1P893, Fill-Rite model #FR112GR)
- Compressed Nitrogen gas tank with appropriate regulator (0 to 15 pounds per square inch (psi). A 300-ft³ tank should be sufficient for discharge of concentrated or non-concentrated kegs and for nitrogen sparging to deoxygenate batch water.
- Pressure washer (or equivalent) for cleaning

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 at least level "D" personal protection equipment (PPE). A Materials Safety Data Sheet (MSDS) is sent with each shipment and should be reviewed before proceeding with installation activities.

Warning

- The BDI PLUS container is pressurized 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 at 949.366.8000. A back up set of quick connects is provided in the packaging material.
3. Check and ensure that all valves are in the CLOSED position.

Storage

If the schedule of bacteria application requires adding the bacteria over a period of more than one day, the keg(s) should be stored at a temperature 2-4 °C, but freezing must be avoided. This can normally be achieved by storing the kegs under ice in the provided coolers. Keg should be pressurized with Nitrogen to pressure 10- 15 psi. before storing to ensure a tight seal on the keg cap.



Culture Keg in Cooler

Shipping

After completion of operation, please, ship cooler with keg and all attachments back to the following address:

Shaw Environmental, Inc.
17 Princess Road, Lawrenceville, NJ 08648

Specific Installation Procedures

1. The BDI PLUS must be added to the previously prepared "oxygen-free" water before it is installed in the subsurface. The desired amount of BDI PLUS should be carefully discharged into the 55-gal drum containing the appropriate amount of "oxygen free" water. The tables provided below indicates the amount of water that a given amount of BDI PLUS should be mixed with. The BDI PLUS must be added to "oxygen-free" water before it is installed in the subsurface. To ensure that the water has reached the desired anoxic state prior to mixing with BDI PLUS an appropriate amount of nitrogen sparging into the 55-gal drum containing a given amount of water at least one hour prior to adding the BDI PLUS. To ensure that a sufficient quantity of "oxygen free" water is available throughout the day, a large trough of "nitrogen sparged" water can be prepared and additional 55-gal drums can be filled from this trough. The water in the trough can be transferred to the 55-gal drums where the BDI is mixed with the water using a primed transfer pump.

Nitrogen sparging is accomplished by a gas sparging device equivalent to a fish tank aerator. Adjust the 300ft³ nitrogen tank pressure regulator to 3-5 psi and immerse the gas sparger to the bottom of the drum or trough. By internal convection and oxygen stripping processes, the oxygen levels should diminish within an hour. Be careful to not consume too much gas and not have nitrogen to empty the kegs. 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.

BDI PLUS Dilution Chart

Volume of BDI PLUS	Volume of Water
5 liters	50 gal
1 liter	10 gal

Volume of BDI PLUS Concentrate	Volume of Water
0.5 liters	50 gal
0.1 liter	10 gal

- The drive rod assembly should be fitted with a disposable tip on the first drive rod and pushed down to the desired depth. This process should be done in accordance with the manufacturer's standard operating procedure (SOP).
- A sub-assembly connecting the delivery hose to the drive rods and pump should be used. The sub-assembly should be constructed in a manner that allows for the drive rods to be withdrawn while the material is being pumped.
- Prior to connecting the hose to the sub-assembly a volume check should be completed to determine the volume and weight of product displaced with each pump stroke.
- After the drive rods have been pushed to the desired depth, the rod assembly should be withdrawn three to six inches so that the disposable tip has room to be dropped.
 - If an injection tool is used instead of an expendable tip, the application of material can take place without any preliminary withdrawal of the rods.
- Fill the annular space of the drive rods with water. This will minimize the amount of air introduced to the system.
- Insert the telescoping suction pipe on the rotary transfer pump into a bung hole on the lid of the 55-gal drum and make sure that the pipe reaches the bottom of the drum. If possible, attach the suction pipe to the bung hole with the 2" bung adapter to ensure that the pump remains securely in place while pumping the Bio-Dechlor INOCULUM mixture from the drum to the pump hopper.
- Attach the hose to the outlet of the rotary transfer pump making sure that the opposite end of the hose reaches the pump hopper. Open the opposite bung hole on the drum lid to prevent a vacuum then pump the desired amount of BDI PLUS solution into the hopper of the pump.
- Connect the hose from the grout pump to the drive rod assembly.
- Start pumping the BDI PLUS product solution.
- The initial volume of BDI PLUS solution pumped should only be enough to displace the water within the drive rods. Once this is done the actual injection can start.
- Begin withdrawing the drive rods, in accordance with the manufacturer's SOP, and start pumping the BDI PLUS solution simultaneously. The dosage should be 0.1 liter per vertical foot or 1 gallon per vertical foot if prepared using the BDI dilution chart. The withdrawal rate should be such that it allows the appropriate quantity of material to be injected into each vertical foot of aquifer being

treated. The withdrawal rate should be slow to avoid creating a vacuum. This vacuum can potentially pull a small volume of material to the surface if the drive rods are withdrawn too quickly.

13. In less permeable soils such as clays and silts, there may be difficulty accepting the volume of estimated material. In this case REGENESIS recommends using a “step-wise” application approach. For this approach we suggest withdrawing the drive rods in one-foot increments and then injecting the quantity of material required per vertical foot.
14. Look for any indications of aquifer refusal such as:
 - Excessive pump noise or application pressure spikes (e.g. squealing)
 - Surfacing of material through the injection point (“blow-by”) If acceptance appears to be an issue it is critical that the aquifer is given enough time to equilibrate before breaking down the drive rods and/or removing the hose. The failure to do this can lead to excessive back flow of the BDI PLUS material on personnel, equipment, and the ground surface.
15. If BDI PLUS solution continues to “surface” after the drive rods have been completely removed from the borehole a plug may be necessary. Large diameter disposable tips or wood stakes have been used successfully for this purpose.
16. Drive rods should be disconnected after one rod (typically 4 feet in length) has been withdrawn. The drive rods should be placed in a bucket (or equivalent) after they have been disconnected.
17. Complete the installation of the BDI PLUS solution at the designated application rate across the entire targeted vertical interval.
18. After the injection is completed, an appropriate seal should be installed above the vertical interval where the BDI PLUS solution has been placed to prevent contaminant migration. Typically, bentonite powder or chips are used to create this seal. However, consultants should review local regulations before beginning field installation activities to confirm that this approach can be used.
19. Complete the borehole at the surface as appropriate using concrete or asphalt.
20. Repeat steps 7 through 19 until the entire application has been completed. If additional drums of de-oxygenated water are required, prepare as suggested in Step 1.
21. Prior to the installation of BDI PLUS, all surface and overhead impediments should be identified as well as the location(s) of any underground structure(s). Underground structures include but are not limited to: utility lines (gas, electrical, sewer, etc), drain piping, and landscape irrigation systems.
22. The planned injection locations should be adjusted in the field to account for impediments and obstacles.
23. The actual injection locations should be marked prior to the start of installation activities to facilitate the application process.
24. Using an appropriate pump to install the BDI PLUS product is very critical to the success of the application as well as the overall success of the project. Based on our experience in the field, REGENESIS strongly recommends using a pump that has a pressure rating of at least 1,000 psi and a delivery rate of at least 3 gallons per minute. If the application involves both HRC and BDI PLUS, two separate pumps may be required to facilitate the process. The pump used to deliver HRC to the subsurface should be in accordance with the specifications outlined in the General Guidelines section of the HRC Installation Instructions.



Additional Information

The internal workings of the grout pump can be cleaned easily by recirculating a solution of hot water and a biodegradable cleaner (e.g. Simple Green) through the pump and delivery hose(s). If additional cleaning and decontamination is required it should be conducted in accordance with the manufacturer's SOP and local regulatory requirements.

Note: REGENESIS assumes that all of the material (microorganisms) sent to a site for installation purposes will be used for that particular project and that no material (microorganisms) will be left over at the conclusion of the installation activities.