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PolySeed® Application Procedure BOD₅ Seed Inoculum

PolySeed® is a blend of broad spectrum bacteria designed specifically as a seed inoculum for the Biochemical Oxygen Demand (BOD₅) test as conducted in accordance to **Standard Methods of the Examination of Water and Wastewater**. PolySeed® is an EPA approved BOD₅ seed inoculum that has been used to seed both municipal and industrial wastes for almost 35 years.

Overview: The following are the most important parts of the BOD₅ test. **First**, the BOD water must be made properly and stored at 20° C. **Next**, the PolySeed® solution must be properly rehydrated and tested to determine its' effect on the test (i.e., the Seed Control Factor – SCF). **Finally**, the seed inoculum must be tested against a known Glucose-Glutamic Acid (GGA) standard. With these tests in order, a very reliable and accepted BOD₅ test can be performed.

1st Step: Control – Dilution Water ("BOD Water"): Prepare the dilution water (some call it "BOD Water" or "Blank Water") in accordance with *Standard Methods*. Be sure to use fresh deionized water and remineralize with the appropriate nutrients and chemicals. Store the Control-Dilution water at 20° C until ready to use. Run a control "**Blank**" on the neat Control-Dilution water at 20° C along with the actual BOD test. To insure an acceptable final test the "**Blank**" must have an oxygen depletion of less than 0.2 mg/liter over the 5-day period. If you have any questions, refer to *Standard Methods*, InterLab's e-Guide Videos or our Frequently Asked Questions ("FAQ") page available at www.polyseed.com.

2nd Step: Seed Solution (i.e. "PolySeed® Solution"): To make the seed solution, place the entire contents of one PolySeed® capsule (discard the gelatin capsule) into 500ml of "DILUTION WATER" prepared in accordance to *Standard Methods* (**do not use DI water by itself**). Normal dilutions are one PolySeed® capsule to 500ml of BOD water; however, the concentration of seed can be adjusted to compensate for variations in BOD water and established internal laboratory testing protocol. This seeded dilution water will be referred to as the "PolySeed® solution". **Note: Bran, which acts as the carrier for the microorganisms, will neither dissolve nor inhibit microbial activity, but must be settled out of the PolySeed® solution prior to use.**

Next, aerate and stir the PolySeed® solution for 1 hour, then let the solution settle for 5 to 15 minutes. Finally, decant the supernatant carefully so as not to allow any bran in the biological solution. Pour the decanted PolySeed® solution in a clean 500ml beaker with a sterile stir bar, place on magnetic stirrer and gently stir for the remainder of the test. (Note: Our lab uses a Nalgene separatory funnel for this purpose) For best results, the PolySeed® solution should be used within 6 hours of rehydration of the capsule.

3rd Step: Seed Control Factor ("SCF"): After following Step 2, carefully draw an aliquot from the PolySeed® solution. It is best to prepare the seed control using 15, 20, 25 & 30ml of PolySeed® solution; however, these aliquots may vary depending upon laboratory procedures. The contribution of the seed to the measured BOD, i.e. SCF in the test bottles should be between 0.6 – 1.0 mg/L (see calculations below).

At the end of the 5-day test period calculate the **SEED CONTROL FACTOR ("SCF")** of the PolySeed® solution per *Standard Methods* by using $[(D1 - D2) \times f]$ where:

D1 = DO of seed control before incubation, mg/L
D2 = DO of seed control after incubation, mg/L and,
f = (Volume of seed in diluted sample)/(volume of seed in seed control)

Note: This can be automatically calculated using InterLab's BOD calculator.

4th Step: Glucose-Glutamic Acid Standard: After the glucose-glutamic acid (GGA) standard solution is prepared (refer to *Standard Methods* or our FAQ page at www.polyseed.com), use 4ml of PolySeed® solution for each BOD₅ bottle. Again, make sure there is no undissolved bran in the pipette. No other seed is required. (Note: PolySeed® solution volume can be adjusted to compensate for variations in DI water, laboratory procedures and established internal laboratory testing protocol.)

5th Step: BOD Sample Analysis: Prepare the live BOD samples in accordance with *Standard Methods*. Insure that the PolySeed® Solution is prepared and stirred in accordance with Step 2 above. Add 4mls of PolySeed® solution (this volume can be adjusted for varying BOD water) to each BOD₅ bottle when preparing the wastewater samples. No other seed is required. Follow *Standard Methods* procedures for incubation, seed correction, GGA, and dilution water preparation. When reporting results using PolySeed® it is best to use the BOD calculator located at www.polyseed.com or hand calculations in accordance with *Standard Methods*.