

# FIVE DAY BIOCHEMICAL OXYGEN DEMAND (BOD)

NUTRIENT ANALYTICAL SERVICES  
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FEBRUARY 2011

Method Description: The five day BOD is an indicator of how much oxygen is utilized by a natural water sample (phytoplankton and bacteria) in five days at 20 degrees C. Low utilization is good.... High utilization not so good.

## Materials & Reagents:

1. 20 +/- 1 degree C incubator
  2. 300 mL BOD bottles (disposable bottles are available and can be reused)
  3. pH meter
  4. air pump with air stone
  5. 10-20 liter carboy
  6. Dilution Water
  7. Chemical Solutions for Addition to Dilution Water
    - a. Magnesium Sulfate            2.25%            (22.5 g/L DHOH)
    - b. Calcium Chloride            2.75%            (27.5 g/L DHOH)
    - c. Ferric Chloride            0.025%            (0.25 g/L DHOH)
    - d. Phosphate Buffer Solution (commercially available from Environmental Express)
- For every liter of dilution water, add 1.0 mL of each of the above.
8. Bacterial Seed (Poly Seed available from Environmental Express)
  9. Glucose/Glutamic Acid (# D1243 from Environmental Express)

## Field Collection

Collect 1-2 liters of whole water and keep chilled.  
Sample must be delivered to the lab within 24 hours of collection

## Method

### Preparation of Dilution Water

1. Add 18 liters of DHOH into carboy and aerate for 2 hours
2. When aeration of DHOH is completed, add (in this case) 18 mL of each of the following solutions; magnesium sulfate, calcium sulfate, ferric chloride and phosphate buffer. Shake well. This is now named **DILUTION WATER.**

## Preparation of Samples

1. Remove the samples from the refrigerator and determine the pH, if not provided. The samples must be in the 6.5 to 7.5 range. If not, adjust to within that range with drop wise addition of sulfuric acid (2.8 mL H<sub>2</sub>SO<sub>4</sub> diluted to 100ml Deionized water).

## Preparation of Incubation Solutions

1. Biological Seed Preparation: Add the entire contents of one capsule into 500 mL of dilution water. Aerate for one hour. **The flocculent material is bran and should not be included in the inoculation!! When inoculating BOD bottles, take the seed from the mid-depth of the beaker using a pipette.**

## Incubation procedure

### 1. Setting up the BOD bottles

**Blank** (2 bottles): Blanks are comprised of dilution water only. Fill two BOD bottles to the brim with dilution water. This is the only treatment that receives NO seed solution.

**Seeded Blank** (3 bottles): Seeded blanks are comprised of dilution water and seed solution. Place 5.0 mL of seed solution in three separate BOD bottles. Fill each bottle to the brim with dilution water.

**2% G and G** (3 bottles): This is a standard and consists of a commercially prepared glucose and glutamic acid sample, seed solution and dilution water. Place a complete vial of glucose/glutamic acid solution in each of three BOD bottles. Add 5.0 mL seed solution to each bottle. Fill each bottle to the brim with dilution water.

**Samples** (3 bottles/sample): For natural water populations, each sample is incubated in triplicate with three different dilution treatments; 40, 50, 60% . For example, the 40% dilution consists of 120 mL sample, 5 mL seed solution and is filled to the brim with dilution water. Each dilution treatment requires 5 mL of seed solution.

## Incubation and Measurement

1. Calibrate dissolved oxygen meter per manufacturer's directions. Take initial dissolved oxygen reading of all bottles per instrument directions.

2 Cap and place in 20 degree C incubator for five days (+/- 3 hours).

3. After five days remove samples from incubator and measure dissolved oxygen.

Don't start processing samples on a Monday or Tuesday unless you want to come in Saturday and Sunday to do the five day readings.

### Calculation of BOD:

1. Subtract the five day DO reading from the initial reading from all bottles.  
This is the corrected value
2. Subtract the mean corrected value of the blanks from all corrected values.
3. Multiply the final corrected values by the dilution of the sample. For example, if the final corrected value of a 50% dilution is 0.64, then the true value is 1.28. For a 60% dilution reading of 3.58, the true value is 5.97 and for a 40% dilution of 0.96, the true value is 2.40.