Biochemical Oxygen Demand Do’s and Don’ts

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ORWEF Short School
March 29, 2017
Classic
Historic
Outline

• Definition and Overview

• Procedure
  – Sampling
  – Blanks
  – Controls
  – Seed
  – Nitrification Inhibitors
  – Dilutions
  – Meter calibration

• Calculations

• Troubleshooting common problems

• Resources, Links...
Approved Methods:

• **Standard Methods 5210 B – 2001**

• If/When new EPA Method Update Rule is published the latest approved BOD method will be 5210B – 2011 (22nd Edition)

• Hach Method 10360 Rev 1.2 – 2011 allows the use of a Luminescence Based sensor.
What does BOD measure?

• The Biochemical Oxygen Demand is an empirical test that measures the amount of oxygen used by bacteria as they metabolize organic matter at 20 °Celsius, in the dark, usually over a 5 day period.

• (pH 6.5 to 7.5 is best)

• Two types of bacterial activity:
  – Carbonaceous
  – Nitrogenous
The BOD Curve

- **First stage:** carbonaceous demand curve
- **Second stage:** combined carbonaceous plus nitrogenous demand curve

**Axis Labels:**
- **Y-axis:** Biochemical Oxygen Demand
- **X-axis:** Time, in days
BOD’s are simple

• Calibrate your DO meter.
• Put samples in BOD bottles, add dilution water and measure DO.
• Put bottles in incubator.

• Repeat again in 5 days
Murphy’s Law

• IF ANYTHING CAN GO WRONG, IT WILL!

• H. Tim Neketin: “Murphy was an optimist.”
Samples

- Clean effluent sampler hoses at least weekly with bleach to remove build up of nitrifying bacteria
- Warm samples to room temp: 20 ± 3°C
- Check pH of samples. If not between 6.0 and 8.5, then adjust to between 6.5 and 7.5 with 1 Normal H2SO4 or 1 Normal NaOH...
- *Recent method says adjust to 7.0 to 7.2*
Bottles, jugs, glassware

• Use **non-phosphate detergent** that leaves glassware sparkly clean.
  
  *Contrad liquid
  
  Neodisher (from Miele) or Tergajet (?)

  **Don’t use Alconox, Versatone**

• To remove dried on film from bottles use 1:1 Sulfuric Acid (or HCl in a hood).
  
  • Don’t use Chromic Acid (honest!)

• *Disposable BOD bottles avoid cleaning problems*
Disposable BOD Bottles
Dilution Water

• Source:
  DI+carbon filtered water
  Steam-distilled commercial water
  Distilled is risky as is straight DI or RO/DI water
  Tap Water is a no-no! (Chlorine, Copper, etc.)

• Aerate & store at room temp. Add nutrients only 1 day ahead of use.

• Bleach storage jugs and hose at least every 2 weeks.
Dissolved Oxygen Meters

(Museum Quality circa 1980…. Please Do Not Touch)
YSI-Polarographic Probe
D.O. Meter and Probe

• **YSI type**: change membrane/cap and filling solution every two to four weeks or when scratched, bubbles under membrane or leaking

• Check for Black AgS on electrode. Soak with 10% Ammonia and buff lightly.

• Store in BOD bottle with a little H2O.

• Keep membrane dry (no water drops) when calibrating.
Luminescent (LDO) Probes
D.O. Meter and Probe

- **LED type:** change cap annually (6-12 mos)

- Store in BOD bottle with a little H20.
  - Check for leaks periodically
  - Replace stirrer as needed

- Keep membrane dry (no water drops).
LED Probe Maintenance
LED Probe Maintenance
Inside LED Cap
Calibration and Checks

• Air calibration.
  – wipe membrane of water drops
  – Allow enough time to stabilize
  – Check against Saturation Tables
    • DO sat is 9.09 mg/l at 20*C and 760 mm Hg

• Check Bottle.
  – Two bottles of aerated dilution water
    • Check DO in Bottle #1 after calibration complete.
    • Run Winkler on Bottle #2. (Agree within 0.5 mg/l?)
    • Check Bottle#1 again at end of readings (within 0.2?)
Seed

- Settled Raw Influent
- Primary Effluent
- Avoid polymer residues, high ammonia
- Aim for seed correction of 0.6 to 1.0 mg/l

- Add to dechlorinated effluent
- Some labs add seed to all industrial discharges
Quality Controls

• GGA:

  150 mg Dextrose, anhydrous,

    +

  150 mg L-(+)-Glutamic Acid per liter

Make your own: pre-measure 5 mls into vials and freeze

or

Purchase already made and refrigerate
Nitrification Inhibitors

• TCMP:

  2-chloro-(TriChloroMethyl)Pyridine, 2.2%
  (powder)

  Available from Hach, North Central Laboratories

• N-Allylthiourea

  (liquid in its pure state)
  (contained in Polyseed NX)

  Available from Hach, other lab suppliers
Set’m Up

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Dilution Water
Calculations

• No seed:

\[(\text{DO}_0 - \text{DO}_5) \times 300\]

(ml sample)

• Seeded:

\[\left(\text{DO}_0 - \text{DO}_5\right) - (\text{seed corr})\] * 300

(ml sample)

Valid dilution has DO depletion of at least 2.0 mg/l

AND

has at least 1.0 mg/l DO left
Calculations

• See Sample Benchsheet
<table>
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<tr>
<th>SAMPLE</th>
<th>BOTTLE #</th>
<th>SAMPLE ML</th>
<th>ML SEED</th>
<th>D.O. in mg/l</th>
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<th>Depletion mg/l</th>
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DO check in, finish | DO check out finish
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TNI Documentation Requirements

- Method ID/SOP ID
- Date/time/Analyst Initials
- Equipment ID
- Before/After Calibration Verification
- Reagent Lot ID’s
- Maintenance (Meter Logbook)
Troubleshooting

- Bad Blanks: depletion > 0.20 mg/l
  - bad dilution water
  - dirty bottles or other labware
  - calibration problems (meter/probe)

Other questions?
- how does reporting limit depend on dilution?
- when do you average results?
Troubleshooting

• Bad GGA controls: $< 167.5$ or $>228.5$ mg/l

  GGA-BOD: $198 +/ - 30.5$ mg/l. source: Std Methods

[GG-A-CBOD: $164 +/ - 26$ mg/l.  source: TNI PT ]

• Bad Seed: source? age?
• Forgot seed, forgot GGA (Steve Martin defense)
• Bad Dilution Water, meter, probe, etc.
My Thanks To:

• James Loftis & Steve Hall, City of Salem
• Chris Dennis, City of Portland, BES
• John Hoppner, City of Corvallis
• Perry Brake
Resources:

Perry Brake:

•  [http://www.perrybrake.com/BODSolutions.html](http://www.perrybrake.com/BODSolutions.html)


Oregon DEQ:

[http://www.deq.state.or.us/lab/techrpts/docs/09lab0071gag.pdf](http://www.deq.state.or.us/lab/techrpts/docs/09lab0071gag.pdf)

If all else fails, then try:

•  [Keith.chapman6023@gmail.com](mailto:Keith.chapman6023@gmail.com)
While the 21st Edition of Standard Methods has not yet been approved for Clean Water Act monitoring, it is worthwhile to know what some of the changes are in the BOD test (SM 5210B). Some of these changes may be allowed or even encouraged by regulators and lab accreditors. This article points out some of the more significant changes in the order they appear in the method. Comments are those of the author.

- **Paragraph 3g(2)** adds allyltiourea (ATU) as an approved nitrification inhibitor.

- **Paragraph 4b(4) and 5b** allow sample temperature prior to dilution to be 20 +/- 3° C rather than +/- 1° C (but the incubation temperature remains 20 +/- 1° C.)

- **Paragraph 4b(5)** adds pretreatment procedures for samples containing hydrogen peroxide.

- **Paragraph 5a** expands guidance on preparation of dilution water and recognizes the fact that source water problems can lead to blanks exceeding 0.20 mg/L depletion, even in the absence of any "contamination" in the test process.

- **Paragraph 5d** adds the following in referring to the amount of seed to add to the seeded bottles: "...if 1 mL of seed...is required to achieve 198 +/- 30.5 mg/L BOD in the glucose/glutamic acid check, then use 1 mL in each [seeded] BOD bottle..." (Comment: Reading the entire subparagraph, one would conclude that if 6 mL of seed were used in the GGA bottle, giving a depletion of 4 mg/L DO, the same amount would be used in each seeded bottle, also causing a depletion of 4 mg/L. This means the seed and not the sample would be by far the major contributor to DO depletion in seeded samples with low BOD, such as effluents. Because of imprecision inherent to the BOD test, the seed contribution to DO depletion could completely mask the sample contribution for low BOD samples. This seems contrary to good reason.)

- **Paragraph 5i** allows a +/- 6-hour variance in the "5-day" incubation period (i.e. it is now 5 days +/- 6 hours).

- **Paragraph 6b** adds "the glucose/glutamic acid check is the primary basis for establishing accuracy and precision of the BOD test..." (Comment: Paragraph 8 confuses this issue by retaining the old statement that "there is no measurement for establishing bias of the BOD procedure." Since "accuracy" as used in 6b contains a "bias" and "precision" component, it appears that paragraph 6b recognizes that the 198 mg/L objective for the GGA test is a bias objective.)

- **Paragraph 7b** establishes the following circumstances under which results should be "identified" in reports:
  - Blank exceeds 0.20 mg/L;
  - GGA result is outside acceptance limits;
  - Test replicates show more than 30% difference between high and low values (Comment: elsewhere, the term "dilutions" is used rather than "replicates" and it is probably "dilutions" that is intended);
  - Seed control samples do not deplete at least 2.0 mg/L, with a retention of at least 1.0 mg/L DO, or
  - The minimum DO retained is less than 1.0 mg/L.

  (Comment: The method does not say what is meant by "identifying" the results, but it probably means mentioning the result(s) in the remarks section of a report. Also, the method does not say results must be "identified" if no dilution depletes at least 2.0 mg/L. This probably means that reporting such results with the "<" less than symbol is sufficient.)

- **Paragraph 8b** establishes a lower detection limit of 0.0 mg/L, replacing the 2.0 mg/L lower limit in previous editions. The 0.0 mg/L is possible when all the DO depletion in a seeded, undiluted bottle is contributed to the seed.
The only substantial change to the BOD test in the most recent (spring 2012) edition of Standard Methods 5210B is that the QC tests applying to the procedure are summarized in a table (5020:1). That table calls for three tests: 1) a lab fortified blank which is the 50:50 glucose/glutamic acid solution required by previous editions of 5210B; 2) a lab fortified matrix, often called a matrix spike; and 3) a lab fortified matrix duplicate. The matrix spike and duplicate are new requirements.
The BOD Song!

• Oh B-O-D, Oh B-O-D
• You are so biochemical
• A little sample and then some seed
• So plain yet still empirical
• To set you up is such a breeze
• Just five short days at 20 degrees
• Oh B-O-D, Oh B-O-D
• Pray the blanks don’t deplete on me.
• First get the bottles all lined up
• Then calibrate the meter
• Aerate the water; prep the seed
• And don’t forget the Winkler
• Pipet your samples in “just so”
• Make sure they have enough D.O.
• Now load the samples on a cart
• Incubate them five days in the dark.
• If Wednesday morn you put them in
• Next Monday all your woes begin
• All the dilutions that you tried
• Were either too low or else too high
• All your controls are out of range
• The calculations just too strange
• Oh B-O-D you are so great
• The test that we all love to hate
Thank You