How are your CBOD's
tips, tricks and troubleshooting

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- CBOD's: The test we love to hate
- Assumed by many to be an "easy" test
- Many variables in performing this test
  - Too many things that can go wrong

CBOD basics

- Carbonaceous Biochemical Oxygen Demand
- Measures the potential of wastewater (and other waters) to deplete the oxygen level of the receiving waters

- CBOD is a subset of BOD
- BOD looks at both carbonaceous and nitrogenous biochemical oxygen demand
- It can be used to monitor plant operating efficiency by determining percent removal of BOD/CBOD between influent and effluent streams

- CBOD test is frequently used to determine the extent a waste stream will contaminate/ pollute a receiving stream by depriving the organisms in the stream of oxygen

- Unfortunately, the BOD/ CBOD test is the ONE test that is most likely to generate invalid data if adequate precautions aren't taken to ensure the quality of the test

- Samples are prepped with dilution water to prevent total depletion of oxygen over the 5 day incubation period
- Nutrients are added to simulate the environment of a receiving stream

- 5 days is the designated time, which allows for about 60 to 70% of available material to be consumed

CBOD Trivia

- The reason for 5 days is supposed to be the length of time it took for water from the river Thames to flow from London to the ocean
  - This was established by the Royal Commission on Sewage Disposal in 1908

- The US determined that at 5 days between 60 and 70% of total BOD was exerted during the incubation period…. after investigating incubations of 1, 2, 5, 10 and 20 days.

- CBOD will never be higher than BOD
- CBOD can be equal to BOD if there is no nitrogenous uptake of oxygen
- There is a correlation between COD and BOD. You can use this determine dilution ranges on new sample sites

To determine CBOD range from COD

- Lowest dilution:
- (2/ COD value of sample) * 300

- Highest dilution:
- (5/COD value of sample) * 300

- This gives a beginning range to set up with

Method Approval

- Standard Methods 5210 B–2001
- Includes use of optical and luminescence DO technology

- Samples are 5 days, not 4 or 6 and “adjusted”

- Pretreat samples if pH isn’t between 6.0 and 8.0 (dilute H_2SO_4 or NaOH). If pretreated, final pH should be between 7 and 7.2

- Pretreat samples so temp. is 20+/- 3C

- Adjust sample DO down if supersaturated
  - (shake or bubble if DO of sample is greater than 9 mg/L)

- Remove any residual chlorine
  - Chlorine will usually dissipate upon standing for 1 to 2 hours)
  - If necessary, perform a titration with Na_2SO_3 to determine required amount needed to remove chlorine. DO NOT ADD TO EXCESS!!

Dilution Water

- Distilled, tap or receiving water
- Free of metals and toxic substances
- Deionized water may contain microorganisms and organics, making it unsuitable for dilution water

- DO of dilution water should be at least 7.5
- Glass is preferred container for storage/ prep of dilution water
- Add 1 mL each of phosphate buffer, Magnesium sulfate, Calcium chloride, and ferric chloride per liter of dilution water

- Temperature should be 20 +/- 3 C
- Prepare dilution water immediately before use unless blanks show water is stable if held longer
- Do NOT add oxidizing agents or expose dilution water to UV light in order to bring depletion into range

Seed Preparation

- Preferred seed is obtained from a biological treatment system processing the waste
- Synthetic seed may be used if there is no viable option of a natural seed source

- Do not filter seed source
- Do not use chlorinated streams as a seed source
- Supernate from settled domestic wastewater, primary effluent, diluted mixed liquor are all acceptable sources of seed

- Seed uptake should be between 0.6 and 1.0 mg/L to allow for seeded samples to fall into the “take 2, leave 1 range”

- Seed controls should be run for every set of BOD/ CBOD

- Ideally, there should be 3 seed control bottles, but at minimum – no less than 2 in order to get an accurate seed value for the run

Sample Prep

- Bring samples to 20 +/- 3C before making dilutions
- Check pH of sample
  - If sample's pH is outside the range of 6.0 to 8.0, adjust with sulfuric acid or sodium hydroxide to a pH of 7.0 to 7.2
  - Always seed samples that have pH adjusted

- When making dilutions, sample volume should not be less than 1% of bottle volume
- If you are using the most common size BOD bottle, it is 300 mL. Therefore, the amount of sample you use to make dilutions cannot be less than 3 mL
- You may need to do a serial dilution to accomplish lower values

Quality Control

- Only bottles that give a minimal depletion of 2 mg/L and have a residual of at least 1.0 mg/L DO can be calculated
- GGA is used to determine accuracy and precision of the BOD and should be included with every batch of samples.

- Seed control check should be 3 bottles, different dilutions so as to allow the smallest dilution to show an uptake of 2 mg/L and the largest to have a 1.0 mg/L residual. These should be included with every batch

- Blank check is used to monitor the dilution water quality and should be set up with every batch of samples as well.
- If your blanks fail, your test data is invalid. Data may be reported out, with a qualifying statement explaining the blank failed.
- Data is still invalid, and corrective MUST be taken

Tips & Tricks

- Always start with clean glassware
  - Make sure the bod bottles, dilution water bottle and pipettes are scrupulously clean
  - Don’t use plastic carboy for dilution water. Plastic leaches into water, and the valve is a breeding ground for bacteria

- Make sure you transfer seed, sample and dilution water to bottles without introducing air.

- Ideally, prep 3 bottles for each sample to allow for a variety of uptakes per site
  - Not all 3 need to meet the take 2, leave 1 rule for valid results.

- Make sure to add nutrient to bottles where there is little to no dilution water present

- If sample volumes are between 150 to 200 mL, add 0.1 mL of each nutrient directly to the bod bottle

- If sample volumes are between 200 to 250 mL, add 0.2 mL of each nutrient directly to the bod bottle

- If sample volumes are between 250 to 300 mL, add 0.3 mL of each nutrient directly to each bod bottle

- Make sure the DO probe is calibrated correctly
- Rinse the probe between every bottle
- Allow the probe to stabilize before recording the reading

- Make sure dilution water is stable, with a good pH and DO
- Samples are incubated for 5 days +/- 6 hours

Troubleshooting

- Final DO of blank higher than initial DO:
  - Check on calibration techniques
  - Check to see if samples are incubating in dark – if too much light gets in, photosynthesis can occur and drive final DO higher.

- Was the dilution water supersaturated?
- Were bubbles introduced into blank when prepping it?

- Too much uptake on blank DO:
  - If there is greater than 0.20 mg/L uptake for blank sample, check the following items:

- Check calibration techniques
  - Are you using the same method every time?
  - If air calibrating, is there a chart showing specific values for each barometric reading?
  - If air calibrating – is barometer set for “real” altitude, not corrected to sea level?

- Check stability of water
  - Are you storing it, and if so…for how long?
  - Do you make it up fresh?
  - When do you add your nutrients?

- Check quality of source water
- Do you use tap water, distilled, deionized?
  - Do you use receiving stream water?
- Where do you get it from?
- Is the system clean?

- Check cleanliness of water storage container
- Check transfer tubing, etc for possible contamination
- Check cleanliness of BOD bottles

- GGA:
  - Glucose- glutamic acid
  - 6 mL solution to 300 mL BOD bottle
  - Average value is 198 mg/L
  - Variance is 30.5 mg/L
  - Range: 167.5 mg/L to 228.5 mg/L

- According to EPA/ NELAC interpretation papers, every lab should determine their own GGA values

- Find the mean and standard deviation of the GGA, use these to determine the actual acceptable range for your lab – your GGA prep, seed, equipment, technician(s)

- LOW GGA:
  - Often occurs when using synthetic seed
  - GGA might be “old” – make fresh if you see values dropping off

- High GGA:
  - Failed to add nitrification inhibitor
  - Inhibitor is “old”
  - Dispenser doesn’t add correct amount
    - TCMP – 3 mg per 300 mL BOD bottle
    - ATU – 0.3 mL per 300 mL BOD bottle

- NOTE:
  - ATU (Allylthiourea) is an alternate form of nitrification inhibitor
  - As a liquid, it goes into solution quickly
  - Not always effective during the 5 day incubation period
  - Added in excess can create false high CBOD results

- TCMP:
  - 2-chloro-6-(trichloromethyl) pyridine
  - Doesn’t dissolve well
  - Most commercial preparations aren’t pure TCMP, allowing them to go into solution better

- High GGA:
  - Bottles may be overseeded.
  - Try setting up the GGA with smaller volumes of seed

- CBOD value decreasing as sample volume increases:
  - Most likely toxicity of sample occurring, killing/ impairing the seed
  - Do more than 3 dilutions on next sample to verify.

- REMEMBER:

- Only 1 of the dilution series for each sample has to meet the take 2, leave 1 rule for valid results for the test

CBOD Math

- BOD/CBOD - unseeded

- $\frac{(D1 - D2)}{P}$

  - D1 = initial DO
  - D2 = final DO
  - P = decimal volumetric fraction of sample used

- Unseeded, slightly easier

- $\frac{(\text{Initial DO} - \text{Final DO}) * 300}{\text{Volume of sample}}$

- Seeded:

- $\frac{(D1 - D2) - ((S1 - S2)SF)}{P}$

- D1 = initial DO of sample
- D2 = final DO of sample
- S1 = initial DO of seed
- S2 = final DO of seed
- SF = seed factor
- P = decimal volumetric fraction of sample used

- Better:
- $\frac{(D - SFC) * 300}{\text{sample volume}}$

- D = sample uptake (initial DO – final DO)
- SFC = average mg/L uptake of the “good” seed bottles, times mL of seed in sample bottle

- Remember:
- If uptake is less than 2.0 for the bottle, don't use that value
- If final DO is less than 1.0, don't use that value
- If dilution set shows non-linear values, don't report the results

- If the BOD values get lower as the sample volume increases, report value from smallest volume(toxicity).
- Set the sample site above up with lower dilutions to verify toxic effect
- Set up matrix spike, and spike dup to meet QC requirements

- Matrix spike can show if sample has an inhibiting effect
- MS/MSD should be evaluated by calculating an RPD

- RPD: Relative Percent Difference

- Absolute value of difference between the dups, divided by the average of the dups…expressed as a percent

QUIZ TIME

- **True or False**
- Consistently high blanks (>0.2 mg/L) can be an indication of poor quality source water (such as distilled water).

- True. Organic material, one cause of high blanks, can be present even after distilling water and also can be leached from deionization columns.

- TRUE or FALSE:
- When a wastewater treatment plant uses ultraviolet light to disinfect effluent, BOD and CBOD samples taken downstream from the disinfection point do not need to be seeded because bacteria in the effluent are not killed by ultraviolet light but rather are unable to reproduce.

- False. Standard Methods 5210B says all samples taken after disinfection must be seeded. Furthermore, unless it is known for certain that sufficient viable bacteria are present in a sample, it must be seeded.

- TRUE or FALSE
- The conversion of ammonia (NH_3) to nitrate (NO_3^-), a process that depletes dissolved oxygen in a sample, does not take place if a nitrification inhibitor is added to the sample in accordance with the method.

- True. This is the basis for the CBOD test and the reason CBOD results should be lower than BOD results for the same sample when the sample includes nitrogen that can be converted to ammonia

- TRUE or FALSE
- The seed control bottle (used to determine the seed correction for seeded samples) must deplete at least 2.0 mg/L of dissolved oxygen to be considered valid.

- True. Standard Methods 5210B says valid bottles must deplete at least 2.0 mg/L DO while leaving a residual DO of at least 1.0 mg/L. It makes no exception for the seed control bottle. (The 21st edition of Standard Methods no longer says the seed control bottle must deplete between 0.60 and 1.00 mg/L of DO.)

- **Multiple Choice**

- **What does the biochemical oxygen demand test measure in a wastewater treatment plant?**
- **A. The concentration of organic material in a waste sample.**
  B. The concentration of organic material and reduced forms of nitrogen (e.g., ammonia) in a waste sample.
  C. The oxygen-depleting capacity of a waste sample.
  D. The dissolved-oxygen concentration of a waste sample.

- c. See Standard Methods Sec. 5210A(1). Concentration of organic material (a) is approximated by the total organic carbon (TOC) test, organic material and reduced forms of nitrogen (b) by the TOC, ammonia and nitrite tests, and dissolved oxygen (d) by the DO test.

- For which test or tests is it acceptable to store dilution water (source water to which nutrients and buffers have been added) in the BOD incubator?
- A. When testing BOD or CBOD.
  B. When testing CBOD, but not BOD.
  C. When testing BOD, but not CBOD.
  D. Only if testing ultimate BOD.

- b. Aging encourages growth of nitrifying bacteria, which would bias BOD and ultimate BOD results. For CBOD analysis, however, nitrifying bacteria are inhibited, so their presence would not have an effect on the test results

- When analyzing effluent BOD from a wastewater treatment plant that uses chlorine disinfection, which of the following reagents is the best choice to ensure excess chlorine is removed before proceeding with the test?
- A. Dilute sulfuric acid.
  B. Dilute sodium hydroxide.
  C. Sodium thiosulfate.
  D. Sodium sulfite.

- d. Sodium sulfite is the best choice. While, sodium thiosulfate and sodium sulfite both remove unwanted chlorine, both also have an oxygen demand that, if added in excess, would bias the BOD result on the high side. Because sodium sulfite's oxygen demand is less than that of sodium thiosulfate, sodium sulfite is the better choice. Sulfuric acid and sodium hydroxide both interfere with the BOD test

- Which of the following options most likely provides the best source water from which to prepare dilution water for a BOD test?
- A. Distilled water put through deionization columns including an activated carbon filter.
  B. Purified drinking water purchased from a drugstore.
  C. Distilled water filtered using a 0.45 micron (e.g., fecal coliform) filter.
  D. Water taken from receiving waters at least 1000 m downstream from a wastewater treatment plant.

- a. Deionization columns including an activated carbon filter that removes impurities that might have made it through the distillation process. Drinking water usually contains additives to give it “taste.” Filtering removes microscopic solid material but not dissolved organic and inorganic material. Water downstream from a wastewater treatment plant (or elsewhere in a stream) would most likely contain materials that would interfere with the BOD test.

- What is the saturation point of dissolved oxygen in water dependent upon?
- A. Temperature, atmospheric pressure, elevation of the lab, and salinity.
  B. Temperature, atmospheric pressure, and salinity.
  C. Temperature and salinity only.
  D. Atmospheric pressure and salinity only

- b. Temperature, atmospheric pressure, and salinity all affect the saturation point of dissolved oxygen in water. Even though the elevation of the lab has an effect on atmospheric pressure, knowing the lab's elevation is not enough, because atmospheric pressure changes from hour to hour and day to day.

- Questions?

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