BOD 201

BOD Analysis: Advanced Techniques & Troubleshooting

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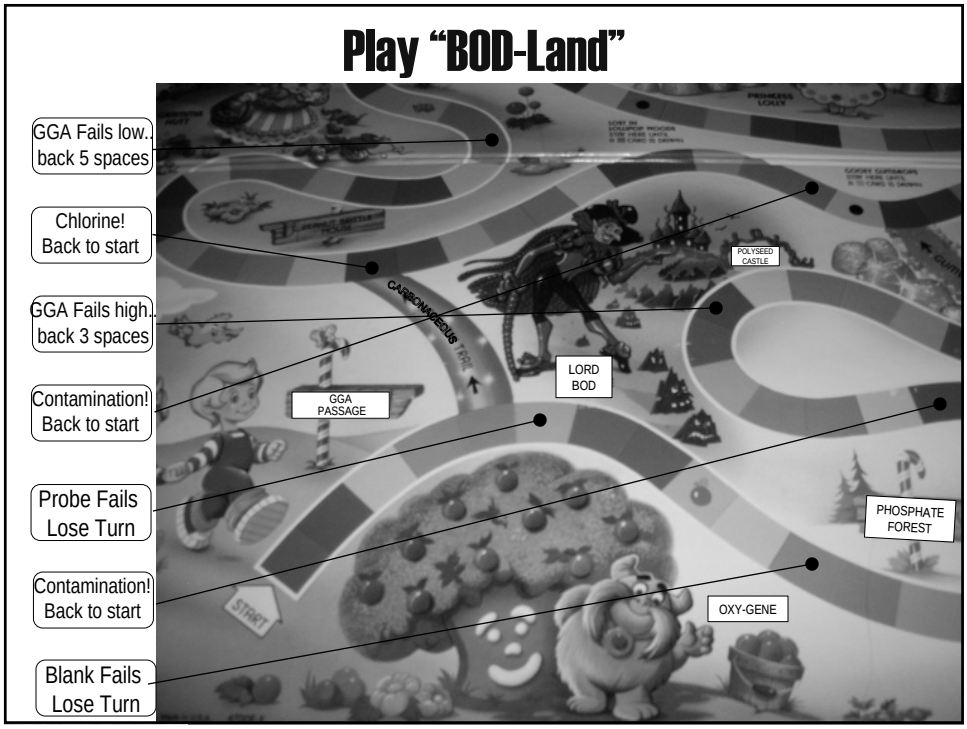
What are you looking for?

- What do YOU want to take away from this session?
- What concepts do YOU want to learn more about?
- What problems are YOU experiencing with the BOD test?

Session Outline

- **Overview**
- **BOD Basics**
- **Calibration**
- **Critical Testing Concerns**
- **Quality Control**
- **Documentation**
- **Reporting**
- **Troubleshooting**


BOD 201
The test everyone loves to hate
O_2
Rick
George


Play "BOD-Land"
GGA Fails low.. back 5 spaces
Chlorine! Back to start
GGA Fails high.. back 3 spaces
Contamination! Back to start
Probe Fails Lose Turn
Contamination! Back to start
Blank Fails Lose Turn
POLYSEED CASTLE
LORD BOD
GGA PASSAGE
PHOSPHATE FOREST
OXY-GENE
START

So….Why BOD?

None of the alternatives provide a better assessment of the bioavailability of a waste like the BOD test.

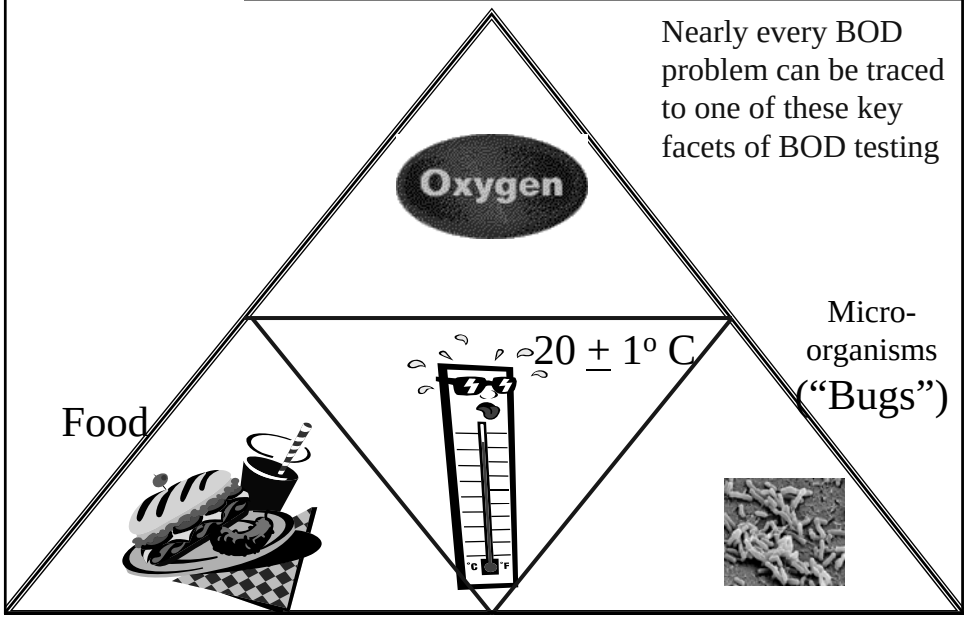
Bottom line: We're stuck with BOD for now!!!!

Is BOD a Pain in the #@$! Test???

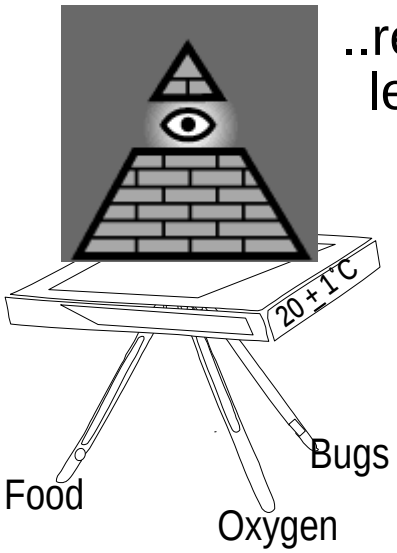
- You bet! But……
- Consistent and reliable BOD results can be produced by any lab if….
  - ✓ they use good laboratory QC practices,
  - ✓ pay attention to details, and
  - ✓ carefully follow the approved method.

BOD Pyramid

Nearly every BOD problem can be traced to one of these key facets of BOD testing



The BOD pyramid is not unlike a table…



..remove any one of its legs, and it falls over

Common Problems of the BOD test

- Sampling and/or preparation related
- Meeting depletion criteria for blanks
- Calibration Issues
- Consistently meeting GGA limits
  - Getting sufficient seed activity
  - Adding the right amount of seed
- D.O. membrane and probe performance
- Sample Size
- Nitrification
- Sample toxicity
- Improper interpretation of results

Sampling & Sample Handling

Sampling Considerations

- Preferable to sample BEFORE any disinfection
- If sampling after <u>any</u> disinfection, samples MUST be seeded

Sample Pre-Treatment

- Composite samples kept at 0 - 6 ^oC
- Recommended Hold Time
  - Grab samples = 6 hr (Std Methods)
  - Composite samples = 48 hr

Oxygen Measurement Techniques

DO Probe
(polarographic)

- ❑ Electrochemical Method
- ❑ Oxygen diffuses through membrane and is reduced at the cathode by the voltage.
- ❑ Produces a current flow, which is proportional to the partial pressure of oxygen.

- No reagents to prepare
- <u>Saves</u> 🕒,$, & labor
- continuous measurement

Winkler titration

- ❑ Titrimetric, wet chemistry test
- ❑ measures O_2 present based on conversion to iodine.

- considered the “Gold Standard”
- <u>Consumes</u> 🕒,$, & labor
- Reagent stability issues

Advances in Probe Technology

Polarographic 1 vs. 2 Thermistor

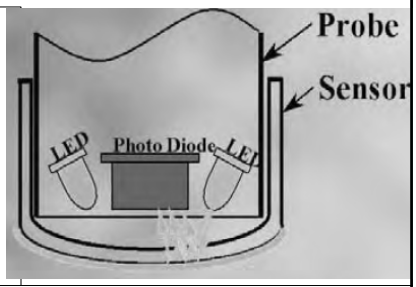
- Added thermistor to electrolyte (in addition to sample sensor). This allows temperature measurement in sample [water or air] AND in electrolyte.
- Air is poor heat sink (***think of cooking in a copper vs. aluminum pot***). People have tendency to calibrate too quickly when doing AIR calibrations.
- Double thermistor monitors differential between air and electrolyte and does not lock in calibration until the two are equal in temperature.
- Bottom Line: provides for more accurate & consistent calibrations

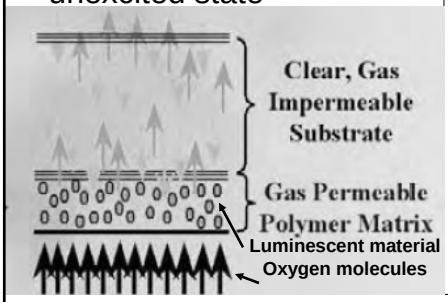
Luminescence

- A whole new technology for environmental chemistry
- Has been in use in medical field for years

Luminescence DO Technology

- Probes utilize a sensor coated with luminescent material
- Blue light is transmitted to the sensor from an LED on the surface
- This blue light excites the luminescent material which in turn emits red light as it returns to its unexcited state





- The elapsed time from excitation till return to steady state is measured
- The more oxygen present, the shorter the time it takes for red light emission
- Time is measured and correlated to oxygen concentration.

Diagrams courtesy of HACH Co.

Features of Luminescence DO Probe

- ❑ Developed by the HACH company
- ❑ Probe uses NO…
  - ☑ Anode ☑ Electrolyte
  - ☑ Cathode ☑ Membrane
- ❑ All of the items listed above are potential sources of error in conventional DO technology
- ❑ Research shows luminescence requires
  - ☑ Less frequent adjustments during calibration
  - ☑ Less maintenance
- ❑ Dis-advantages of luminescence probe
  - ☑ Requires "goofy" funnel and magnetic stirrer
  - ☑ Funnel difficult to use; poses cross-contamination risk

BOTTOM LINE: This is 1st generation instrument; suggest holding off for 2nd generation to "fix" a number of bugs

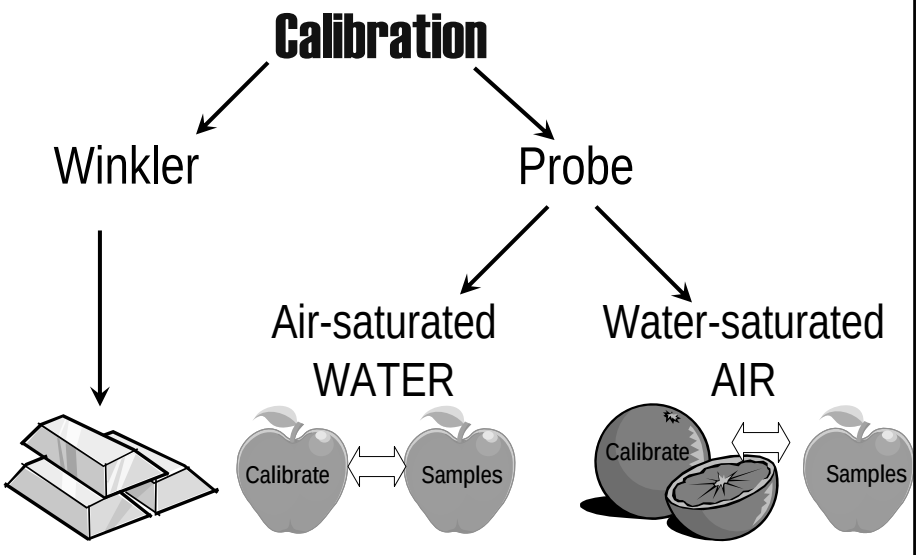
Can I use the Luminescence probe for my wastewater compliance testing?

- The short answer is:
  "…*technically*….not yet"
- The national EPA Office of Water has recommended EPA approval based on HACH data. ***But…***
- There are two big glitches to resolve:
  - ☑ It's not approved under the Clean Water Act (40 CFR Part 136)
  - ☑ It's not approved under Wisconsin's NR 219
  - …and it cant be approved in NR 219 until it gets approved under 40 CFR Part 136
- Currently, the only option would be to apply for acceptance as "Emerging Technology" under NR 149.12 (2)

BOD - Winkler Calibration

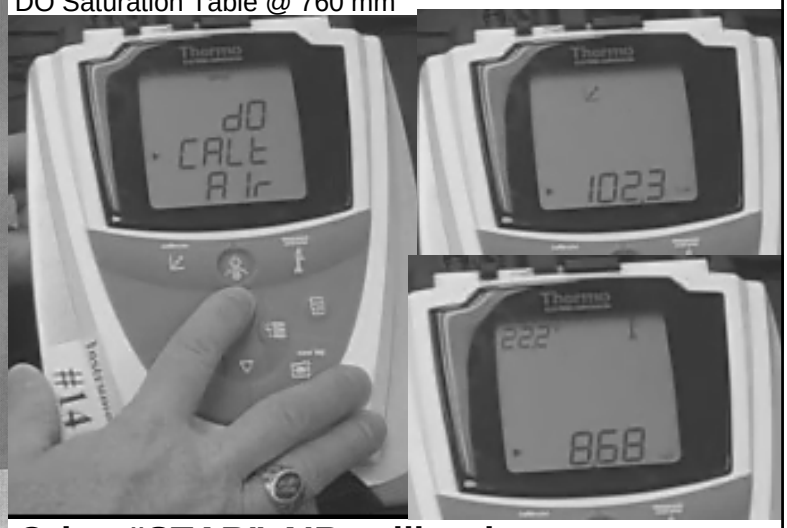
- Use air-saturated dilution water
- Use fresh reagents
- Standardize titrant
- Perform Winkler titration
- Check Winkler result against theoretical saturation
- Set meter
- Document your procedure!

Calibration-related errors are the #1 source of blank failures.



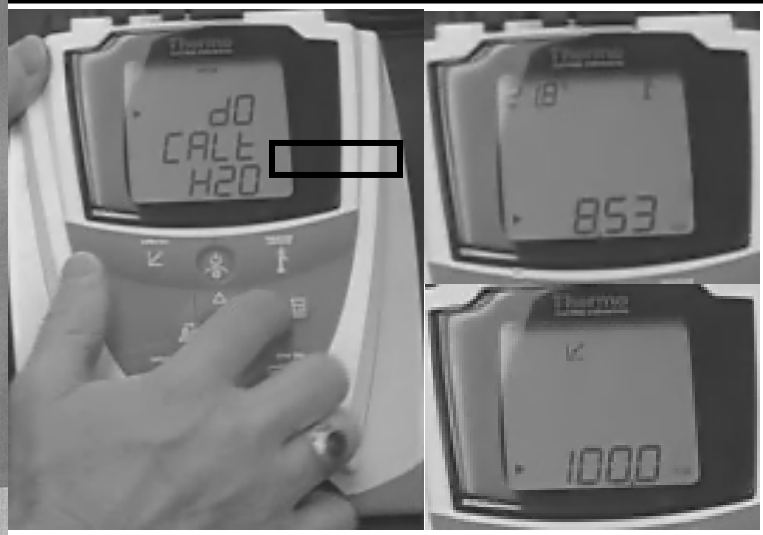
Apples to Oranges?
Or Macintosh to Grannysmith?

DO Saturation Table @ 760 mm



Orion "STAR" AIR calibration:
DO = 8.68 mg/L Saturation is at 102.3%!!!

Apples to Oranges?
Or Macintosh to Grannysmith?



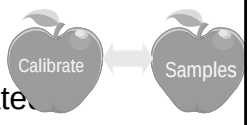
Orion "STAR" WATER calibration:
DO = 8.53 mg/L Saturation is at 100.0%!!!

Apples to Oranges? Or Macintosh to Grannysmith?

- Water vs. air calibration using the same sample
- Air calibration is higher by 0.15 mg/L (8.68 vs. 8.53) *NOTE: 8.68/8.53 = 101.7%*
- This is due to the "correction factor" which accounts for equilibrium in air vs. water
- Note that ORION manual erroneously states the correction factor to be 101.7%.
- The instrument however, ALWAYS reads 102.3% saturation when air calibrating. This is correct for polarographic probes.
- Orion STAR algorithm overcompensates during air calibration (i.e., reads 2.3% high) so it will read properly in water.
- Bottom line: the value shown after air calibration will be high but if immediately placed in water will read correctly.

Calibration & the problems it poses

- Supersaturation
  - ☑ Initial DO > 9.0 mg/L
  - ☑ Initial DO > saturation point (based on Temp & Pressure)
- Calibration approach
  - ☑ Winkler – reagent quality
  - ☑ Probe – is water (or air) saturated



- Blanks and the Calibration
  - ☑ Blanks can fail simply due to calibration problems

Common Sources of Error in the Water Saturated Air Calibration

- Temperature: thermistor must equilibrate to air temp. in the BOD bottle before calibration
  - ☑ It takes time for the probe equilibrate initially after removing the droplets from the tip
    - *It takes longer to equilibrate in air than water! That's why some have more success with the "air saturated water" approach.*
- Not allowing the air to become saturated with water…it takes at least 30 minutes.
- Not allowing the meter and probe to warm-up for at least 30 minutes
- Probe maintenance
  - ☑ Change the membrane regularly
  - ☑ Remove sulfide deposits from anode & cathode
- Check and calibrate the on-board barometer

Probe –calibration in water-saturated AIR

- Place the probe in a BOD bottle with about 3 cm of water
  - ☑ Shake BOD bottle prior to inserting probe to assure saturation. We recommend leaving the stirrer on (*although manufacturer says it's not necessary*) ---it speeds up equilibration.
  - ☑ The probe may need to sit in the bottle for 30-35 minutes in order to match the temperature of the air.
  - ☑ Determine barometric pressure and adjust meter's internal barometer as necessary
  - ☑ Check temp. of the air to be sure the probe thermistor is working correctly
- Use the meter's auto-calibration function to calibrate the probe and meter

Key to successful water saturated air calibration

- **Consistency is the key**
  - ☑ Meter warm up time (at least 30 mins)
  - ☑ How droplets removed from the probe tip (Shake? Dab?)
  - ☑ Amount of water in the BOD bottle (~1 inch)
  - ☑ How long you let the probe sit in the BOD bottle or the calibration chamber before calibration (≥ 30 mins.)
  - ☑ Consistent temperature conditions in Lab
  - ☑ MUST be consistent from day 0 to day 5
  - ☑ Get into a routine and STICK WITH IT!
- **How important is consistency?**
  - ☑ Your calibrations will likely work even if you don't wait for the air to be 100% saturated with water as long as you do your calibration the same EVERYDAY!

Consistency

One operator's SOP for consistency:

- 30 minute warm-up for meter
- Allow 1-hour in bottle after wiping probe tip
- New membrane every 2 weeks

Result: Has successfully met blank requirements for several years

Probe –calibration in air-saturated WATER

- Place the probe in a BOD bottle filled with air-saturated (well-shaken) water
  - ☑ Leave probe in the water w/ stirrer operating long enough for the probe temperature to equalize with the water temperature
  - ☑ Determine barometric pressure and adjust meter's internal barometer as necessary
  - ☑ Check temp. of source water to be sure the probe thermistor is working correctly
- Use a detailed DO saturation table to determine the theoretical DO concentration
- Adjust the meter to read the DO concentration determined from the saturation table.

Air-saturated WATER vs. Water-saturated

- There IS a difference when calibrating in air vs. measuring samples in water!
- Manufacturers often program a correction factor to account for the difference between oxygen diffusion in air vs. water
- This is often seen as a saturation of 102.3% (polarographic) when calibrating in air vs. calibrating in water.
- Which technique is best??????
  - ☑ WHATEVER WORKS!

Summary of Calibration Techniques

- The Winkler calibration takes longer than the other calibration techniques… with no net gain in quality.
- Calibration with air saturated water takes less time because the probe's temperature equilibrates quicker in water than air. **Water is a more effective heat sink**
- Obvious advantage: You don't have to worry about droplets on the probe tip when calibrating in air saturated water (DUH!).
- All three methods work. The results of the seed control and GGA were the same even though the IDO's and DO_5's were different. Consistency is the key to good results regardless of calibration technique.

How do you KNOW the DO meter is accurate?

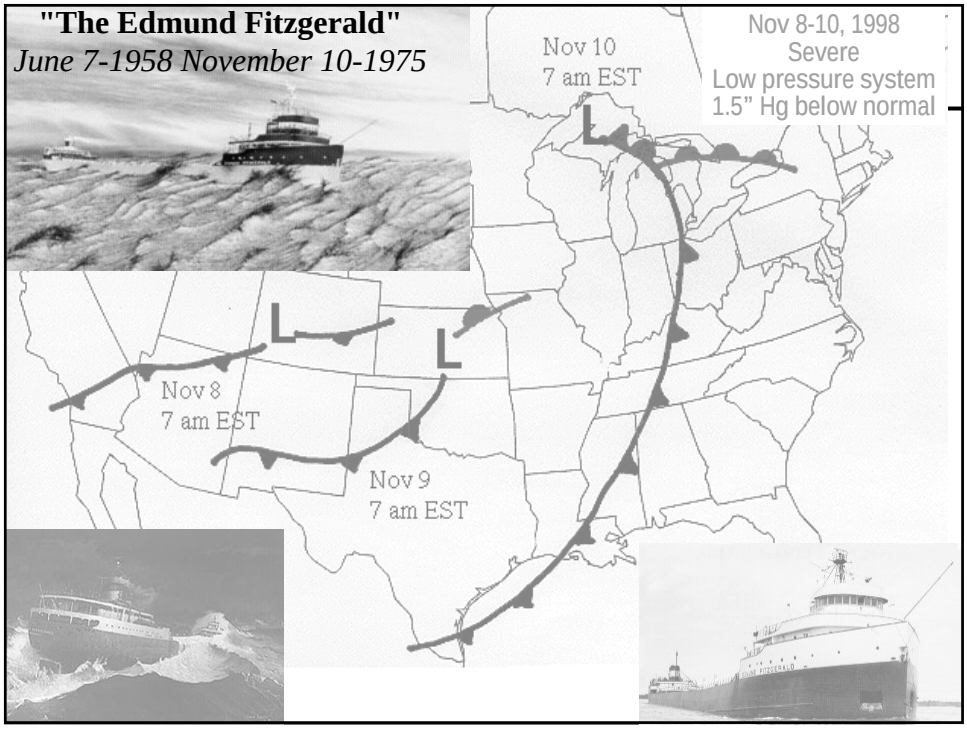
Check the barometer!

- Internal barometer
- External barometer

- check reading against local TV station, radio, or airport at least monthly
- Compare apples to apples (sea level?)
- Make adjustments as necessary
  - ☑ Temperature ± 0.5 ˚C translates to 0.1 mg/L DO
  - ☑ Pressure ± 5 mm translates to 0.06 mg/L DO
- Analyze a "known" standard…the IDO (DO_I) of your daily blank IS a known standard

Saturation Conversions – Rice Lake, WI

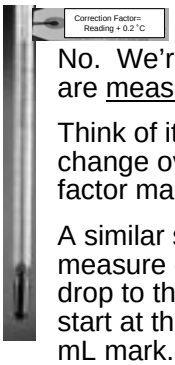
Your facility's altitude is **1140** ft ASL	$\frac{760 - (\mathbf{1140} \times 0.026)}{760} = \frac{760 - 29.6}{760} = \frac{730.4}{760}$ = 0.9611 = baro. pressure correction
Radio station says pressure is **30.21** ↑ inches (but that's corrected to sea level)	**30.2** in. x 0.9611 = 29.03 in (true un corr. BP) x 25.4 mm/inch = 737.4 mm
MY lab's air temperature is **22.4 ºC**	Saturation at 760 mm & **22.4 ºC** = 8.65 mg/L
So… saturation at MY Temp. and altitude=? Standard O2 sat. tables are set to 760	Correction= $\frac{737.4}{760} = 0.9703$ = 8.65 x 0.9703
What should I set the meter at?	**= 8.39 mg/L**



How do you KNOW DO measurements are accurate?

- ❑ You need a "known" standard
  - ☑ Air saturated water (YOUR daily blank IDO!)
- ❑ You need some basic physical data
  - ☑ Temperature
  - ☑ Absolute barometric pressure
- ❑ Use physical data to determine standard "true" value
  - ☑ Determine theoretical TRUE value for oxygen in mg/L (i.e. saturation point)
  - ☑ If measured value = True value ± 0.2 mg/L; calibration is accurate

Calibration only has to be ± 0.2 mg/L??? But…won't that cause me to fail blanks?

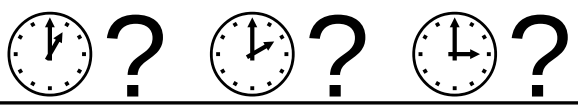


No. We're not actually interested in absolute DO measurements. We are measuring the change in DO from Day 0 to Day 5.

Think of it like using a thermometer with a correction factor that may change over time. The reading may not be accurate, but the correction factor makes it so….and it's CONSISTENT from Day 0 to Day 5

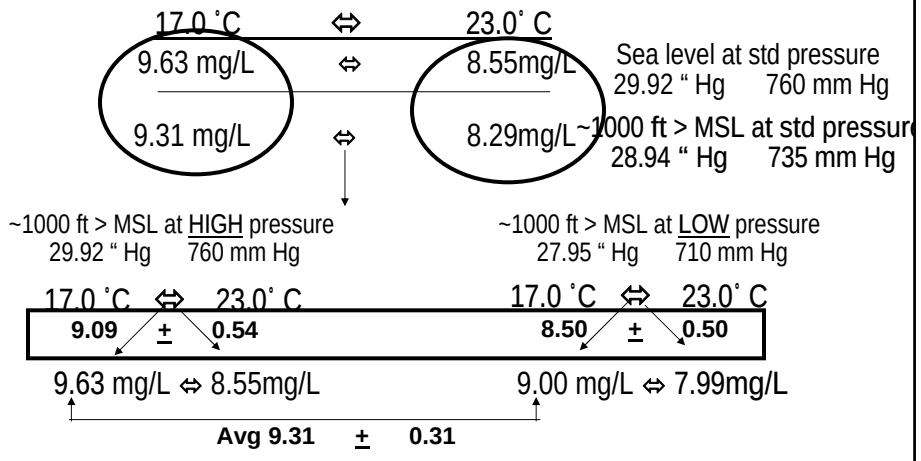
A similar situation can be seen when using serological pipets. To measure out 6 mLs, you CAN start at the "0" line and allow the fluid to drop to the 6 mL mark. But, it doesn't have to start at "0". You can start at the 2.5 mL mark and still deliver 6 mLs by dropping it to the 8.5 mL mark.

Another way to look at it is if you travel to a different time zone. Your watch may not show the actual time, but you can still tell when an hour or any number of minutes has passed. You may be in a new time zone, but for 5 days you're consistently off by that hour.



Oxygen saturation variability

- Oxygen saturation varies with pressure & temperature



- Oxygen saturation varies + 0.3 mg/L with normal pressure range
- Oxygen saturation varies + 0.5 mg/L with normal temp. range

Limit of Detection (LOD)

BOD detection limits are theoretically based.

- Assumption: the LEAST depletion allowable is 2 mg/L.
- Based on the highest volume of sample used in a dilution series.
- This technique doesn't consider seed correction.

$$\text{LOD mg/L} = 2 \text{ mg/L} \times \frac{300 \text{ mL}}{\text{mL sample}}$$

BOD bottle maximum volume!

If the highest sample volume used is:	The LOD for **that** sample is:
300 mL	**2**
200	**3**
100	**6**
75	**8**
50	**12**

Using a full bottle STILL seem to be an issue

Method Details – Potential Trouble Spots

Prepare seed

Preliminary testing

Determine dilutions

Measure out samples

Add seed to those that need it

Incubate 5 days (± 4-6 hrs)

Determine BOD

Preliminary Testing - pH

- **Test for proper pH** "pH extremes" kill bugs
  - pH extremes defined as < pH 6 or > pH 8.5
    - (*pH extremes undefined in previous editions*)
    - NOTE: 21st ed: pH 6 – 8; adjust to pH 7.0 to 7.2 (to match international standards)
  - If undiluted sample is < 6, adjust pH to 6.5 - 7.5 and seed all dilutions!
  - Phosphate buffer addition often results in acceptable pH
    - ✔ *As needed, neutralize with 1N H_2SO_4 or 1N NaOH.*
    - ✔ *Do not dilute sample by >0.5% (1.5 ml in a 300 ml bottle).*
- Diluted sample pH must be between 6.5 & 7.5.
- ALWAYs seed samples that have been pH-adjusted

Preliminary Testing – Disinfection

Chlorine

☠ **Test for chlorine residual!** Disinfection kills bugs

If any chlorination process is employed

(1)Quench the chlorine residual;

(2) SEED the sample(s)

Other Disinfection (UV)

If **ANY** disinfection process is employed (UV)

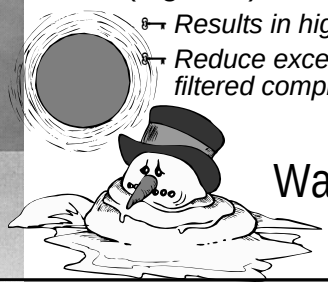
SEED the sample(s)

Preliminary Testing – Super Saturation

☠ **Check for super-saturation (of O_2)!**

Result = high bias

- Know the saturation point at your facility/your conditions
- Definitely a problem if DO_i > 9.0 mg/l at 20°C,
- Can occur during winter months (cold water)
- In localities where algae are actively growing (lagoons)



- *Results in high bias (quickly lost during incubation)*
- *Reduce excess DO (shake sample(s) or aerate with filtered compressed air)*

Warm to room temperature!!!!

(20 ± 3˚C)

Checklist: Sample Storage/Pre-Treatment

	Sample Storage and Pretreatment	Citation
1	Are BOD samples set up within 2 hours or stored at ≤ 6 ºC prior to analysis?	NR 219; Table F
2	Are samples set up within hold time (≤48 hours)?	NR 219; Table F; 5210 B
3	Are samples checked for residual chlorine?	5210 B; 4.e.(2)
4	If residual chlorine is found is the sample neutralized?	5210 B; 4.e.(2)
5	Is the pH of samples checked prior to set up?	20th 5210B; 4.e.
6	Are samples pH adjusted to pH 6.5 - 7.5 (if not in pH 6.0 - 8.5 initially)?	20th 5210B; 4.e.
7	If pH adjustment is done is the amount of acid or base used limited to ≤ 0.5% of sample volume?	5210B; 4.e.(1)
8	Are samples warmed to 20 +/-3 ºC before analysis? (18th and 19th say 20 +/-1 ºC)	20th 5210B; 1.b. 18th/19th 5210B; 4.e.(5)
9	Are samples over the 100% DO saturation value identified and treated for super saturation?	5210B; 4.e.(4)

Seed Preparation

Source

- ⇨ **DO NOT RECOMMEND**: Effluent from a biological treatment system.
  - ⇨ *nitrification inhibition is recommended*
- ⇨ Domestic WW supernatant; settled at 20º C >1 h but <36 h.
- ⇨ Commercial seed (BOD seed, Polyseed)
  - ⇨ *may need to mix longer/differently than manufacturer recommends*

DO NOT mix seed in distilled or deionized water!

Delivering seed

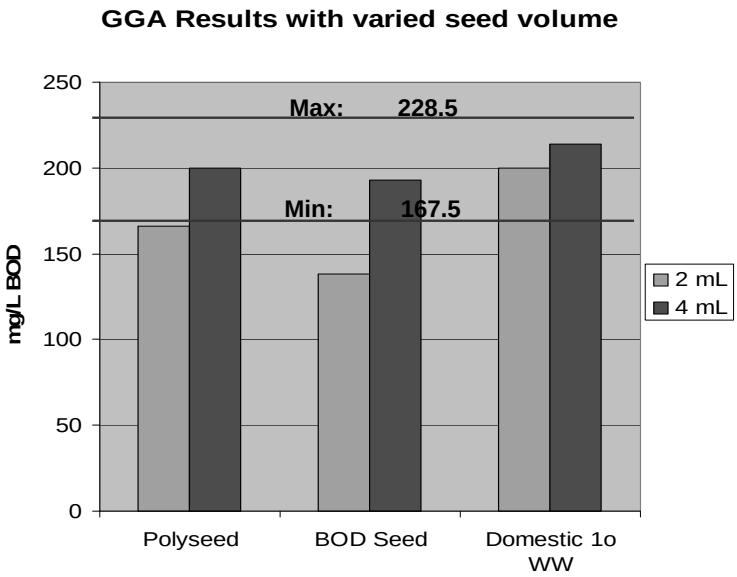
We recommend decanting and stirring vs. drawing individual aliquots off top

Seed: dilution water? samples directly?

Either is fine…

Seeding dilution water ensures all samples seeded

2 mLs seed doesn't always work!



Seeding Summary

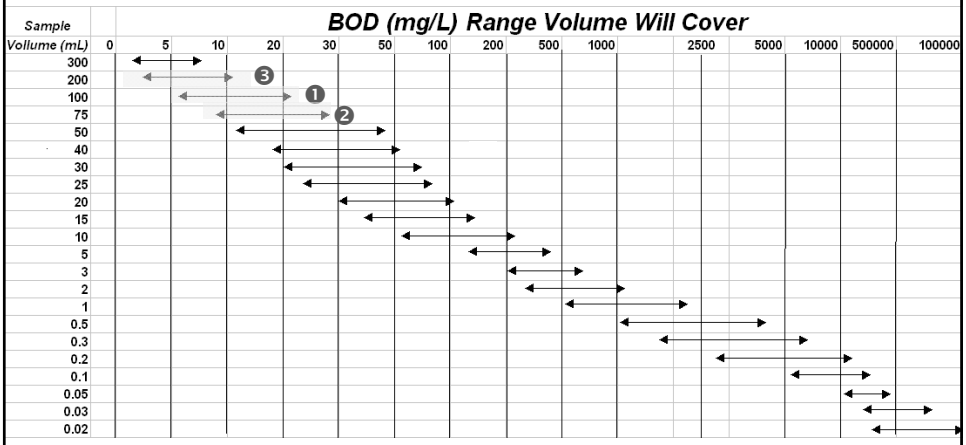
- Good GGA results were achieved when larger volume of the commercial seed was used.
- The recommended seed control depletion criteria of 0.6 to 1.0 mg/l were exceeded in order to achieve better results for the GGA.
  - ☑ It's GUIDANCE! Adjust seed volume to meet GGA acceptance criteria
- Domestic primary wastewater seed seemed to provide the most consistent GGA results.
  - ☑ Note however that ANY wastewater seed strength can change with conditions

Determine Dilutions

BOD Volume Estimation Chart

Assuming:8.5 mg/L DO_i; meets method depletion requirements

Example: if sample BOD expected to be about 5 to 25 mg/L



Making initial dilutions ...if you need to use < 3 mLs

Recommend: make initial 10-fold dilution

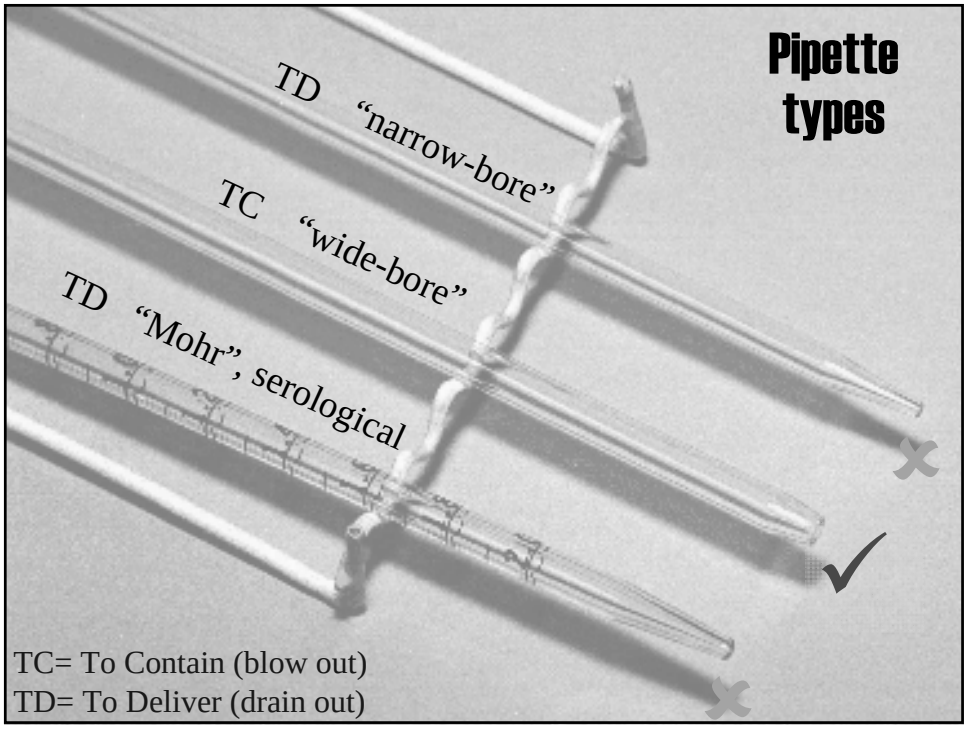
10 mLs sample to 100 mLs total volume (*with dilution water*)
25 mLs sample to 250 mLs total volume (*with dilution water*)
50 mLs sample to 500 mLs total volume (*with dilution water*)
100 mLs sample to 1000 mLs total volume (*with dilution water*)

make all dilutions with large-bore volumetric pipets and flasks!

mLs of 10X dilution	=	**mLs of Original sample**
5		**0.5**
10		**1.0**
20		**2.0**
25		**2.5**
50		**5.0**

Measure out samples

- ROTATE BOD bottles!!!!!
- Volumes from 3 mLs to 100 mLs- use wide-bore pipettes
- *Can* use a graduated cylinder for volumes > 100 mL
- Don't let samples sit too long between dilution and DO
- Standard Methods suggest no longer than 30 mins.
- Impact of a long delay on samples w/ rapid demand…
  - *- you will lose that instantaneous measure*
  - *- if assess user fees, instantaneous BOD can ⇩ fees*



Measuring out samples - some tips

When using pipets

- Use only ONE pipet for a given sample
  - Ex. For 25 mLs, don't use 20 mL + a 5 mL pipet
  - ☞ Use a 25 mL pipet
- DON'T fill a pipet twice to obtain a volume
  - Ex. For 200 mLs, don't pipet 100 mL twice
  - ☞ Use a graduated cylinder

If going to use serological pipets, make sure (1) they are wide-bore and (2) use them ONCE. Re-fill after each pipetting.

When using graduated cylinders (> 100 mL)

- DON'T agonize over "getting exactly to the mark"
  - Pour quickly;
  - get as close to target volume as you can;
  - record actual volume used

Large volumes - Need extra nutrients???

CURRENT GUIDANCE: SM 20th ed

- When a bottle contains more than 67% of the sample (> 200 mL) after dilution, nutrients may be limited and subsequently reduce biological activity.
- In such samples, add the nutrient/buffer solutions (¶ 3*a* through 3*e*) directly to each BOD bottle at a rate of 1 mL/L (0.33 mL/300-mL bottle) or use commercially prepared solutions/pillows designed to dose the appropriate bottle size.
- When individual nutrient pillows are used, it's OK to use dilution water

NOTE: It's easier to just add 1 Hach nutrient buffer pillow (Cat# 14160-66)

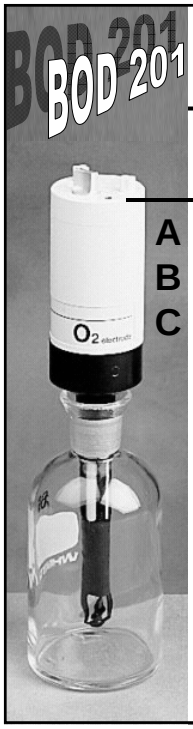
Add seed to those that need it *

* REMEMBER! If you sample downstream of ANY disinfection, you MUST seed.

BOD SEED DILUTION GUIDELINES

Estimated seed BOD	Dilutions for Seed Control	# mL seed/ BOD bottle
30	15, 25, 50	6 - 10
50	15, 25, 50	4 - 6
100	**5, 10, 15**	**2 - 3**
150	5, 10, 15	1 - 2

- ☑ adjust amount of seed to BOD bottle to obtain GGA results in the 198 + 30.5 range;
- ☑ Never pipet seed material into a dry BOD bottle.
- ☑ Always have some dilution water in first.
- ☑ Adding seed to DI water can rupture (lyse) cells!!!



Seed correction: add seed directly to bottles

Seed Correction Sample Calculation

	DO_i	DO_f	Depletion	mLs seed	Deplete/mL
A	8.5	0.3	8.2	30	**--.---**
B	8.4	1.6	6.8	20	0.34
C	8.4	4.3	4.1	10	0.41

Bottle A is not used due to the insufficient final DO

$$\frac{(0.34 + 0.41)}{2} = 0.375 \frac{\text{mg/L DO}}{\text{mL seed}}$$

If 2 ml undiluted seed added to each sample bottle, seed correction =

$\frac{0.375 \text{ mg/L DO}}{\text{mL seed}}$ X 2 ml seed = 0.75 mg/L DO

Checklist: Seeding

	Sample Seeding	Y	N	Citation
15	What is the seed source and which samples are seeded?	NA	NA	5210B; 4.d.
16	Is the seed properly prepared?			5210B; 4.d.(1)
17	Are industrial, disinfected (UV or chlorine), or pH-adjusted samples seeded?			5210B; 4.e.(1&2)
18	Are at least two seed controls run? (should have at least two that meet depletion criteria and recommend a seed correction factor between 0.6 to 1.0 mg/L)			5210B; 4.d.(2)
19	Are seed correction factors properly calculated and used to adjust results of seeded samples?			5210B; 4.d.(2)

A word on Nitrogenous Oxygen Demand

If you have Nitrogenous Oxygen Demand (NOD) you should consider analyzing CBOD vs. BOD

Reduced
Nitrogen + Oxygen → Nitrite (NO_2) → Nitrate (NO_3)

NH_3 + 1.5 O_2 → HNO_2 + H_2O + cells

HNO_2 + 0.5 O_2 → HNO_3 + cells

NH_3 + 2 O_2 → HNO_3 + cells

Theoretically 1 mg/L of NH_3-N requires 4.57 mg/L O_2 to oxidizeof NH3 to NO3-N

NH_3-N in dilution water can contribute up to 1.9 mg NOD x dilution factor to a BOD sample. Thus a 200 mL sample yields 1.9mg/L x 1.5 or 2.85 mg/L BOD

Source: Jim Young, Midwest Environmental Laboratory Stakeholders Summit, Dec. 2005

How do I know if nitrification is occurring?

- ❑ If BOD is always significantly higher than TSS, nitrification is likely occurring.
  (e.g., TSS 10, BOD 25)
- ❑ Confirm by performing side-by-side BOD tests with and without nitrification inhibitors.
- ❑ If the inhibited (carbonaceous)BOD results are significantly lower and closer to the TSS results, nitrification is occurring.
- ❑ Repeat side-by-side tests to confirm your findings.
- ❑ Contact your DNR wastewater engineer to see if your discharge permit can be changed from total to carbonaceous BOD.
- ❑ ***NOTE: Always seed samples when nitrification inhibitor is used***.

Carbonaceous BOD (CBOD)

Samples that may require nitrification inhibition include:

- biologically treated effluents,
- samples seeded with biologically treated effluents,
- river waters.

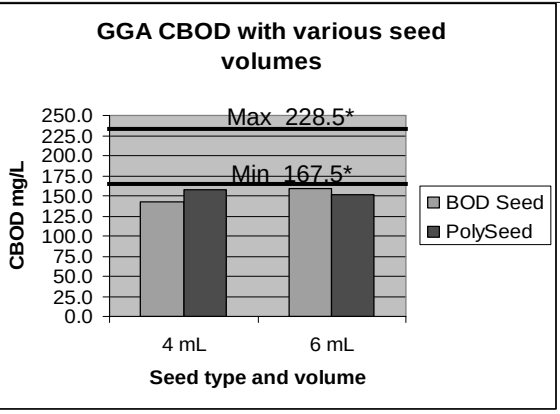
Note the use of nitrogen inhibition in reporting results

*** ONLY allowed if specified in your permit ***

Nitrification Inhibition & Toxicity

Q: Is TCMP toxic to the carbonaceous BOD reaction?

A: No scientific tests have shown any evidence of toxicity when TCMP is used properly, but the BOD Task Group recommends that all inhibited samples be seeded to reduce the possibility of interference.



How else to explain lower results for inhibited GGA?

Source: Jim Young, Midwest Environmental Laboratory Stakeholders Summit, Dec. 2005

Sample Toxicity

- Often referred to as "sliding" BODs
- BOD drops as sample volume increases (less dilute)
- Occurs frequently in systems receiving industrial waste
- Amounts to killing off (or severe shock to) "the bugs"
- Results in UNDER-reporting the BOD of a waste
- Failure to mix sample b/w dilutions can APPEAR as toxicity
- Even pH adjustments can result in this effect
- Poor technique (pipetting, pouring samples)
- Sometimes we just can't determine (isolated cases)

If nitrification IS occurring

(*remember : dilution water contains* ***NH_3***)

…as dilution ↑ , available NH_3 ↑ ==> final BOD ↑

...if sample has lots NH_3, can see the opposite effect

Sample Toxicity

Sample mLs	Depletion (mg/L)	BOD mg/L
25	7.2	86.4
50	5.1	30.6
100	2.6	7.8
		41.6

Report?
41.6 ?
86.4 ?
____ ?

➔ DO NOT report the "average" of dilutions (41.6)
➔ DO NOT report the highest value (86.4)
➔Best answer: report ">" plus the highest BOD (> 86)
➔ MUST qualify these results as exhibiting "toxicity"
➔ Should repeat w/ additional dilutions (**e.g., 5, 10 mLs**)

Another viewpoint: Jim Young on sliding BOD data

Q: How should the following data be interpreted?

Sample volume/bottle, ml =	25	75	200	300	AVG
Replicate #1, $CBOD_5$	29	23	9	6	17
Replicate #2, $CBOD_5$	26	18	10	6	15
Replicate #3, $CBOD_5$	34	16	10	6	16
Average $CBOD_5$, mg/L	30	19	10	6	

Do these results indicate a toxic condition in the sample?

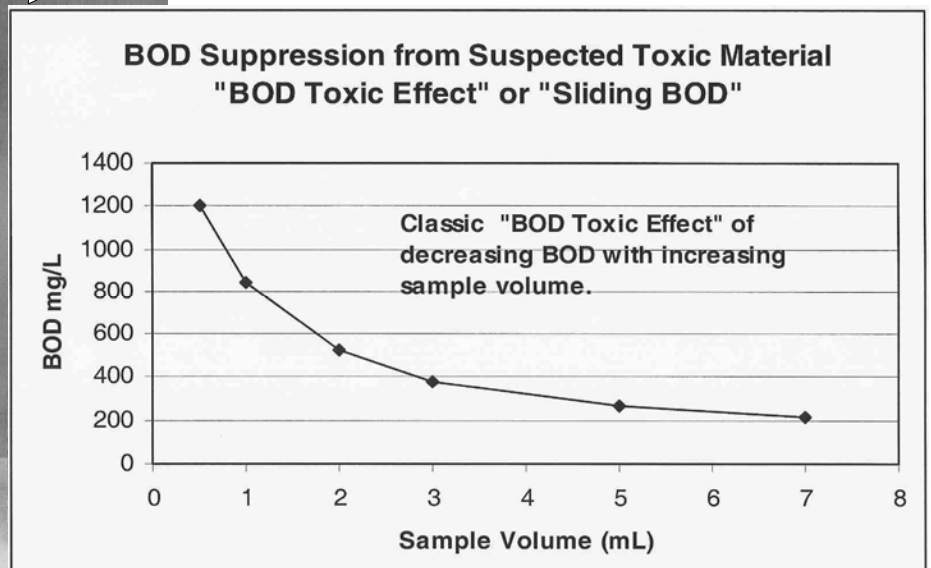
A: There is no evidence that the above sample contains toxic materials because this was an effluent from a well-operating plant that essentially provided complete nitrification. If the same amount of seed was added to each bottle, the lower dilution could have a higher relative reaction rate. In any case, the results of all dilutions should be averaged if all meet the minimum DO and minimum depletion criteria, and none is a qualified outlier.

Note that while we appreciate Jim's perspective, we disagree on his decision to average all data. We'd suggest reporting: "> 34" and qualify the data.

* Jim Young is the Chairman of the Standard Methods Joint Task Force for BOD

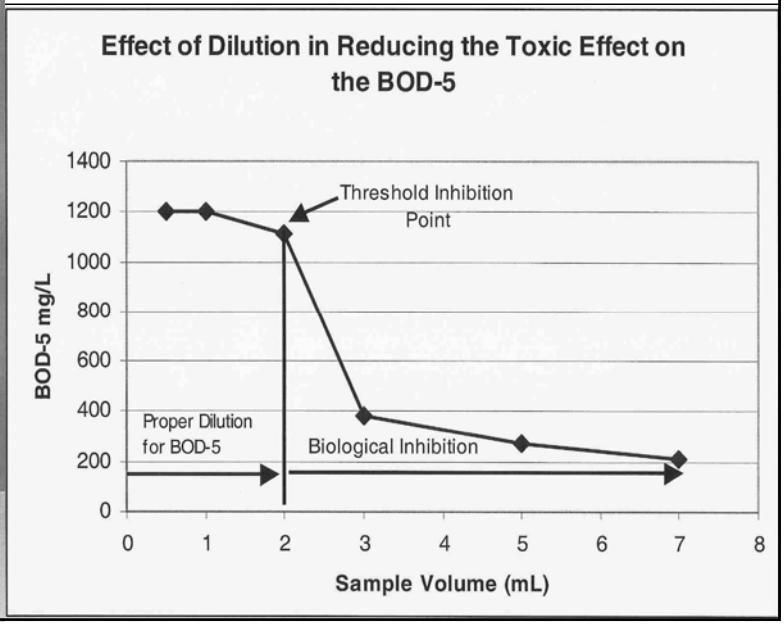
BOD 201

Toxicity Data



BOD 201

Dealing with Toxic Samples



Reporting suspected toxicity

20th edition, Standard Methods:
If more than one sample dilution meets the depletion criteria **and** there is no evidence of toxicity at higher sample concentrations or the existence of an obvious anomaly, **average results**

21st edition, Standard Methods:
Identify samples in test reports when serial dilutions show more than 30% between high and low values.

Contamination? Calibration? Or Seed?

Contamination?
- Can affect BOTH blanks and GGA
- Tends to be LARGE effect
- Tends to be HIGH bias (GGA high)

Calibration?
- Mainly affects blanks
- Tends to be SMALL effect
- Can be LOW or HIGH bias (blanks deplete > 0.2 mg/L or GAIN > 0.2 mg/L)

Or Seed?
- Mainly affects GGA
- Tends to be LARGE effect
- Tends to be LOW bias (GGA low)

Contamination? Bugs or Dirty Dishes?

Remember: All “legs of the table” must be present for a “BOD” to be determined:

You must have bugs, oxygen, and a food source

You can have bacterial contamination, general contamination (food source) or BOTH.

Ex.1: GGA fails high but blanks are perfect

- The contamination could be “bugs”, possibly from a bad filter in the DI system.
- Blanks are likely fine because glassware is clean and there is no “food source” to keep bugs going and expending oxygen.
- GGA fails high due to the extra oxygen consumed by the bugs as they attack the GGA

Contamination? Bugs or Dirty Dishes?

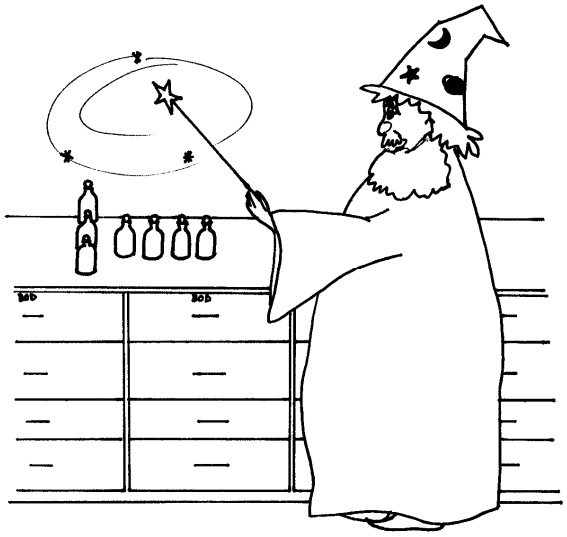
Ex.2: GGA fails high but blanks are perfect

- The contamination could also be just “dirty glassware”, providing a food source.
- Blanks are likely fine because –despite availability of a food source (the “crud”)--- there is no source of bugs and therefore no oxygen can be used.
- GGA fails high due to the extra oxygen consumed by the bugs as they attack both the GGA and the “crud”

Ex.3: GGA & blanks fail high (blanks deplete 1-2 mg/L)

- The contamination is likely a combination of “dirty glassware”, dilution water, and “bugs”.
- Blank(s) and GGA fails high because not only is there a food source (“crud”) but also there are “bugs” that shouldn’t be there.

You CAN be successful at BOD….



….it's NOT just a mystical art

Troubleshooting:
Excessive depletion in Dilution Water

Possible Causes:

- Tubing is constructed of oxygen-demand leaching material
- Correct tubing not being used throughout the lab
- Slime growth in delivery tubing
- Poor water quality/improperly maintained system
- Poorly cleaned BOD bottles or dilution water storage container.
  - NOTE: Glass is best!
- Contamination during aeration
- Poorly calibrated DO Probe

Solving: Slime Growth in delivery tube

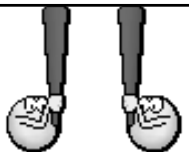
Disinfect delivery tube **weekly**

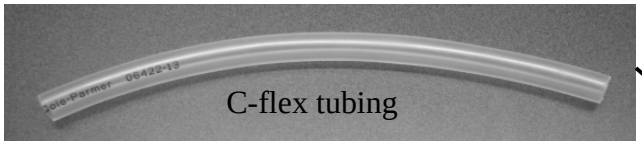
- (50mL bleach/2L)
- dilute solution of HCl (100 mL HCl/ L water)

NOTE:

1. DO NOT mix acid with bleach!
   Chlorine gas is produced in this reaction. Even in small quantities, exposure to chlorine gas can be fatal.
2. Use reinforced nylon tape around larger bottles for safety
3. Nothing should be immersed in water except Teflon or glass

Tubing types

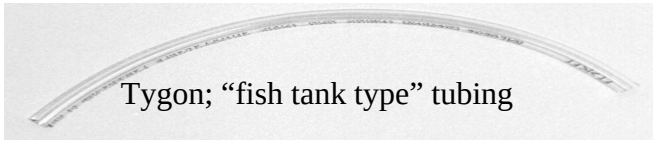




C-flex tubing

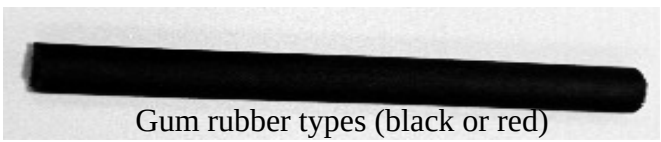
Yes

Surgical latex



Tygon; "fish tank type" tubing

Maybe



Gum rubber types (black or red)

NO

Solving: Water Quality issues

- **Avoid "grocery store" distilled water.**
  -- plastic bottles often leach oxygen demanding materials
- **Don't have to "age" if using high quality water**
- **In-lab auto-dispensing deodorizers.**

Solution: Don't use them!

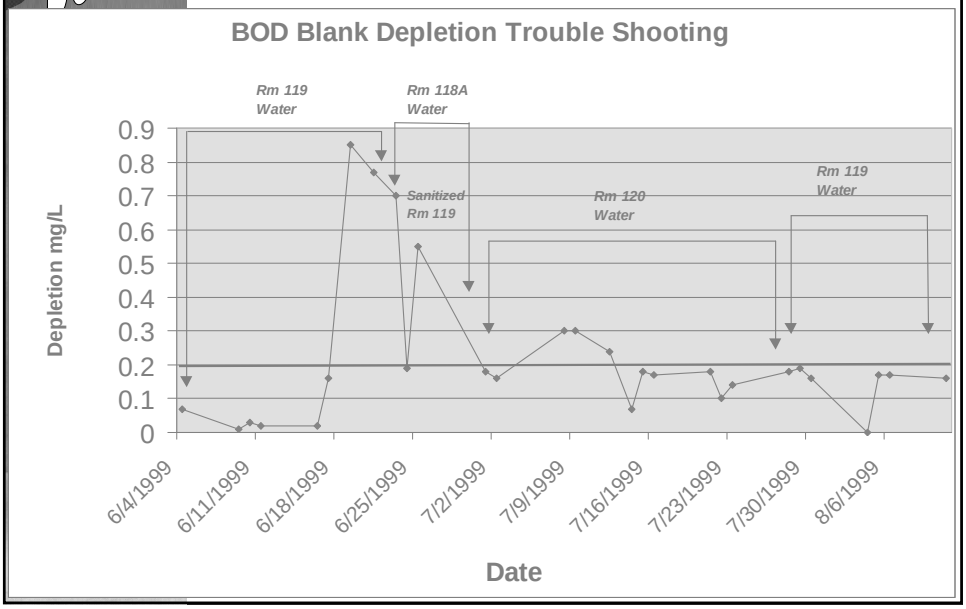
Solving: Water System issues

- **Follow manufacturer's recommendations for cleaning and disinfecting stills, etc**
- **Simple deionizer systems can work well but can quickly be overgrown with bacteria and mold.**
  Can leach organics if not maintained regularly.
- **If using simple deionizer system, use nuclear-grade or virgin resin.**
  *i.e., Lower grade or "re-used" resins **WILL** leach organic matter and cause problems.*

Caution: Charcoal can become contaminated with bacteria and cause problems as well (at least one lab's experience").

BOD 201

SLH's dilution water experiences



BOD 201

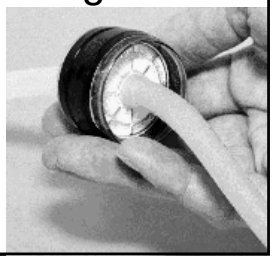
Dilution water- simplest solutions

- Obtain water from another laboratory or vendor.
- Purchase water from a source that has proven success.
- Buy an all glass laboratory still and distill your own water.
- Buy a bench-top water RO and polisher combo that will produce ASTM Type I water.

Note: These systems are expensive (about $1000) and must be maintained regularly to be effective.

Solving: aeration-related contamination

- Don’t leave dilution water open to the air
- Never use an air stone
- Never put "fish tank" tubing directly in dilution water
- Filter compressed air through a filter or glass wool
- Use an in-line air filter



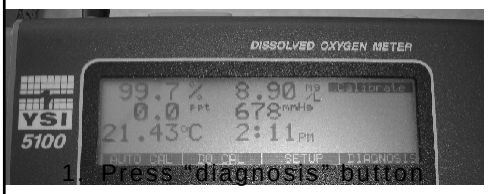
DO Probe Maintenance

- ❑Electrolyte replenishment
- ❑Membrane failure
  - ☑Membrane rupture
  - ☑Membrane fouling
- ❑Cathode and anode cleaning
  Follow manufacturer recommendations for interval & procedure

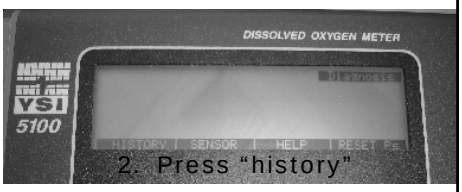
For best results, replace every 3-4 weeks

Troubleshooting DO Probe Problems: see NCL website for great tool for diagnosing DO probe problems. Some information included at the end of this presentation.

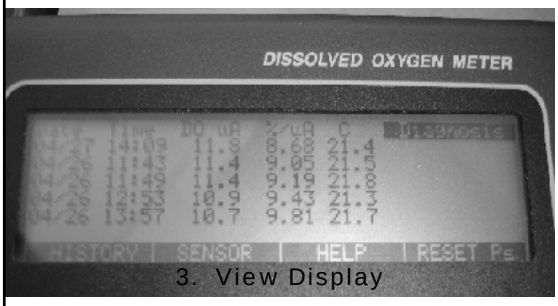
YSI 5100 On-board tools for monitoring membrane problems



1. Press "diagnosis" button



2. Press "history"



3. View Display

- DO µA should be from 8.0 – 17.0
- %/ µA should be from 5.9 – 12.6%
- Replace membrane if outside this range.
- Good tool for preventative maintenance

Tips for Maintaining the YSI DO Probe

- ❑ Examine membrane daily
  - ☑ Replace if air bubbles, wrinkles or "crud" on or under the membrane
- ❑ If gold tip (cathode) is tarnished, clean it
  - ☑ The gold should have a bright "matte" finish with fine scratches-DO NOT polish too much!
  - ☑ Clean with sanding disk provided in the YSI cap kit

Materials with smooth surfaces appear glossy, while very rough surfaces reflect no specular light and therefore appear matte.

Probe Maintenance

- The silver anode should have a light silver color
- Clean if anode is dark
- Clean by soaking overnight in household ammonia solution
- Rinse thoroughly with tap water, DI water and electrolyte

Figure 1. On the right is a properly prepared cathode and anode. Note the light silver appearance of the onode and matte finish of the gold cathode.

Source: YSI Technical Note - How to Ensure Successful & Accurate BOD Measurements
http://www.ysi.com/extranet/EPGKL.nsf/d73e4c089382db1485256a49005dd58c/1f24980cddee02ac85256cef00763ec1/$FILE/ET603.pdf

Troubleshooting:

Consistent high bias in GGA

Seed source selection is critical; if recycling final into primary clarifiers, could be adding nitrifiers to the seed

- To determine if nitrification is occurring, try adding a nitrification inhibitor.
- Compare GGAs seeded with domestic wastewater vs. commercial (Polyseed, BOD seed)
- If you don't warm the GGA before use, results will be consistently high

If nitrification **is** occurring:

- Select another source (that does **not** receive final wastewater)
- Use commercial seed

Troubleshooting:
Consistent low results for GGA

Not enough seed - adjust the amount used until you consistently achieve GGA results in the acceptable range.

Poor seed quality - try another seed source(mixed liquor; primary; another WWTP; commercially prepared seed) Recall the previous slide showing 2 vs. 4 mL of seed

GGA too old or contaminated - discard expired or contaminated solutions

Try another GGA source - Several different types / vendors (NCL, Fisher, other scientific specialty companies)

Troubleshooting:
Poor Precision (samples)

- Characterized by wide variation among dilutions
- BOD is a bioassay technique ….thus
  - inherently less precise than instrumental tests
  - like ammonia and total phosphorus
- Look into sample measuring technique
- Look for “chunks” that might still be visible
- More concern with poor precision in final vs. raw

Checklist – Equipment & Reagents

	Equipment	Y	N	Citation
10	Are all necessary reagents and glassware available? Reagents purchased_____or prepared_____?			5210 B; 2.& 3.
11	Is the DO meter properly calibrated on each analysis day? Water sat.air_____ Air sat. water____ or Winkler_____			NR 149.14 (3)a.
12	Does the incubator maintain samples at 20 +/- 1 ºC during the 5 day test period?			5210B; 2.b.
13	Is the room temperature sufficiently controlled to meet the test requirements of 20+/- 3 ºC?			20th 5210B; 4.
14	Is the room temperature sufficiently controlled to meet the test requirements of 20 +/- 1ºC?			18th/19 5210B; 4.

Checklist – General BOD Procedure

	General Procedural Observations	Y	N	Citation
20	Are the proper reagents prepared_____ or purchased_____ for dilution water preparation?			5210B; 3.
21	Are all reagents properly labeled and in good condition?			5210B; 3.
22	Is the dilution water properly made and stored?			5210B; 4.a.& b.
23	For sample dilutions of greater than 1:100 is a preliminary dilution done? **NOTE: This means if using < 3 mLs sample**			5210 B; 4.f.(2)
24	Are sample volumes adjusted so that depletion criteria are met as often as possible?(d*epletion of > 2 mg/L DO & remainder of > 1 mg/L DO)*			18th/19th 5210B; 4.f.
25	Do at least two sample volumes meet the depletion criteria?			20th 5210 B; 4.f.
26	Are at least two sample dilutions run for each sample?			5210B; 4.f.

Checklist – General BOD Procedure

	General Procedural Observations	Y	N	Citation
27	For samples over 201 mL are additional nutrients added? Are the nutrients powder ____ or liquid____(0.33 mL per 300 mL)?			20th 5210B; 4.f.
28	Are sample bottles water sealed prior to incubation?			20th 5210B; 4.f. 18th/19th 5210B; 2.a.
29	If nitrification inhibitor is used, does the lab have certification or registration for CBOD?			NR 149.04 (1)
30	Are CBOD samples properly labeled and the results reported as CBOD?			149.06 (1)
31	Have sliding BODs been observed?			5210B; 4.e.(3)
32	If sliding BODs have been observed have steps been taken to identify the source of the toxicity?			5210B; 4.e.(3)
33	Are BOD values properly calculated for all samples?			5210B; 5.

Checklist – General QC

	Quality Control	BOD	Citation
3	Is a replicate run after the analysis of 20 samples of each matrix type (at least 1 replicate for 20 samples)?		NR 149.14 (3)e.
4	Are quality control (QC) limits for replicates calculated for each matrix (unless lab has < 20 QC results/year then they can set QC limits)?		NR 149.14 (3)g.
5	Are QC limits used to assess replicate performance each time replicates are analyzed?		NR 149.14 (3)g.
10	When QC limits for standards, replicates, spikes or blanks are exceeded is corrective action taken?		NR 149.14 (3)h.
11	Are blind standards analyzed three times a year with 3 to 5 month spacing between each set?		NR 149.14 3(j).
12	When a blind standard result fails is a new standard ordered and analyzed after taking corrective action?		NR 149.14 3(j).

Checklist – Specific QC Requirements

	Glucose-Glutamic Acid (GGA) Standard	Y	N	Citation
34	Is GGA standard properly prepared or commercially purchased?			5210B; 3.h.
35	Is GGA standard analyzed at a 2% dilution (6 mL to 300 mL) using a concentration that yields 3 mg/L glucose and 3 mg/L glutamic acid in the GGA test bottle?			5210B; 4.c.
36	Are GGA standards analyzed after every 20 samples or weekly at a minimum (if < 20 samples are run in a week)?			NR 149.14 (3)(c)4
37	Are seed controls run and correctly applied to GGA data?			5210B; 4.d.(2)
38	Do GGA results meet the 198 +/- 30.5 mg/L BOD standard? *(167.5 - 228.5)* Multiple GGA standards cannot be averaged.			5210B; 6.

Checklist – Specific QC Requirements

39	Do all samples, standards and seed controls used to calculate results meet the depletion criteria?			5210B; 5.
40	If criteria are not met are data excluded from calculations or qualified if there are no acceptable dilutions to use?			5210B; 5.
41	Is a dilution water blank run with each batch of samples and/or batch of dilution water?			5210B; 4.h.
42	Do dilution water blanks meet the depletion limit of < 0.2 mg/L DO?			5210B; 4.h.

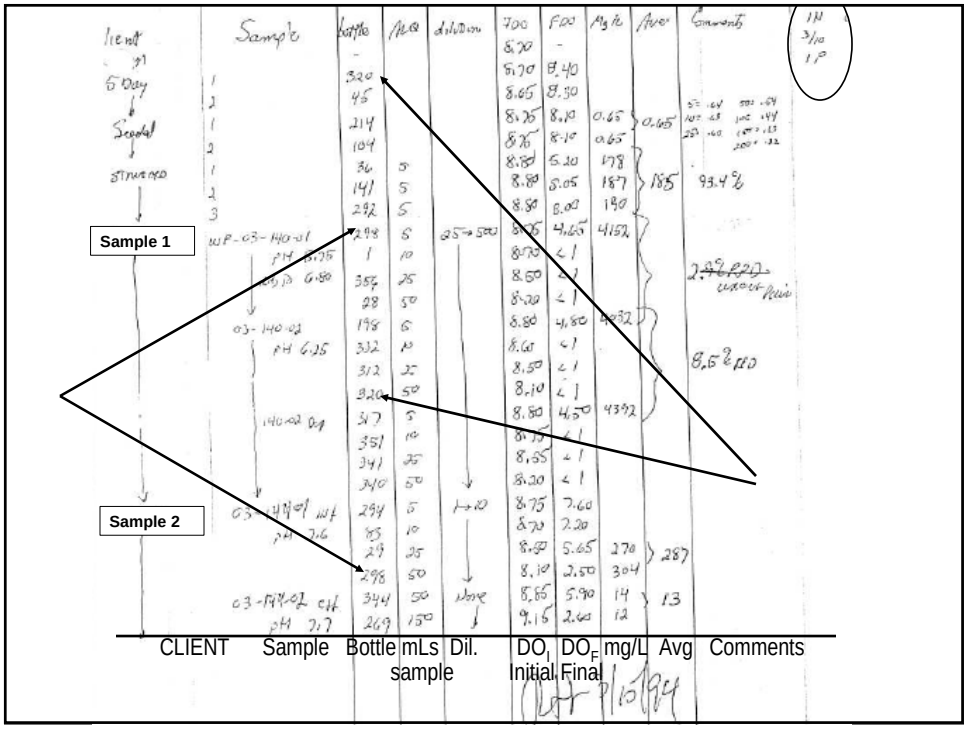
A long time ago…

…right here in our own galaxy,

The state of

BOD documentation

… was pretty grim



Progress! 6 years later!

DAILY BOD / SUSPENDED SOLIDS WORK SHEET

SAMPLE DATE 02-01-00 RECD. BY: GKS
TIME RECD: 720
SAMPLE COLLECTION DATE 02-02-00 RECD. FROM: DANAD

0 - Day Analysis — Tested
Date 02-02 Time 1010 By GKS
Flow 6047 Temp. 19 D.W. Jug # 1
28.81 / 29.92 X 100 = 96.3
Barometric Pressure — Meter Calibration Factor %

5 - Day Analysis — Tested
Date 02-07 Time 745 By GKS
28.92 / 29.92 X 100 = 96.7
Barometric Pressure — Meter Calibration Factor %

Sample	Bottle #	Volume (ML)	Dilution Factor	Initial D.O.	Final D.O.	D.O. Drop	BOD (MG/L)	Avg. BOD (MG/L)	BOD #/day
Dilution Water	16	300	1	8.62	8.42	-.20	-.20		
	32	300	1	8.62	8.43	-.19			
**Effluent Composite Sample	35	200	1.5	8.69	6.31	2.38	3.57	3.54 3.5	
	43	300	1.0	8.86	5.34	3.52	3.52		
**Effluent Weekly Duplicate									
Glucose Standard 198 +/- 30.5	76	6	50	8.68	4.43	4.25	212.50	213 - 17 = 196	

Amount of effluent used = 100 ML unless otherwise noted + 900 ML dilution water (for glucose std.)

Glucose standard correction:
Avg. Effluent BOD (mg/l) x 4.9000 correction factor
Avg Glucose standards BOD - correction factor = corrected value

Documentation

Have available for any inspection

- ✍ **Any preliminary testing (pH, chlorine residual)**
- ✍ **Sample temperature & barometric pressure**
- ✍ **Time & date in (and out) incubator** ***(military or am/pm)***
- ✍ **Incubator temp. - each day samples in progress**
- ✍ **ALL sample-related information and raw data**
- ✍ **Seed source, which samples seeded, & how much**
- ✍ **Clearly show any initial dilutions (*vs. writing "0.5 mLs"*)**
- ✍ **Calculations and data associated w/ control limits**
- ✍ **Control limits used over time (most recent 3 years)**
- ✍ **Any Corrective Action (including maintenance)**

The nitty gritty checks

- ❑ Residual Chlorine
  - ☑ If sample U/S of chlorination, have documentation (plant schematic)
  - ☑ If not using chlorine (e.g., UV) note such on benchsheet
  - ☑ Otherwise, should somehow document that sample was checked, how, and results.
- ❑ pH
  - ☑ Need documentation someplace that pH of ANY sample analyzed is within requirements
  - ☑ If the whole sample meets pH requirements, so will any dilution
  - ☑ OK to use short range pH paper for this check
- ❑ Temperature
  - ☑ Need documentation someplace that temperature of ANY sample analyzed is within requirements (20 ± 3° C)
  - ☑ If the whole sample meets temperature requirements, so will any dilution.
  - ☑ OK to use DO meter thermometers that have been verified and corrected (if necessary)

Checklist: Record Keeping

	Records	BOD	Citation
13	Are all records available for last 3 years of analysis?		NR 149.06 (1)
14	Are records kept in secure manner, recorded in ink or stored electronically w/ safeguards?		NR 149.06 (5)
15	Are sample results traceable to analyst, date collected, and method used including raw data, calculations, results and final report?		NR 149.06(1)a.
16	Are sample collection records complete? (i.e. sample dates, location, sampler, sample condition, preservation etc.)		NR 149.06 (1)
17	Is the raw data (i.e. absorbance, millivolts) recorded for all samples and standards?		NR 149.06(1)a.
18	Are sample results clearly traceable to the calibration curve that was used to generate them?		NR 149.06(1)a.

Checklist: Record Keeping

	Records	BOD	Citation
19	Are equipment maintenance records for all analytical equipment kept?		NR 149.06 (1)
20	Are clear records of replicates and associated control limits available and current?		NR 149.06 (1)
21	Are clear records of spikes and associated control limits available and current?	NA	NR 149.06 (1)
22	Are records associated with blind and reference samples available?		NR 149.06 (1)
23	Are records of corrective actions taken in response to QC failures available?		NR 149.06 (1)
24	Does corrective action include qualification of data on data report or DMR?		NR 149.14 (3)h.

New BOD benchsheet (for new NR 149)

SAMPLE BOD BENCHSHEET

Sample Location	Type	Date	Sample Time
Influent	24-hr Comp	12/2/04	6:55 AM
Effluent	24-hr Comp	12/2/04	6:30 AM

Date in: 12/2/04 Time in: 8:05 AM Incubator Temp: 21

Date out: 12/7/04 Time out: 8:10 AM Incubator Temp: 20

Sample ID	Bottle ID	Sample mLs	CBOD	Seed, mLs	pH	Cl2	Temp	DO, initial	DO, final	Difference	Seed Correction	BOD (mg/L)	Average BOD	Reported BOD, mg/L	Comment
Blank	1						19.8	8.9	8.8	0.1		0.1			OK
												Depletion /mL			
Seed Control	10	5						8.9	6.7	2.2		0.44			
Seed Control	11	10						8.9	5.5	3.4		0.34			
Seed Control	12	12						8.8	4.3	4.5		0.38			
											Average:	0.39			
LCS (GGA)	23	6		2				8.9	3.3	5.6	0.8	239.5		240	Qualify LCS
Influent 12-2-04	A	3		2	7.2	No	19.8	8.8	6.8	2.0		200.0	203.0	203	
Influent 12-2-04	BC	5		2	7.2	No	19.8	8.8	5.2	3.6		216.0			
Influent 12-2-04	D	7		2	7.2	No	19.8	8.9	4.4	4.5		192.9			
Effluent 12-2-04	E	200		2	7.3	No	21.5	8.8	6.8	2.0		3.0	2.6	3	
Effluent 12-2-04	GG	250		2	7.3	No	21.5	8.8	6.6	2.2		2.6			
Effluent 12-2-04	H	300		2	7.3	No	21.5	8.8	6.5	2.3		2.3			

DO Meter Calibration:

Test Start Meter Calibration — Date: 12/2/04 Time: 7:15 AM Analyst: Joe Q. Public

Technique

■ Water-Saturated Air		□ Air-Saturated Water		□ Winkler Titration	
DW Temp (ºC):	19.8	DW Temp (ºC):		mL Titrant:	
Pressure (mm Hg):	740	Pressure (mm Hg):			
DO Calibration:	8.86	DO Calibration:		DO Calibration:	

Test End Meter Calibration — Date: 12/7/04 Time: 8:00 AM Analyst: Joe Q. Public

Technique

■ Water-Saturated Air		□ Air-Saturated Water		□ Winkler Titration	
DW Temp (ºC):	20.2	DW Temp (ºC):		mL Titrant:	
Pressure (mm Hg):	720	Pressure (mm Hg):			
DO Calibration:	8.81	DO Calibration:		DO Calibration:	

Another option – What the SLH has generated

5S-DAY BOD SET-UP LOG
ESS INO METHOD 260.1

Set-up Date: _____
Analyst: _____
Batch # _____

DPD Code: _____
Neutralizing Code: _____
Incubator: _____
RO Dilution Water: _____

TCMP: _____
GGA Code: _____
Seed Code: _____
Nutrient Code-Carboys: _____
Nutrient Code-300-ml pillows: _____

Sample & bottle ID	Matrix[a]	Temp (°C)	BOD est (mg/L)	pH	Adjusted pH	DPD check for CL (+ or -)	Seed (S)	Other sample treatments[b]	Set-up volumes (mL)	Comments/Action

Corrective Action

Corrective action documentation for quality control (QC) failures within analytical runs will include: a) identifying the QC failure and why it occurred (if known); b) noting specific corrective actions that were taken or attempted; c) stating the next action that will be taken.

The following analytical items will be checked routinely. If the analyst cannot pinpoint a specific problem, they will note, "Analytical Checks OK--Unknown cause" on the bench sheet.

System Problems	Sample Problems	Analyst Error Problems
PC Hardware	Matrix Interference	Dilution Error
PC Software	Sample not Homogeneous	Sample Position
Probe		Pipet Error

[a] Matrix: "EF" = effluent; "IF" = influent; "MW" = monitoring well; "OW" = other waste; "SU" = surface water.
[b] Other Treatments: "N" = neutralized sample with either an acid or base; "I" = inhibited sample for CBOD; "F" = filtered sample.

Summary

- ☑ Discussed the "whys" of BOD
- ☑ Reviewed common problems with the test
- ☑ Discussed the art of calibration
- ☑ Reviewed the method in detail
- ☑ Highlighted QA/QC requirements
- ☑ Provided resolutions to common problems
- ☑ Discussed what documentation is required

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http://www.slh.wisc.edu/outreach/

DNR's LabCert homepage:
http://www.dnr.state.wi.us/org/es/science/lc/

- Please note additional information after this point in your packet.

Sample Data I

		A		B	C	D	E	F		
		Sample	Seed			Depletion		DF	BOD	
Sample	BotL#	mLs	mLs	DO_I	DO_F	B-C	SCF	300/A	F x (D-E)	REPORT
Dil'n Blank	X	300	0	8.5	8.4	0.1				
	U	300	0	8.5	8.4	0.1				
Seed Control	AA		5	8.5	6.2	2.3	0.46			
	C		10	8.5	4.7	3.8	0.38			
	H		15	8.5	1.9	6.6	0.44			
GGA	L	6	2	8.5	3.4	5.1	0.85	50	212.3	
	T	6	2	8.5	3.5	5	0.85	50	207.3	
	B	6	2	8.5	6.1	2.4	0.85	50	77.3	
Sample 1	VV	3	0	8.5	6.5	2	0	100	200.0	
	F	5	0	8.4	4.3	4.1	0	60	246.0	
	AN	10	0	8.4	3.2	5.2	0	30	156.0	
Sample 2	P	10	0	8.3	4.9	3.4	0	30	102.0	
	G	25	0	8.3	2	6.3	0	12	75.6	
	D	40	0	8.4	2.4	6	0	7.5	45.0	

Sample Data II

		A		B	C	D	E	F		
		Sample	Seed			Depletion		DF	BOD	
Sample	BotL#	mLs	mLs	DO_I	DO_F	B-C	SCF	300/A	F x (D-E)	REPORT
Dil'n Blank	X	300	0	8.5	8.1	0.4				
	U	300	0	8.5	8	0.5				
Seed Control	AA		5	8.5	7.9	0.6	0.12			
	C		10	8.5	7.1	1.4	0.14			
	H		15	8.5	6.2	2.3	0.15			
GGA	L	6	2	8.5	5.0	3.5	0.28	50	161.2	
	T	6	2	8.5	4.8	3.7	0.28	50	171.2	
	B	6	2	8.5	4.6	3.9	0.28	50	181.2	
Sample 3	VV	50	0	8.5	6.5	2	0	6	12.0	
	F	75	0	8.4	4.4	4	0	4	16.0	
	AN	100	0	8.4	1.9	6.5	0	3	19.5	
Sample 4	P	50	0	8.3	6.3	2	0	6	12.0	
	G	75	0	8.4	1.0	7.4	0	4	29.6	
	D	100	0	8.4	3.7	4.7	0	3	14.1	

Sample Data III

		A		B	C	D	E	F		
		Sample	Seed			Depletion		DF	BOD	
Sample	BotL#	mLs	mLs	DO_I	DO_F	B-C	SCF	300/A	F x (D-E)	REPORT
Dil'n Blank	X	300	0	9.6	9.5	0.1				
	U	300	0	9.4	9.3	0.1				
Seed Control	AA		5	9.5	5.0	4.5	0.90			
	C		10	9.6	1.1	8.5	0.85			
	H		15	9.5	<< 1.0					
GGA	L	5	6	9.4	0.9	8.5	5.25	60	195.0	
	T	5	6	9.5	1.1	8.4	5.25	60	189.0	
	B	5	6	9.5	0.8	8.7	5.25	60	207.0	197.0
Sample 5	VV	200	0	10.6	2.8	7.8	0	1.5	11.7	
	F	100	0	10.1	6.3	3.8	0	3	11.4	
	AN									**11.6**
Sample 6	P	50	0	9.5	9.1	0.4	0	6	2.4	
	G	75	0	9.5	8.6	0.9	0	4	3.6	
	D									**3.0**

BOD FAQs

- How is the 5-day "incubation" period defined?

  The book, "Laboratory Testing for BOD and CBOD", by Brake and Raynovic, is the only reference that discusses an absolute definition (± 2 hours). Both Standard Methods and the EPA are silent on the issue. We believe that you should strive to stay as close to the actual 5 day incubation period as you can, but certainly stay within 5 days ± 4-6 hours.

- How are weekend incubator temperatures verifed?

  There are several options, including a digital minimum/maximum thermometer, and an electronic monitor that send real-time data to a computer for storage. Alternatively, if someone is available, they can always come in and read the thermometers "the old-fashioned" way. Note that incubator temps are only required over weekends when samples are in the incubator.

BOD FAQs - 2

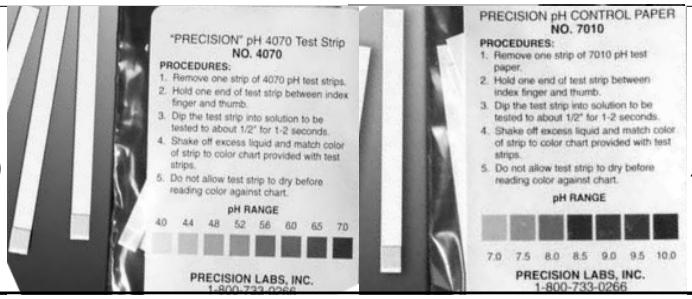
- How should the pH of samples for BOD testing be checked?

  The original, undiluted sample must be checked to determine whether the natural pH is outside the range at which a natural population of microrganisms would be expected to be viable.

  If pH of the original sample is outside of this range, pH adjustnment of the original sample and seeding of any sample dilutions is required.

  Can you use pH paper? Yes…provided it's "narrow range" (vs. 1-14). We need paper that covers the range from 6 to 9. You may need two sets of paper

pH
4.0 to 7.0



pH
7.0 to 10

BOD FAQs - 3

- If my GGA fails, what do I have to do?

  1. Qualify results on benchsheet and on the DMR back to the date when you last analyzed an acceptable GGA.

  2. Take Corrective Action – try to determine what may have caused the failure (was it high? Or low?). Take action to resolve the problem.

  3. We recommend that you prepare another GGA the next time you set up samples for BOD testing.

- Do I need to record the temperature of my lab?

  If you intend to analyze samples for BOD, you must maintain a lab temperature of 20 $\pm$ 3 ˚C.

  Remember also that if you perform ammonia analysis using ISE, there is a 1-2% error per degree C change in temperature (between temperature of calibration standards and room temp).

  Consequently, you need to be able to control lab temperature.

BOD FAQs - 4

- I reference the 20th ed. Of Standard Methods for BOD and routinely analyze 3 dilutions per sample. For one sample, only one dilution met depletion criteria…what do I need to do?

  We do not see this as a "QC Exceedance per se. Therefore it does not need to be qualified on the DMR or noted in the "QC Exceedance" box on the DMR.

  That being said….the method does require that at least two dilutions meet the depletion criteria. Therefore, we recommend that you document the occurrence on your benchsheet and strive to set up the most appropriate dilutions for your sample to ensure that at least two of them meet depletion criteria. This may mean using additional dilutions or altering the dilutions used historically.

- Can I use the new disposable ("plastic") BOD bottles?

  Yes, as long as you use them once and throw then away and all QC meets requirements.

  Note that attempts to wash and reuse these bottles can remove the polycarbonate protective coating which can lead to leaching BOD

BOD FAQs - 5

- Can I throw out a dilution if it doesn't agree with others?

  What does Standard Method say?

  - ☑ 18th , 19th and 20th are mute on the point
  - ☑ 21st Edition provides the following guidance:

    "…Identify samples in test reports when serial dilutions show more than 30% between high and low values. Exceptions occur for highly treated wastewaters and natural waters having BODs less than 20 mg/L."

  Here is what we recommend:

  - ☑ DO NOT discard dilutions without evaluating the data
  - ☑ Identify problem dilutions and try to determine cause (e.g., "chucks", high solids, etc.)
  - ☑ If you discard the a dilution, you must qualify results and be prepared to defend your actions.

Sample Data IA

Blank OK

SC OK

GGA Most likely failed to seed 3rd one

S1 1. poor precision?
2. sub-sampling problem? Wrong pipet?
Too slow to transfer?
3. sample ”chunky" (heterogenous)

S2 sliding BOD
toxic sample?
Nitrification?
inadequate mixing b/w dilutions?
more solids in earlier dilutions

Sample Data IIA

Blank Excessive depletion in blank

SC seed too weak

GGA GGA fails…low bias!
not enough seed
seed not strong enough

S3 sliding BOD
probably mixing problem
inadequate mixing b/w dilutions

S4 contaminated 75 mL pipet?
sub-sampling probably (chunk!)

Sample Data IIIA

Blank Bad calibration
Since DOf is still high, cant be cold

SC VERY active seed

GGA Seed too active; overdepletes
Not enough GGA
Data probably OK
Can't average GGA

S5 Needs extra nutrients
Supersaturated (200 mL)
Dilution water dropped DOi

S6 Insufficient depletion
Need to use more sample
LOD is 8 so should report "< 8"

Troubleshooting: DO Probe malfunctions

1. Allow ≥ 2 hr after membrane change for probe to stabilize. Overnight is better.
2. Warm-up instrument. Calibrate.
3. Observe readings continuously for 2 mins. w/probe in bottle.
4. Be sure the temperature is constant.
5. Watch the readings carefully.

DON'T just record initial reading & come back 2 minutes later . You need to actually see what happens over the time period.

- If readings drifts slowly DOWN, a longer warm up time is required.
- If readings JUMP AROUND, the probe is not functioning properly.
- If readings STABLE in the air calibration bottle, sensor is probably OK.
- If readings stable in the air calibration bottle but not in solution, the membrane is probably defective.)

Troubleshooting: DO Probe malfunctions

Zero Oxygen Check (Response check):

- Dissolve 0.5-1 grams of Sodium Sulfite in 300 ml of water.
- Stir slowly-avoid “tornadoes”; slowly pour into a BOD bottle.
- Calibrate your DO probe as you normally would.
- Place probe in the "Zero Oxygen" solution
- **Observe!**
- Meter should read "0" within two minutes.

  (With some older YSI systems, readings below 1.0 mg/l are considered zero.)

Solving: Glassware cleanliness problems

- Use a lab-grade non-phosphate detergent and bleach
- Rinse thoroughly with tap water ten with distilled water
- Allow to dry before storing.
- Always cover glassware & store in a clean, dry place.

*** Alternate Cleaning Method w/o Bleach ***

- Use a good laboratory grade non-phosphate detergent
- Rinse well with tap water followed dilute HCl
  (10% solution; 100 mL HCl per liter of water).
- Rinse again w/ tap water followed by distilled water.
- Allow to dry before storing.
- Always cover glassware & store in a clean, dry place.

Warning**: DO NOT MIX HCl and bleach: It will produce poisonous chlorine gas!!!!**

Corrective Action

Situation	Corrective Action
Dilution water depletes > 0.2 mg/L	1) Check probe performance (*incl. calibration*) 2) Using "grocery store" water in poly jug 3) Clean glassware/tubing 4) Evidence of growth in nutrient solutions?
Seed Control depletion not 0.6 to 1.0 mg/L	1) Re-evaluate seed strength 2) Use more seed 3) Consider another seed source 4) ***GGA performance good & consistent?
Replicates exceed control limits	1) Check for errors, sample problems 2) Review control limits 3) Run another replicate on next analysis day 4) Qualify results on DMR back to last pass

Corrective Action

Situation	Corrective Action
GGA failing **HIGH**	1) Check probe performance/calibration. 2) Look for sources of contamination. 3) Change in seed source? 4) Possibility of nitrification? 5) Run another GGA next time 6) Qualify data on DMR back to last good GGA.
GGA failing **LOW**	1) Check probe performance/calibration. 2) Using enough seed?? 3) Seed from your plant; change in the process? 4) Old/expired GGA? Discard. 5) Run another GGA next time 6) Qualify data on DMR back to last good GGA.

Setting up an effective QA Plan

- Tables are better than lots of text!
  - ✓t "a picture is worth 1000 words" concept
  - ✓Tables FORCE you to be brief

3 keys for tabular QA Plan

What am I evaluating? (parameter)

How do I evaluate it (criteria)

What if it doesn't meet specifications?

(Corrective Action)

Putting it all together - your QA Plan

Evaluating?	Criteria	Corrective Action
Dilution Water Blank	< 0.2 mg/L depletion	1) Identify source 2) Correct Problem 3) Qualify data
GGA	198 ± 30.5 mg/L = 167.5 to 228.5 mg/L = 84.6% to 115.4%	1) Check prep. data 2) Analyze another next run 3) Qualify data
Replicates	Within Control Limit(s)	1) Homogeneous sample? 2) Analyze known std. 3) Qualify data

BOD 201 — Saturation Conversions – Rice Lake, WI

Your facility's altitude is **1140** ft ASL	$\frac{760 - (\mathbf{1140} \times 0.026)}{760} = \frac{760 - 29.6}{760} = \frac{730.4}{760}$ = 0.9611 = baro. pressure correction
Radio station says pressure is **30.21** ↑ inches (but that's corrected to sea level)	**30.2** in. x 0.9611 = 29.03 in (true un corr. BP) x 25.4 mm/inch = 737.4 mm
MY lab's air temperature is **22.4 ºC**	Saturation at 760 mm & **22.4 ºC** = 8.65 mg/L
So… saturation at MY Temp. and altitude=? Standard O2 sat. tables are set to 760	Correction= $\frac{737.4}{760}$ = 0.9703 = 8.65 x 0.9703
What should I set the meter at?	**= 8.39 mg/L**