

Operation's Challenge 2012 Laboratory Event

EVENT: Perform the following steps of a BOD analysis using the YSI ProOBOD Optical BOD probe.

General NOTES:

1. Team Captain tells the Lead judge they are ready to begin and the Lead judge says "START" to signal the beginning of the event. The Lead judge and one other judge will be the timekeepers.
- 2. Event is complete when all tasks have been completed and Team Captain hands in the work sheets to the Lead judge and says the team is finished.**
3. To ensure a fair contest and to avoid challenges, judges will not speak to contestants while the event is being performed.
4. The Event Coordinator will settle disputes with input from the event judges.
5. All team members must participate in the event, but are not limited to performing only one task.
6. After the event, the Event Coordinator may explain to the Team Captain what was done incorrectly, but will NOT reveal penalty points or total score.
7. Team members may ask judges questions before the beginning of the event, but the judge may choose not to answer the question, depending on the type of question asked.

ALL STEPS OF THE PROCEDURE MUST BE PERFORMED FROM MEMORY. NO BOOKS OR PRINTED MATERIALS ARE ALLOWED IN THE LABORATORY COMPETITION AREA.

MATERIALS REQUIRED:

Teams will be required to wear safety glasses and gloves during the event. Teams will be responsible for providing their own safety glasses and calculators.

YSI ProOBOD Optical BOD Probe part# 626400

YSI ProODO meter part# 626281

YSI pH 100 meter part# pH100

YSI pH/Temp Combination Electrode part# 100-1

YSI Electrode stand part# 605170

Sample (in 1L plastic bottle)

GGA Solution (in 250 ml plastic bottle)

pH Buffer Solution 7

Stir Plate and Stir bars

Stir Bar retriever

Deionized Water (DI) in 1L Wash Bottle (1)

Kim Wipes

Beakers-100-250mL

Nitrile or Latex Disposable Gloves

300ml BOD bottles
Liquid Seed Material (in 500 ml beaker)
Waste Containers
Pipet Washer (basket only)
BOD Dilution Water in a carboy with spout (will be elevated)
1 L Wash bottles containing BOD Dilution Water (2)
2, 5, 10, and 25mL transfer pipets
6mL volumetric pipet
Pipet bulbs, safety type
50mL graduated cylinder
Glass BOD bottle stoppers
Plastic BOD bottle caps
Timers with 20 second reset capability
Calculator (Teams must provide their own)
Sharpie Markers

SETUP:

Teams will have **two minutes** before beginning the event to organize items on the tables. These items are limited to Sample, GGA Solution, wash bottles, kimwipes, paper towels, beakers, BOD bottles, waste containers, pipets, pipet bulb, 50mL graduated cylinder, glass BOD bottle stoppers, plastic BOD bottle caps and sharpie marker. The lead judge will time setup. At the end of the setup time the judge will say “TIME”, team members must remove their hands from the table. Judges will then place bench sheets facedown on the tables. See “General Notes #1” above for instructions on starting the event.

Teams will start from the inside of the table set up.

Numbering and Labeling Bottles:

This must be completed before any sample, seed material or dilution water can be placed in each BOD bottle.

All BOD bottles must be labeled and/or numbered using a sharpie marker according to the numbers on the bench sheet.

1. Label one BOD bottle as “BLANK” and with the bottle number according to the bench sheet provided.
2. Label four BOD bottles as “SEED 1”, “SEED 2”, “SEED 3” and “SEED 4” and with the bottle number according to the bench sheet provided.
3. Label one BOD bottle as “GGA” and with the bottle number according to the bench sheet provided.
4. Label the remaining **four** BOD bottles to be used for sample planting with the bottle number **only** according to the bench sheet provided.

Determine pH of sample is between 6.00 and 8.00:

Sample pH must be determined before the sample can be planted

1. Mix the sample by gently inverting* 5 times (5X). Rinse a beaker one time with a small amount of sample and pour approximately 50-75 mL of the sample into a beaker.
2. Remove pH probe from soaking solution; rinse with DI and blot dry using a kimwipe.
3. Place the beaker containing the sample on the stir plate, add a stir bar, stir gently, and lower the probe into sample.
4. Press “MEA./EFF.” key. The word “Wait” will flash until a stable reading is obtained. (All teams will be required to wait 20 seconds before recording a reading. The reading may be taken after 20 seconds even if the “Wait” sign is still flashing).
5. Press “START” on the timer provided to begin a 20-second stabilization time.
6. Record the pH reading (to two places after the decimal point), unit of measure (su), date, time, analyst on worksheet
7. Remove the pH probe from sample, rinse with DI, blot dry and return to soaking solution.
8. Retrieve the stir bar from sample, rinse it off, pat dry, and return to the table.

* A single inversion consist of taking a bottle from an upright position and turning it a full 180° to an upside down position, then back into the upright position.

Once the pH of sample has been determined to be 6.00-8.00, the sample is ready to be planted.

Preparing a Blank and Seed Correction Series:

Penalties will be assessed for blowing out pipets.

BLANK PREPARATION

1. Rinse the bottle labeled as “BLANK” one time with dilution water.
2. Fill the bottle with dilution water from the carboy without entraining air by tilting the bottle on a 45-degree angle. Fill only half way up the frosted neck of the bottle.

SEED CORRECTION SERIES PREPARATION

1. Rinse the bottles labeled as “SEED 1”, “SEED 2”, “SEED 3”, and “SEED 4” one time with dilution water.
2. Using a 5mL transfer pipet and bulb, rinse the pipet once with seed material, then transfer 5mL of seed material from the beaker on the stir plate into the “SEED 1” bottle
3. Using a 10mL transfer pipet, rinse the pipet once with seed material, then transfer 10mL of seed material from the beaker on the stir plate into the “SEED 2” bottle
4. Using a 25mL transfer pipet, rinse the pipet once with seed material, then transfer 15mL of seed material from the beaker on the stir plate to the “SEED 3” bottle

5. Using the same 25mL transfer pipet, transfer 20mL of seed material from the beaker in the stir plate to the "SEED 4" bottle
6. Place all dirty pipets in the pipet washer.
7. Fill the bottles with dilution water from the carboy without entraining air by tilting the bottle on a 45-degree angle. It is acceptable to fill bottles only partially out of the carboy then top off with the wash bottle containing dilution water to prevent loss of seed material during filling. Fill bottles half way up the etched glass neck of the bottle. Penalties will be assessed for overfilling bottles.

These bottles can now have the Initial DO readings recorded. The DO meter must be air calibrated before taking any Initial DO readings.

Plant, seed and fill bottles for the GGA Standard and BOD sample:

**Sample cannot be planted until the pH has been determined to be 6.00-8.00.
Penalties will be assessed for blowing out pipets.**

GGA STANDARD

1. Rinse the bottle labeled "GGA" one time with dilution water.
2. Gently mix GGA standard by inverting 5 times, then rinse a small beaker one time with the GGA standard, pour approximately 50mL into the beaker.
3. Using a 6mL volumetric pipet, rinse one time with GGA standard then transfer 6mL of the GGA standard into the BOD bottle.
 - The outside of the pipet must be wiped dry with a kimwipe before dispensing into the bottle.
 - Pipet must be allowed to drain into the bottle- do not blow out.
 - Touch tip of pipet to the side of the bottle to ensure the appropriate volume has drained.
 - Proper use of a volumetric pipet will result in a small amount of solution remaining in the pipet.
4. Using a 2mL transfer pipet, rinse one time with seed material, then transfer 2mL of seed material from the beaker on the stir plate containing seed material into the GGA bottle.
5. Fill the bottle with dilution water from the carboy without entraining air by tilting the bottle on a 45-degree angle. It is acceptable to fill bottle only partially out of the carboy then top off with the wash bottle containing dilution water to prevent loss of seed material and standard during filling. Fill bottle to half way up the etched glass neck of the bottle. Penalties will be assessed for overfilling bottles.

SAMPLE:

1. Rinse bottles numbered for planting the sample once with dilution water.
2. Gently mix sample by inverting 5 times, rinse a large beaker one time with the sample, then pour approximately 200 ml of the sample into a large beaker.
3. Align the bottles numbered for sample planting from lowest to highest number.
4. Using a 5ml transfer pipet, rinse one time with sample, then transfer 5mL of the sample from the beaker to the first bottle (lowest number) using a transfer pipet and bulb.

5. Transfer 2mL of seed material from the beaker on the stir plate containing seed material into the bottle using a using the same 2mL transfer pipet used to seed the GGA standard.
6. Fill the bottle with dilution water from the carboy without entraining air by tilting the bottle on a 45-degree angle. It is acceptable to fill bottles only partially out of the carboy then top off with the wash bottle containing dilution water to prevent loss of seed material and sample during filling. Fill bottle to half way up the etched glass neck of the bottle. Penalties will be assessed for overfilling bottles.
7. Using the next bottle, repeat steps 4-6 using a 10mL pipet to transfer 10 ml of sample.
8. Using the next bottle, repeat steps 4-6 using a 25mL pipet to transfer 20mL of sample.
9. Using the last bottle, repeat steps 4-6 using a 50mL graduated cylinder that has been rinsed with sample one time to transfer 35mL of sample.
10. Place all dirty pipets in the pipet washer.

These bottles can now have the Initial DO reading recorded. DO meter must be air calibrated before taking any Initial DO readings.

Calibrating the DO meter and determining Initial DO readings:

CALIBRATION

1. The meter will already be turned on and the DO probe will be in the calibration vessel
2. Perform Air calibration:
 - Press Calibration button (cal), highlight “DO”, press “enter”
 - Highlight “DO%”, press “enter”.
 - Press “START” on the timer provided to begin a 20-second stabilization time.
 - Under “Actual Readings” the DO% & Temperature will be displayed. After 20 seconds, highlight “Accept Calibration” & press enter.
 - The meter will read “Calibrating Channel...” then read “Calibration Successful”.
 - The meter is now ready to take readings.

DETERMING INITIAL DO:

- **Blank, Seed Series, GGA and Sample bottles must all be read**
- **The DO probe must remain in each bottle for at least 20 seconds before the Initial DO can be recorded.**

1. Remove the DO probe from the calibration chamber and rinse with dilution water.
2. Place the probe in the BOD bottle labeled “BLANK”.
3. Turn the probe stirrer on using the flip switch on top of the probe.
4. Press “START” on the timer to begin the 20-second bottle reading time.
5. Record the initial DO in mg/L on the bench sheet in the appropriate space after the probe has been in the bottle at least 20 seconds as indicated by the timer.
6. Turn off the probe stirrer, remove the probe from the blank bottle and place it in the first “SEED” bottle. Press “START” on the timer to begin the 20-second bottle reading time.
7. While the reading for the “SEED” bottle is stabilizing complete Step 8 for the “BLANK”

8. Stopper and cap the bottle:

- Using the wash bottle containing dilution water refill the bottle to half way up the etched glass neck of the bottle without creating bubbles or entraining air.
- Gently place glass stopper in bottle without causing bubbles or entraining air.
- Using the wash bottle containing dilution water fill the neck of the bottle to create a water seal if there is no water in the neck above the stopper.
- Place a plastic cap over the glass stopper to create an air seal.

9. Complete steps 2-8 for all other “SEED” bottles.

10. Once all “SEED” bottles have been completed, rinse the probe with the dilution water wash bottle over the waste container before continuing.

11. Place the rinsed probe in the “GGA” bottle. Repeat steps 3-5 and 8. Rinse the probe with the dilution water wash bottle over the waste container before continuing.

12. Place the rinsed probe in the first “SAMPLE” bottle. Repeat steps 3-8 for each bottle until all sample bottles have been completed.

13. After all bottles have been read, rinse the probe with dilution water using the wash bottle gently blot the membrane and lower assembly of the probe dry and replace in the air calibration chamber.

14. Complete benchsheet for the samples you have just set up, filling in Date, Time of Completion and Analyst

Calculating BOD Blank, Seed Correction and Sample Values:

A worksheet will be provided for samples that were set up 5 days earlier. This worksheet will have initial DO (IDO) values and final DO (FDO) values for a Blank, a seed correction series, and at least one sample. All results are to be reported in mg/L. **Only calculate bottles that have depleted at 2 mg/L or greater DO (IDO-FDO >2), and has 1 mg/L or greater DO remaining (FDO >1), except for the BLANK.** Volume of seed material used in GGA and sample bottles will be 2mL. Bottle volume is 300 mL.

BLANK

Blank depletion = IDO-FDO

SEED CORRECTION

Seed Correction bottle = (IDO - FDO) x volume of bottle (300) / mL seed material used in bottle.

Only calculate bottles meeting depletion criteria

Seed Correction (SC) = Average of all seed correction bottles calculated / volume of bottle (300) x mLs of seed used per sample & GGA bottles (2)

GGA AND SAMPLE

BOD in mg/L = [(IDO-FDO) - SC] x volume of bottle / mLs of Sample planted or GGA used in bottle.

Final Sample Result = Average of all results calculated for the sample