Biochemical Oxygen Demand (BOD) Troubleshooting

State Hygienic Laboratory
at the University of Iowa

Dustin May
Analytical Chemist

Presentation Overview

- SHL BOD Analysis
- Why Do We Test for BOD?
- How are Samples Analyzed for BOD?
- Scientific Method
- Sources of Issues
- Troubleshooting Examples

SHL Three Locations


Ankeny Laboratory


Lakeside Laboratory - Milford


Coralville Laboratory

High Sample Load

- Ankeny Lab – ≈3100 BOD analyses/year
- Coralville Lab – ≈2200 BOD analyses/year
- Lakeside Lab – ≈100 BOD analyses/year
- ≈ 5400 Analyses/year

Why Do We Test for BOD?

- BOD has been performed for a long time
- Lots of historical data
- Required for discharge into surface water in accordance with EPA NPDES permit program and Iowa Surface Water Quality Standards

Why Do We Test for BOD?

- BOD is used to measure a solution's impact on dissolved oxygen
- Solutions with a high BOD can lead to adverse environmental impact
- Low dissolved oxygen levels, algae blooms, fish kills, etc.

Why Do We Test for BOD?

- Dissolved oxygen is consumed by bacteria and wild yeast present in solution
- Oxygen is consumed in biological processes such as cellular respiration and reproduction
- Oxygen is also consumed via nitrification, the conversion of ammonium ions (NH_4^+) to nitrite ions (NO_2^-), and then from nitrite ions to nitrate ions (NO_3^-)

How are Samples Analyzed for BOD?

How are Samples Analyzed for BOD?

- Samples are brought to room temperature and checked for pH
- Visual, olfactory, and historical inspection is used to determine an estimate for the BOD result
- Multiple subsamples are diluted at varying concentrations with water fortified with nutrients and a buffering solution

How are Samples Analyzed for BOD?

- Control blanks and duplicates of samples are prepared
- A nitrification inhibitor is added to samples requesting carbonaceous BOD analysis
- An initial measurement of the dissolved oxygen concentration is taken
- The bottle containing the dilution of the sample is sealed and incubated at 20°C for 5 days

How are Samples Analyzed for BOD?

- A final measurement of dissolved oxygen concentration is taken and the BOD result is calculated as follows,

$$\frac{DO_i - DO_f}{V_S/V_T}$$

where,

DO_i = Initial dissolved oxygen concentration
DO_f = Final dissolved oxygen concentration
V_s = Volume of sample used
V_T = Final diluted volume

How are Samples Analyzed for BOD?

- If the samples are highly acidic or alkaline, or contain chlorine, neutralization and seeding are required due to lack of bacteria and yeast
- A seeding solution is used to introduce aerobic microbes to the sample
- Control solutions to correct for and evaluate seed are prepared along with the samples.

How are Samples Analyzed for BOD?

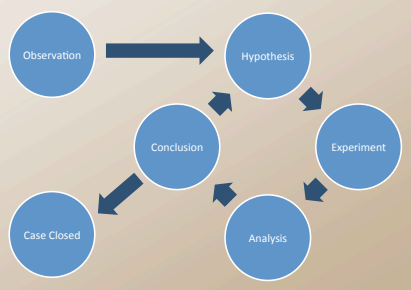
- The BOD result for seed samples is calculated as follows,

$$\frac{(DO_i - DO_f) - (DO_{is} - DO_{fs})f}{V_S/V_T}$$

where,

DO_i = Initial dissolved oxygen concentration, mg/L
DO_f = Final dissolved oxygen concentration, mg/L
DO_{is} = Initial dissolved oxygen concentration of seed control, mg/L
DO_{fs} = Final dissolved oxygen concentration of seed control, mg/L
V_s = Volume of sample used
V_T = Final diluted volume
f = Ratio of seed in sample to seed in seed control

Scientific Method



Scientific Method: Observation

- Something you want to explain
- Helpful to think of in the form of a question
- Could be a problem/issue or could be related to process improvement

Scientific Method: Hypothesis

- A theory or explanation that could explain your observation
- Think of it as the answer to the question you formulated in relation to your observation
- ONE AT A TIME!

Scientific Method: Experiment

- Devise an experiment to test your hypothesis
- Repeat to be sure your results are unlikely to be affected be random chance
- May need to test multiple different variables
- Test only one variable at a time

Scientific Method: Analysis

- Evaluate the experimental results
- What do the results show?
- Try to avoid bias
- The answer is not known, this is why we experiment

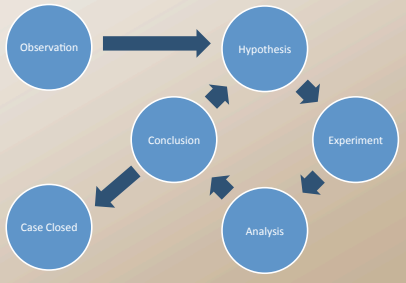
Scientific Method: Conclusion

- Draw conclusion from the analysis of the experiment as it relates to your hypothesis and your observation
- If the results do not support the hypothesis start again
- If the results support the hypothesis the case closed and the observation can be addressed

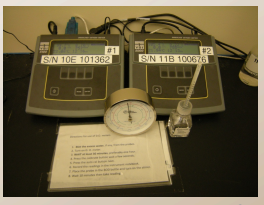
Scientific Method: Conclusion

- Either outcome results in knowledge gained
- THERE IS NO SUCH THING AS A FAILED EXPERIMENT!

Scientific Method

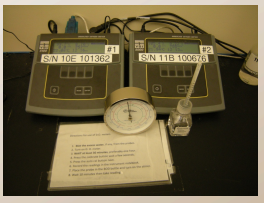


Sources of Trouble: Meter



- Dissolved oxygen meter can be affected by changes in environmental conditions
- Changes in temperature (especially outside of the 17-23°C range) and pressure can impact calibrations

Sources of Trouble: Meter



- Avoid large temperature swings
- Adjust atmospheric pressure to a NIST-traceable barometer
- Meters tend to drift over time

Sources of Trouble: Probes

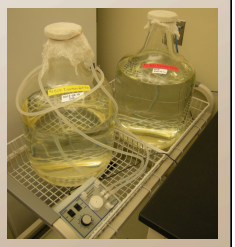
- Membrane caps can become loose over time.
- Membranes can also be damaged
- Condensation on membranes will yield erroneous calibrations
- Regular preventative membrane cap replacement is usually a good idea
- Air calibrated probes may drift slightly when first placed in water

Sources of Trouble: Probes

- Electrodes become less efficient over time
- Need to be cleaned periodically with ammonium hydroxide to remove silver chloride buildup
- Cleaning shortens the life of the probe, must be done sparingly

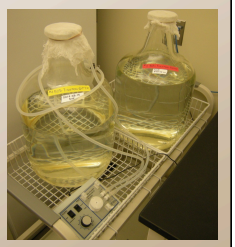
Sources of Trouble: Dilution Water

- Dilution water needs to be prepared from a clean, reliable source
- Most problems with blanks can be traced back to the water
- Carboys containing water must be cleaned periodically to remove any possible mold growth or ferric chloride build-up



Sources of Trouble: Dilution Water

- Tubing should be replaced when it starts to discolor
- Food grade silicone tubing lasts longer and is more resistant than PVC
- Water must be adequately oxygenated prior to use



Sources of Trouble: Reagents

- Reagents need to be labeled properly and tracked
- If one doesn't know what reagents were used in a given batch problems with reagents cannot be traced back to their source

Sources of Trouble: Reagents

- Reagents should be monitored visually for changes (i.e. mold growth) and stored appropriately (i.e. room temperature vs. refrigerated)
- Method specified amounts must be adhered to; consistency is important

Sources of Trouble: Bottles and Glassware

- Bottles and pipettes must be cleaned and stored properly
- In dusty environments clean bottles should be covered

Sources of Trouble: Bottles and Glassware

- Bottles and stoppers should be rinsed immediately after use and washed with an appropriate laboratory detergent, rinsed thoroughly, and allow to dry completely
- Incorrectly cleaned and/or stored bottles can lead to erratic results

Sources of Trouble: Seeding and G/GA Standards

- Seed should be added at a rate high enough to yield consistent and satisfactory results (per method specifications) for glucose/glutamic acid standards
- The range of 0.6 to 1.0 mg/L for seed control depletion given in the method may not be enough
- If the seed volume works for your standards it should work for your samples

Sources of Trouble: Samples

- Samples should be mixed thoroughly, immediately before subsampling
- Subsamples should be taken from a central, repeatable place in the sample bottle
- Some samples contain chemicals and/or organisms that can create a toxic, inhibitory effect
- Non-homogenous samples can cause variation in duplicates and dilutions, larger subsamples can minimize this variation

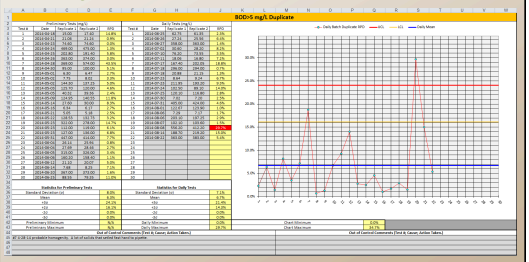
Quality Control

- Operating conditions of equipment needs to be recorded
- If something isn't recorded, you can't prove it happened
- Incubator temperature, equipment calibration, and repairs/maintenance of equipment needs to be recorded

Quality Control

- Results of quality control samples (i.e. blanks, glucose/glutamic acid standards, and duplicates) should be tracked, reviewed and evaluated on a regular basis
- This allows for trends in the data to be discerned
- This information can help one stop emerging problems before they become major issues

Quality Control



	A	B	C	D	E	F	G	H	I	J
1										
2	Preliminary Tests (mg/L)					Daily Tests (mg/L)				
3	Test #	Date	Dilution 1	Dilution 2	RPD	Test #	Date	Dilution 1	Dilution 2	RPD
4	1	2013-10-18	10.00	11.60	14.8%	1	2014-06-03	82.70	81.30	2.1%
5	2	2013-10-21	41.00	43.00	4.9%	2	2014-06-04	164.00	160.00	2.5%
6	3	2013-10-22	75.00	71.00	5.6%	3	2014-06-05	252.00	252.00	0.0%
7	4	2013-10-23	660.00	671.00	1.6%	4	2014-07-02	28.00	28.00	0.0%
8	5	2013-10-24	203.00	191.00	5.8%	5	2014-07-03	76.00	73.00	4.1%
9	6	2013-10-25	860.00	874.00	1.6%	6	2014-07-07	18.00	18.00	0.0%
10	7	2013-10-28	1000.00	1000.00	0.0%	7	2014-07-08	286.00	284.00	0.7%
11	8	2014-04-02	93.00	100.00	5.1%	8	2014-07-09	286.00	284.00	0.7%
12	9	2014-05-01	6.00	6.47	7.7%	9	2014-07-10	20.88	21.33	1.1%
13	10	2014-05-02	100.00	100.00	0.0%	10	2014-07-14	11.00	10.00	9.5%
14	11	2014-05-03	144.00	147.00	2.0%	11	2014-07-15	11.00	10.00	9.5%
15	12	2014-05-05	115.50	120.00	4.0%	12	2014-07-16	193.50	88.50	14.0%
16	13	2014-05-06	100.00	100.00	0.0%	13	2014-07-17	7.00	7.00	0.0%
17	14	2014-05-08	114.00	95.00	11.8%	14	2014-07-21	7.00	7.00	0.0%
18	15	2014-05-14	27.00	30.00	8.3%	15	2014-07-31	405.00	424.00	4.6%
19	16	2014-05-15	5.00	5.00	0.0%	16	2014-08-01	7.00	7.00	0.0%
20	17	2014-05-21	5.00	5.43	7.7%	17	2014-08-05	7.00	7.17	1.7%
21	18	2014-05-22	158.55	162.75	2.7%	18	2014-08-06	193.30	187.20	3.2%
22	19	2014-05-23	313.00	276.00	12.7%	19	2014-08-07	193.30	187.20	3.2%
23	20	2014-05-27	127.00	140.00	9.7%	20	2014-08-08	166.30	166.30	0.0%
24	21	2014-06-02	127.00	148.00	8.8%	21	2014-08-12	188.70	218.00	13.0%
25	22	2014-06-03	147.00	152.00	7.7%	22	2014-08-13	163.00	160.00	1.1%
26	23	2014-06-04	20.14	21.76	2.8%	23				
27	24	2014-06-05	252.00	252.00	0.0%	24				
28	25	2014-06-06	210.00	224.00	2.0%	25				
29	26	2014-06-09	160.00	158.40	1.1%	26				
30	27	2014-06-10	7.00	7.00	0.0%	27				
31	28	2014-06-12	7.00	8.00	2.0%	28				
32	29	2014-06-20	367.00	373.00	1.8%	29				
33	30	2014-06-24	38.00	38.00	0.0%	30				
34										
35	Statistics for Preliminary Tests					Statistics for Daily Tests				
36	Standard Deviation (s)				8.0%	Standard Deviation (s)				9.1%
37	Mean				8.0%	Mean				10.7%
38	+3s				24.1%	+3s				21.4%
39	-3s				-8.1%	-3s				-10.3%
40	+2s				16.1%	+2s				18.2%
41	-2s				-0.0%	-2s				-0.0%
42	Preliminary Minimum				0.0%	Daily Minimum				0.0%
43	Preliminary Maximum				26.7%	Daily Maximum				29.7%
44						Chart Minimum				0.0%
45						Chart Maximum				30.1%
46	Date of Control Comments / Root & Cause / Action Taken:					(Date of Control Comments / Root & Cause / Action Taken:				
47	07-08-14 is probably incorrect. A set of results that seem the same.									
48										
49										
50										

Troubleshooting Examples

- **Observation: Some dilution water blanks are out of control, but others are perfectly fine. There seems to be no discernable pattern.**
- Hypothesis: Some bottles may not be getting cleaned properly, leading to some bottles being clean and others being contaminated.

Troubleshooting Examples

- Experiment:
  - Randomly select bottles that have been used recently and set up a series of blanks.
  - Modify cleaning procedures on a batch of bottles to be more rigorous (i.e. more manual washing, longer detergent contact time, more rinses).
  - Set up a series of blanks with the new bottles keeping all other conditions the same.

Troubleshooting Examples

- Analysis and Conclusion
  - If there are significantly fewer or no blanks out of control in the rigorously cleaned batch relative to the regular batch of blanks, it is likely that the hypothesis posed was correct. New bottle cleaning procedures will need to be implemented.
  - If the number of out of control blanks in both batches are about the same it is unlikely to be the cause and a new hypothesis will need to be devised.

Troubleshooting Examples

- **Observation: All dilution water blanks are consistently out of control.**
- Multiple hypotheses may be devised and tested to expedite corrective action
- Hypothesis 1: The source of the dilution water has become contaminated.
- Hypothesis 2: The reagents used to prepare the samples have become contaminated.
- Hypothesis 3: Tubing used to distribute dilution water has become contaminated.

Troubleshooting Examples

- Experiment 1:
  - Prepare two sets of blanks using different sources to prepare dilution water, but use the same equipment and reagents.
- Experiment 2:
  - Prepare two sets of blanks using different reagents with the same equipment and dilution water source.
- Experiment 3:
  - Prepare two sets of blanks using different equipment with the same reagents and dilution water source.

Troubleshooting Examples

- Analysis and Conclusion
  - If any of the experimental results support their respective hypotheses, proceed with corrective action to replace the offending item.
  - If the experimental results to not support respective hypotheses, a new set of hypotheses will need to be devised.

Troubleshooting Examples

- Batching hypotheses and experiments in this way is extremely important in determining corrective action quickly
- The nature of the method for determining BOD occurs over a long time
- It would be prohibitive to test only one possibility at a time

Conclusion

- Consistency in procedures can minimize issues
- Preventative maintenance can prevent issues from arising
- Tracking reagents and changes to equipment allow for sourcing issues
- Questions?