Biochemical Oxygen Demand

(BOD)

VT WSMD Wastewater Program Lab Manual Section #12

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Biochemical Oxygen Demand

Background

We perform the BOD analysis to determine the amount of oxygen required to biochemically break down the organic material in wastewater and the oxygen used to oxidize inorganic materials such as sulfides and ferrous iron. Unless an inhibitor is used, the BOD test may also measure the amount of oxygen required to convert ammonia to nitrates. This is referred to as the nitrogenous BOD.

In the BOD analysis proper dilutions of samples are prepared. Initial dissolved oxygen readings are then taken. After a five-day incubation period at 20℃ +/- 1℃, Dissolved Oxygen readings are again taken, and calculations are performed based on the difference between these readings and readings taken before incubation.

NOTE: Some operators use the Chemical Oxygen Demand (COD) analysis to estimate the BOD. The ratio of COD to BOD varies greatly between facilities and even within a facility, especially at facilities with varying industries discharging to them. Whereas a BOD analysis depends upon the microbiology (bugs) to break down the organic matter, the COD “burns” organic matter using strong acid(s) and high temperature. The great advantage of this analysis (COD) is that it can be completed in a matter of hours as opposed to the BOD which takes 5 days. What might be considered a disadvantage is that the COD, by nature of “burning” all organics in a short period of time, includes organic materials (such as many solvents) that would not be metabolized (broken down) in the 5-day period allowed for in the BOD analysis. Further, by “burning” all organics in such a short period, the analysis does not allow for the nitrogenous demand that would be observed in a 5-day BOD. Even accounting for these inherent differences, the COD results can be obtained in an hour and can be a good indicator of BOD results that aren’t available for 5 days. Further information regarding the analysis of Chemical Oxygen Demand can be found in section 5220 of the 23rd edition of Standard Methods for Analysis of Water and Wastewater

Or at this link from the Hach® company: DOC312.53.94012_1Ed_LCK314 (1).pdf

BOD Sampling and Preservation

Samples for analysis of BOD are generally required to be collected as 24-hour composites (a sample composed of a number of discrete grab samples collected over the entire sampling period). The composite samples should be properly flow proportioned (representative of the volume and nature of the discharge over the sampling period). Some Vermont facilities such as lagoons and industries that operate only during a single shift, are allowed by permit to collect 8-hour composites. The same rules apply to this sample (i.e. must be properly flow proportioned based on an 8-hour sampling period.) Flow proportioning is required if the hourly flow varies by more than 10% over the sampling period.

If, for process control purposes, analysis of a grab sample is desired, this sample does not need to be refrigerated if analyzed within 2 hours. Samples collected for analysis per the facility’s NPDES permit. MUST be maintained at a temperature at or below 6 °C, but not freezing, for the entire sampling period and until the analysis is begun.

If using an automatic sampler for collection of a composite sample the unit must be maintained at 6 °C during the entire composite period.

If manually taking the discrete samples for a composite, each discrete sample must be maintained at 6 °C until compositing takes place. The composite must then be kept at 6 °C until analysis takes place.

The BOD analysis must begin no more than 48 hours from the time the last discrete sample is collected. It is preferable to perform the analysis within six hours from the time the last sample is collected.

Just before analysis, the sample must be warmed to 20 °C. This can be accomplished by placing the refrigerated sample container into a sink or other basin partially filled with warm water to bring the temperature up fairly quickly but not so fast as to adversely affect the nature of the sample (approximately 15 minutes). The sample **MUST** be brought to 20 °C +/- 2 °C before beginning the analysis.

Equipment

- **Air Pump**. Used as a means of aerating the dilution water. Shaking also works depending on the volume of dilution water required. (It's difficult to shake 10 gallons of dilution water!)
- **Beakers, Assorted Sizes**. Always pour reagents into small beakers before pipetting to avoid contamination of the entire reagent bottle.
- **Carboy**. To contain the dilution water. The size needed depends on the number of samples you expect to run. We attach a rubber hose to this for delivery of the dilution water into the cylinders.
- **Dissolved Oxygen Bottles**. These bottles must have a flared mouth with ground glass stoppers and are generally 300 mL in volume.
- **Dissolved Oxygen Meter**. With a stirrer type probe, specially designed to fit into D.O. bottles. Alternatively, a magnetic stirrer can be used but this necessitates the removal of the magnetic bar before incubation or disposal.
- **Filter**. Used as a means of aerating the dilution water.
- **Graduated or volumetric Cylinders, Assorted Sizes**. For measuring the sample, etc.
- **Incubator**. The incubator must be capable of maintaining a constant temperature of 20 °C (plus or minus just 1 °C).
- **Mixer**. The Propeller type mixer can be used to mix the sample just before delivering into the D.O. bottles.
- **Overcaps**. Placed over the flared mouth of the D.O. bottles to reduce evaporation of the water seal during incubation.
- **pH Meter**. The pH of the sample must be 6.5 to 7.5.
- **Pipette**. For measuring small volumes of sample and reagents.
- **Pipette Bulb**. For drawing reagents or sample into pipette. Never mouth pipette.
- **Refrigerator**. Capable of maintaining 6 °C. For storage of reagents and sample.
- **Stand**. A stand or shelf used to set carboy on for elevation, which is helpful in delivering the dilution water into the BOD bottles.
- **Thermometer**. Thermometers must be NIST traceable or annually calibrated against either an NIST or NIST traceable thermometer.
- **Tubing**. Used as a means of aerating the dilution water.

NOTE: The following four equipment pieces are necessary if using the Iodometric Method for either D.O. measurement or meter standardization.

- **Burette and Burette Stand**. A 25 mL burette with 0.1 mL graduations is used for titration of the sodium thiosulfate solution.
- **Dissolved Oxygen Bottles With Auto Pipettes**. These are used to store the reagents for D.O. analysis. The auto pipettes can be set to consistently deliver 1 mL of each of the reagents.
- **Dropping Bottles**. These bottles with dropper caps are handy for storing the starch and back titrant solutions.
- **Erlenmeyer Flasks**. 500 mL wide mouth flasks are used during the titration process.

Reagents

NOTE: For accuracy, Class A volumetric flasks/pipettes must be used when making reagents.

The phosphate buffer, magnesium sulfate, calcium chloride and ferric chloride solutions listed below can be purchased in premade/combined powder pillows or reagent packets. Use of these pre-made reagents is highly recommended.

- Phosphate Buffer Solution:
  Dissolve 8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4 \cdot 7H_2O$ and 1.7 g NH_4Cl in about 500 mL distilled water and dilute to 1 L. The pH should be 7.2 without further adjustment. Discard reagent (or any of the following reagents) if there is any sign of biological growth in the stock bottle.
- Magnesium Sulfate Solution:
  Dissolve 22.5 g $MgSO_4 \cdot 7H_2O$ in distilled water and dilute to 1 L.
- Calcium Chloride Solution:
  Dissolve 27.5 g $CaCl_2$ in distilled water and dilute to 1 L.
- Ferric Chloride Solution:
  Dissolve 0.25 g $FeCl_3 \cdot 6H_2O$ in distilled water and dilute to 1 L.
- Acid and Alkali Solutions, 1N:
  For neutralization of caustic or acidic water samples.
  - Acid - Slowly and while stirring, add 28 mL conc sulfuric acid to distilled water. Dilute to 1 L.
  - Alkali - Dissolve 40 g sodium hydroxide in distilled water. Dilute to 1 L.
- Sodium Sulfite Solution, 0.025N:
  Dissolve 1.575 g Na_2SO_3 in distilled water and dilute to 1 liter (or 0.788 g to 500 mL). This solution is not stable; prepare daily.
- Nitrification Inhibitor, 2-Chloro-6-(Trichloro methyl) pyridine:
  This can be purchased from the Hach Chemical Company or other laboratory supply vendors.
- Glucose-Glutamic Acid Solution:
  Dry reagent-grade glucose and reagent-grade glutamic acid at 103 °C for 1 h. Add 150 mg glucose and 150 mg glutamic acid to distilled water and dilute to 1 L. Prepare fresh immediately before use. This can be purchased pre-made. Glucose-Glutamic Acid (GGA) may be purchased in ampules.

- Ammonium Chloride Solution:
  Dissolve 1.15 g NH_4Cl in about 500 mL distilled water, adjust pH to 7.2 with NaOH solution, and dilute to 1 L. Solution contains 0.3 mg N/mL.

If performing the Winkler Titration Method for determination of initial and final Dissolved Oxygen readings or for standardizing the D.O. meter, all reagents listed under the heading in Section 11 on Pages 14 - 15 are needed.

Dilution Water Preparation

Dilution water consists of high-quality distilled water to which we add nutrients and a buffering solution (to optimize the conditions for bacteriological growth). The use of deionized water is not recommended as organic compounds can leach through from the resins. It is extremely important that the oxygen depletion of the dilution water is 0.2 mg/L or less. Several suggestions for producing dilution water of this quality are offered in the 23rd Edition of Standard Methods.

To the carboy containing distilled water add one milliliter each of calcium chloride, magnesium sulfate, ferric chloride, and phosphate buffer solutions per liter of distilled water, then aerate using the air pump, filter, and tubing. Alternatively, powder pillows/packets prepared for specific dilution water volumes can be used.

Take the samples out of the refrigerator and begin the process of bringing the sample temperature up to 20°C. Place the carboy in an elevated location to facilitate draining.

While the sample is warming up, turn on the D.O. meter, prepare the G.G.A. and sodium sulfite solutions, decide what dilutions of sample to use, and set up and label the glassware to be used.

Dissolved Oxygen Meter Standardization

Please refer to the Dissolved Oxygen Section of this manual for instructions describing meter standardization methods

Sample Pretreatment

pH

The pH of the sample must be between 6.5 and 7.5. Samples can be neutralized using either sulfuric acid or sodium hydroxide.

Temperature

The sample temperature must be brought up to 20 °C before beginning the analysis.

DO NOT BEGIN THE ANALYSIS UNTIL THE SAMPLE HAS WARMED TO 20 °C.

Dechlorination

The dechlorination procedure consists of adding Sodium Sulfite in sufficient quantity to destroy the residual chlorine. NOTE: If the chlorine residual is less than 0.5 mg/Liter it might be possible to remove

the residual by shaking the sample, then removing the cover to allow the gas to escape. Repeat until the residual is removed. **Addition of Sodium Sulfite solution should be avoided if possible because this solution acts as an oxygen scavenger and if too much is added to the sample it can significantly increase the oxygen demand in the sample!**

> **NOTE:** Untreated dairy effluent samples often contain high levels of chlorine from equipment cleaning practices. Always check these samples for chlorine residual and pH before proceeding. These samples should also be seeded.

The method of dechlorination is as follows:

To a 100 mL volume of sample add 1 mL of 1:1 acetic acid or 1 mL of 1:50 sulfuric acid (1:50 means 1 part sulfuric acid to 50 parts distilled water). Add 1 mL of potassium iodide solution (10 g/100 mL) and 2 to 3 drops of starch indicator solution. Titrate with 0.025N Sodium Sulfite solution until the colorless end point is reached. Record this amount of solution used in the titration. Then add this amount of sodium sulfite solution per 100 mL of sample remaining. Let it set for 10 to 20 minutes and recheck for chlorine residual.

Review

At this point the following tasks have been performed:

1) Sample warmed to 20 °C.
2) pH adjusted to 6.5 to 7.5.
3) Sodium sulfite solution prepared (if dechlorination is necessary).
4) Sample dechlorinated if necessary.
5) GGA solution prepared. (powder pillow/packet can be used)
6) Glassware labeled.
7) D.O. meter standardized.

Sample Dilution

This chart can be used as a rough guide to proper dilutions. Once you become familiar with the procedure and the quality of samples analyzed, the dilutions used can be adjusted to more closely correspond to your sample requirements.

Choose the proper dilutions for your samples. As a guide:

SAMPLE TYPE		DILUTION	BOD mg/L
Dilution Water	=	100%	
Good Secondary Effluent	=	10%, 20%, 50%	4 - 75
Poor Secondary Effluent	=	5%, 10%, 20%	10 - 150
Very Poor Secondary or Primary Effluent	=	2%, 5%, 10%	20 - 375
Influents	=	1%, 2%, 5%	40 - 750
GGA	=	2%	198 +/- 35
Dairy Wastes	=	.01, .03, .1, .3, 1% (depending on the strength)	200 - 75,000
Meat Packing Wastes	=	.2, .7, 2%	100 - 750
Paper Wastes, Untreated	=	1%, 2%, 5%	40 - 750
Paper Wastes, Treated	=	2%, 5%, 10%	20 - 275

We strongly suggest that you always use three dilutions for each sample. By doing this you reduce the likelihood of not producing the required oxygen **depletion** of at 2.0 mg/L (the oxygen depletion of the sample after the five-day incubation period MUST be at least 2.0 mg/L or the results are considered invalid. Also the oxygen **residual** at the end of the five-day incubation period MUST be at least 1 mg/L). This is referred to as the **R1-D2 rule**. It is very important that this requirement be met. The terms depletion and residual are defined below.

Depletion

Refers to the amount of oxygen "used up" during the incubation period. To determine the depletion, we simply subtract the average of the two final D.O. readings from the initial D.O. reading.

Example:	Initial D.O.	= 8.5 mg/L
	Average Final D.O.	= 5.5 mg/L
	Depletion	= 8.5 - 5.5 mg/L
	Depletion	= 3.0 mg/L

Residual

Refers to the amount of dissolved oxygen remaining in the sample at the end of the incubation period. In other words, Final D.O. = Residual.

Sample Dilution

Assume a fairly good quality secondary effluent will be used as a sample with sample concentrations of 20, 60 and 90%.

Using 300 milliliters as the total volume 60, 180 and 270 milliliters of sample are required respectively.

The **blank** consists of dilution water only. Simply fill a 300 milliliter BOD bottle with dilution water. Be careful not to entrain air. Dispense the dilution water slowly along the inside of the glass.

Next, measure the 60 mL sample using a 100 mL graduated pipette or graduated cylinder. **Remember to shake the sample first.** Slowly dispense the sample into the BOD bottle by touching the pipette to the inside of the bottle and allowing the sample to run down the inside of the glass to avoid air entrainment. If using a graduated cylinder pour the sample volume slowly down the inside of the glass.

NOTE: When using small quantities of sample it's a good idea to add dilution water to each of the cylinders before adding sample.

Measure and dispense the 180 and 270 milliliter samples in the same manner. It is always best to use the measuring device that is the closest in volume to the desired volume of sample. For the 180 milliliter sample a 200 milliliter graduated cylinder would be preferable while it might be necessary to use a 500 milliliter cylinder for the 270 milliliter sample. Ideally, an auto pipette capable of delivering the exact volume desired should be used.

Seeded Samples

The SEED is added next. The purpose of the seed is to ensure that a sufficient number of microorganisms is present to oxidize the biodegradable organic matter in the sample. The seed must be used if the sample has been disinfected or is comprised of dairy wastewater.

Primary effluent, or settled influent, is commonly used as seed. A commercially prepared seed can also be used. The same volume of seed is added to each of the sample dilutions and the 2% GGA solution.

The seed volume is determined by using this formula for 300 milliliter BOD bottle.

0.8 divided by the expected seed BOD multiplied by the total bottle volume (300 mL) = mL of seed

0.8 is used because it is the average of the acceptable range of seed contribution (0.6 – 1.0).

EXAMPLE:

If the influent being used as a seed usually produces a BOD of 250 mg/L we would divide 0.8 by 250 and then multiply this number by the total bottle volume (300 mL).

$$\frac{0.8}{250\frac{mg}{L}} * 300\ mL = 0.96$$

We would round this number off to 1 and use 1 mL of seed.

Add the amount of seed calculated as in the example above, to each of the dilutions of the effluent sample and to the 2% GGA solution.

Next, prepare the seed sample dilutions. If we are using settled influent as seed, we might use the typical influent dilutions of 1%, 2% and 5%. To prepare these dilutions we would measure out 3, 6 and 15 milliliters of settled influent respectively and add these volumes to the appropriate bottles. Then fill each bottle into the neck with dilution water.

Replace the ground glass stoppers.

> **NOTE:** If nitrification inhibition of the effluent sample is desired, for consistency, the inhibitor is added to each of the "blank" bottles as well as to each of the effluent sample bottles. Or if desired a separate blank containing trichloromethyl pyridine (TCMP) (may be set up along with the "uninhibited" blanks. The U.S. EPA allows use of nitrification inhibitor only if the facility's NPDES permit specifically requires Carbonaceous Biochemical Oxygen Demand (CBOD).

Determination of the Initial D.O.

1) Before beginning to read initial D.O.'s, we should once again check the meter reading of the distilled water bottle set aside. It should be very close to the original reading.
2) Now after dumping the excess liquid in the well, we simply remove the cap of the first of the two "blank" bottles. Place the D.O. probe into the bottle, being careful not to entrap air in doing so. Turn on the stirrer.
3) Check for air bubbles in the sample. If there are no air bubbles record the reading If there are air bubbles, reinsert the probe carefully eliminating all air.
4) Place the plastic cover over each of the two bottles and place them into the box to be incubated.
5) Repeat this procedure for the remaining dilutions of effluent, seed and GGA.
6) Always move from the least concentrated to most concentrated solutions to reduce the chances for contamination.
7) If necessary, fill the bottle “well” (the portion between the glass stopper and the flared mouth) with dilution water. This helps ensure the airtight seal. Then place the plastic cap over the bottle
8) Bottles are to be incubated at 20 °C plus or minus 1 °C, for five days.

Determination of the Final D.O.

After five days (±3 hours) of incubation, Dissolved Oxygen remaining in the bottles is determined. Again, the meter is standardized. Remove the bottles from the incubator. Check each bottle for air bubbles. Discard those bottles which contain bubbles. Determine the residual D.O.'s of the remaining bottles. D.O. residuals of each dilution are read and recorded on the bench sheet. The D.O. results of the two bottles used for averaging the final D.O. should not vary by more than 0.5 mg/L. Large variations in D.O. readings between these bottles containing the same concentration of sample are indicative of either contamination or use of a non-homogenous sample.

Blank Depletion

The D.O. depletion of the blank after the five-day incubation period should not exceed 0.2 mg/L. There is no "Blank Correction" calculation. If the Blank demonstrates a depletion of greater than 0.2 mg/L, the cause of the problem must be identified and corrected. Only the highest quality distilled water should be used when making up the dilution water. Sources of contamination must be eliminated.

Calculation of Unseeded BOD

When no seed has been used, the BOD calculation is simply:

$$BOD\ \left(\frac{mg}{L}\right) = \frac{\left(Initial\ DO\ \left(\frac{mg}{L}\right) - Final\ DO\ \left(\frac{mg}{L}\right)\right) * BOD\ Bottle\ Volume\ (mL)}{Sample\ Volume\ (mL)}$$

EXAMPLE: A 30 milliliter concentration of sample diluted in a 300 mL BOD Bottle yields these results:

- Initial D.O. = 8.5 mg/L
- Average Final D.O. = 6.0 mg/L

$$BOD\ \left(\frac{mg}{L}\right) = \frac{\left(Initial\ DO\ \left(\frac{mg}{L}\right) - Final\ DO\ \left(\frac{mg}{L}\right)\right) * 300\ mL}{Sample\ Volume\ (mL)}$$

$$BOD\ \left(\frac{mg}{L}\right) = \frac{\left(8.5\frac{mg}{L} - 6.0\frac{mg}{L}\right) * 300\ mL}{30\ mL}$$

$$BOD\ \left(\frac{mg}{L}\right) = \frac{2.5\frac{mg}{L} * 300\ mL}{30\ mL}$$

$$BOD\ \left(\frac{mg}{L}\right) = 25\frac{mg}{L}$$

Don't forget the R1-D2 rule!! Let's try another example where the rule applies.

A BOD sample yields the following results:

Sample volume (mL)	Initial D.O. mg/L	Average Final D.O. mg/L	Depletion mg/L
30	8.0	7.0	1.0
60	8.0	6.0	2.0
150	8.0	3.0	5.0

In this example we must first calculate the results for each concentration that fits the rule and then average these results.

The 30 mL. sample is not included in the calculation because the DEPLETION is less than 2.0.

The result of the 60 mL. sample is (8-6) * 300 / 60 = 2mg/L x 300 mL / 60mL = 10 mg/L

The result of the 150 mL. sample is (8-3) * 300 / 150 = 5 x 300 / 150 = 10 mg/L

The average of the two is 10 mg/L so we would report the BOD as 10 mg/L.

Calculation of Seeded BOD

The calculation of seeded BOD's is a bit tougher. In Vermont, we require that the BOD of the seed be established in the same manner as the sample (recommended three dilutions). Therefore, we calculate the BOD of the seed material using the calculation we just described. We use these results to calculate the seed correction factor which is then applied to the overall BOD calculation of the effluent sample.

The actual BOD calculation for a seeded sample is:

$$BOD\left(\frac{mg}{L}\right) = \frac{\left(\left(I.D.O.\left(\frac{mg}{L}\right) - F.D.O.\left(\frac{mg}{L}\right)\right) - Seed\ Correction\ Factor\right) * BOD\ Bottle\ (mL)}{Sample\ Volume\ (mL)}$$

Where:

- I.D.O. = Initial Dissolved Oxygen reading before incubation.
- F.D.O. = Final Dissolved Oxygen after incubation
- Seed Correction Factor = $\frac{Actual\ Seed\ BOD\left(\frac{mg}{L}\right) * Seed\ Used\ (mL)}{Total\ Volume\ of\ Sample\ After\ Dilution\ (mL)}$
- Since dilutions are being made in 300 mL BOD bottles we would use 300 mL as a total volume.

The BOD calculation for a seeded BOD can be performed in four steps.

1) Calculate the depletion (Initial minus the average final D.O.'s.)

EXAMPLE: If we had an average initial D.O. reading of 8.6 mg/L and an average final D.O. reading of 5.6 our answer would be 3.0 mg/L. We get this answer by subtracting 5.6mg/L, from the initial D.O. reading of 8.6mg/L.

2) Calculate the Seed BOD. Since the seed is essentially an unchlorinated influent sample we use the calculation for unseeded BOD's:

NOTE: We must actually physically determine the seed BOD by running a BOD analysis of the seed material itself along with the whatever sample we are analyzing. This number cannot just be estimated or represent a BOD run on the seed material a month ago!!

$$Seed\ BOD\ \left(\frac{mg}{L}\right) = \frac{\left(I.D.O.\left(\frac{mg}{L}\right) - F.D.O.\left(\frac{mg}{L}\right)\right) * 300\ mL}{Sample\ Volume\ (mL)}$$

EXAMPLE: Using a 3 mL volume of influent sample (this influent will serve as our seed material) if our average Initial D.O. reading was 8.5mg/L and our average final D.O. reading was 5.5 mg/L our Seed BOD would be 300mg/L. We arrive at this number by applying the formula:

$$Seed\ BOD\ \left(\frac{mg}{L}\right) = \frac{\left(8.5\left(\frac{mg}{L}\right) - 5.5\ \left(\frac{mg}{L}\right)\right) * 300\ mL}{3\ mL}$$

$$Seed\ BOD\ \left(\frac{mg}{L}\right) = \frac{3\frac{mg}{L} * 300\ mL}{3\ mL}$$

$$Seed\ BOD\ \left(\frac{mg}{L}\right) = \frac{900\ \frac{mg * mL}{L}}{3\ mL}$$

$$Seed\ BOD\ \left(\frac{mg}{L}\right) = 300\frac{mg}{L}$$

***Note:** The (mL) cancel out in the equation leaving us with only mg/L in the final answer

Seed BOD = 300 mg/L

3) Perform the calculation for the Seed Contribution. For our example assume that we used 1 mL of seed in each dilution of sample:

$$Seed\ Contribution\ Factor = \frac{\left(Seed\ BOD\ \left(\frac{mg}{L}\right) * Seed\ Used\ (mL)\right)}{Total\ Volume\ of\ Sample\ Dilution\ (mL)}$$

$$Seed\ Contribution\ Factor = \frac{\left(300\frac{mg}{L} * 1\ mL\right)}{300\ mL} = 1.0\frac{mg}{L}$$

***Important Note:** The seed contribution should be between 0.6 and 1.0 mg/L

4) Plug these numbers into the equation.
Following our example above, we have a 30 mL sample, with an initial D.O. of 8.6 and an average final D.O. of 4.6. Our seed correction factor as calculated in Step 4 above is 1.0.

$$BOD\ \left(\frac{mg}{L}\right) = \frac{\left(\left(I.D.O.\left(\frac{mg}{L}\right) - \ F.D.O.\left(\frac{mg}{L}\right)\right) - Seed\ Correction\ Factor\right) * 300\ mL}{Sample\ Volume\ (mL)}$$

$$BOD\ \left(\frac{mg}{L}\right) = \frac{\left(\left(8.6\frac{mg}{L} - 4.6\frac{mg}{L}\right)\ - 1.0\frac{mg}{L}\right) * 300\ mL}{30\ mL}$$

$$BOD\ \left(\frac{mg}{L}\right) = \frac{\left(4.0\frac{mg}{L}\ - 1.0\frac{mg}{L}\right) * 300\ mL}{30\ mL}$$

$$BOD\ \left(\frac{mg}{L}\right) = \frac{3.0\frac{mg}{L} * 300\ mL}{30\ mL}$$

$$BOD\ \left(\frac{mg}{L}\right) = \frac{900\ \frac{mg * mL}{L}}{30\ mL}$$

$$BOD\ \left(\frac{mg}{L}\right) = \ 30\frac{mg}{L}$$

The other effluent dilutions which meet the R1-D2 rule would then be calculated using the procedure shown above and the results averaged to determine the final BOD result to be reported.

*__Note__: BODs should be reported only to the nearest whole number 30mg/L NOT 30.02mg/L or 29.98mg/L

Perform a BOD of the Glucose Glutamic Acid (GGA) and Calculate the BOD of the solution

The GGA is used to check on possible dilution water toxicants and seed source reliability. For example, distilled water could be contaminated by copper causing the seed to be relatively inactive. These factors can often yield inaccurate lower BOD results. Therefore, dilution water quality, seed reliability and analytical technique needs to be checked by measuring BOD on pure organic compounds.

A 2% dilution (6 mL) of GGA is prepared the same as any sample. The 2% GGA solution is seeded with the same volume of seed used in all the samples dilutions.

This seeded 2% glucose-glutamic acid solution should yield a BOD in the range of 198+/- 30 mg/L.

Note: If the BOD of the GGA is slightly below the desired range this suggests that perhaps insufficient seed was used. Consider increasing the volume of seed used in future analyses. Conversely, if the GGA reading is slightly higher than the desired range consider using less seed in future analyses. If the BOD of the GGA is significantly outside the desired range, it is important to find the possible sources of error, correct the problems and perform the analysis again. Remember to calculate the GGA BOD using the "seeded BOD" formula.

Remember the R1-D2 rule!! Remember that the seed contribution should be 0.6-1.0 mg/L

Good Luck!

- **Remember, if samples are sent to another lab for BOD analysis it is still the responsibility of the permittee to ensure that the results are acceptable.**
    - **Be sure to read the lab results carefully and make sure all the rules have been satisfied. (all temps, initial DO, R1-D2, blank depletion, seed contribution 0.6-1, etc.)**
    - **The Permittee needs to resolve the situation with the lab and, if there is uncertainty about permit compliance, resampling may be needed. Coordinate with the permitting analyst for your facility to determine the next step forward.**
    - **Applicable NODI codes should be used if the facility does not have reliable data to report.**

SAMPLE BENCH SHEET #1

FACILITY: Maple City
SAMPLING: **DATE:** 02/15/2021 **COLLECTED BY:** AB
CHLORINATED: No **Other Disinfection:** None
Composite: 1/hour **Start Time:** 7 a.m. **Duration:** 8 hours **Grab Sample**__________ **Time**________

In to incubator: Date: 02/16/2021 **Time** 11 a.m. **Temp.** 20.5C **Analyst** CD **Seed Used** None

Out of incubator: Date: 02/21/2021 **Time** 1 p.m. **Temp.** 20C **Analyst** AB

D.O. VALUES

Sample	1 sample volume	2 Bottles Numbers	3 Initial	4 Residual	5 Average Residual	6 Depletion	7 Seed Depl.	8 Corrected Depletion	9 BOD mg/L	10 Final BOD
Blank	300 mL	1,2,3	8.50/8.50	8.40/8.40	8.40	0.10			----	
GGA	6 mL	4,5,6	8.55/8.55	4.30/4.40	4.35	4.20			210	
Inf	3 mL	10,15,18	8.50/8.50	5.90/6.10	6.00	2.50			250	
Inf	6 mL	19,20,21	8.45/8.45	2.40/2.50	2.45	6.00			300	
Inf	15 mL	25,28,30	8.50/8.50	0.80/0.50	0.65 (R<1)					
Eff	30 mL	31,32,33	8.45/8.45	8.40/8.40	8.40	0.05 (D<2)				
Eff	60 mL	34,35,36	8.55/8.55	7.10/7.00	7.05	1.50 (D<2)				
Eff	150 mL	37,38,39	8.55/8.55	4.55/5.45	5.50	3.05			6.1	
Eff Dup	30 mL	40,43,44	8.50/8.50	8.45/8.50	8.48	0.02 (D<2)				
Eff Dup	60 mL	46,48,50	8.55/8.55	7.20/7.10	7.15	1.40 (D<2)				
Eff Dup	150 mL	100,98,96	8.55/8.55	4.60/4.60	4.60	3.95			7.9	

(INCLUDE ACTUAL CALCULATIONS ON REVERSE SIDE)

CALCULATIONS: (To accompany bench sheet)

- Influent 3 mL (8.5mg/L – 6mg/L) *300mL / 3 mL = 250 mg/L
- Influent 6 mL (8.45mg/L – 2.45mg/L) *300 mL /6mL = 300 mg/L
- **Average BOD** = 250mg/L + 300 mg/L /2 **= 275 mg/L**
- Influent 15 mL ---> Average residual less than 1
- Effluent 30 mL ---> Depletion less than 2
- Effluent 60 mL ---> Depletion less than 2
- Effluent 150 mL ---> (8.55mg/L – 3.50 mg/L) * 300mL / 150 mL = 6.1mg/L
- Effluent Duplicate 30 mL ---> Depletion less than 2
- Effluent Duplicate 60 mL ---> Depletion less than 2
- Effluent Duplicate 150 mL ---> (8.55mg/L – 4.60mg/L) * 300mL /150 mL = 7.9 mg/L
- **Average BOD** = (6.1mg/L + 7.9mg/L) / 2 = **7 mg/L**

SAMPLE BENCH SHEET #2

FACILITY: Maple City
SAMPLING: **DATE:** 02/15/2021 **COLLECTED BY:** AB
CHLORINATED: No **Other Disinfection:** Ultraviolet
Composite: 1/hour **Start Time:** 7 a.m. **Duration:** 8 hours **Grab Sample**__________ **Time**________
Into incubator: Date: 02/16/2021 **Time** 11 a.m. **Temp.** 20.5C **Analyst** CD **Seed Used** 1 mL Primary Effluent
Out of incubator: Date: 02/21/2021 **Time** 1 p.m. **Temp.** 20C **Analyst** AB

D.O. VALUES

Sample	1 Dilution/ sample volume	2 Bottles Numbers	3 Initial	4 Residual	5 Average Residual	6 Depletion	7 Seed Depl.	8 Corrected Depletion	9 BOD mg/L	10 Final BOD
Blank	100%/300 mL	1,2,3	8.50/8.50	8.40/8.40	8.40	0.10			---	
GGA	2%/6 mL	4,5,6	8.55/8.55	4.00/4.00	4.00	4.55	0.825	3.725	186.25	186
Seed	1%/3 mL	10,15,18	8.50/8.50	5.90/6.10	6.00	2.50			250	
Seed	2%/6 mL	19,20,21	8.45/8.45	2.40/2.50	2.45	6.00			300	
Seed	5%/15 mL	25,28,30	8.50/8.50	0.80/0.50	0.65(R<1)					
Eff	10%/30 mL	31,32,33	8.45/8.45	8.40/8.40	8.40	0.05(D<2)				
Eff	20%/60 mL	34,35,36	8.55/8.55	7.10/7.00	7.05	1.50(D<2)				
Eff	50%/150 mL	37,38,39	8.55/8.55	5.55/5.45	5.50	3.05	0.825	2.225	4.45	
Eff Dup	10%/30 mL	40,43,44	8.50/8.50	8.45/8.50	8.48	0.02(D<2)				
Eff Dup	20%/60 mL	46,48,50	8.55/8.55	7.20/7.10	7.15	1.40(D<2)				
Eff Dup	50%/150 mL	100,98,96	8.55/8.55	4.60/4.60	4.60	3.95	0.825	3.125	6.25	

(INCLUDE ACTUAL CALCULATIONS ON REVERSE SIDE)

CALCULATIONS FOR SEEDED BOD

Seed 3mL (8.5mg/L – 6.0mg/L) * 300mL / 3 mL = 250 mg/L

Seed BOD = 250 + 300 = 275 mg/L
2

Seed 6 mL 8.45mg/L - 2.45mg/L * 300mL / 6mL = 300mg/L

Seed 15 mL ---> Average residual less than 1

Seed Correction/Contribution = 275mg/L x 1mL = .9 mg/L

300mL

Effluent 30 mL ---> Depletion less than 2

Effluent 60 mL ---> Depletion less than 2

Effluent 150 mL---> ((8.55 mg/L – 5.5 mg/L) –(0.9mg/L)) *300mL /150 mL = 4.3 mg/L

Effluent Duplicate 30 mL ---> Depletion less than 2

Effluent Duplicate 60 mL ---> Depletion less than 2

Effluent Duplicate 150 mL ---> ((8.55mg/L - 4.6mg/L) – (0.9mg/L)) *300mL /150 mL = 6.1mg/L

Avg Effluent and Effluent duplicate = 4.3mg/L + 6.1mg/L / 2 = **5.2mg/L**

Biochemical Oxygen Demand Troubleshooting Guide

PROBLEM	LIKELY CAUSE	SOLUTION
BLANK DEPLETION (unseeded dilution water) is greater than the allowable .2 mg/L.	Contamination of BOD bottles, carboy, or syphon.	Clean bottles, carboy and syphon thoroughly with hot 2% Micro solution. Rinse thoroughly with hot tap water followed by distilled water rinses. If the problem persists use chromic acid to clean the bottles, followed by several rinses of hot tap water and finally several distilled water rinses. Use an appropriately sized brush to aid in thorough cleaning.
	Contaminated distilled water.	Thoroughly clean the distilled water storage tank. Place a 0.4-micron organic filter at the end of the system, especially if deionizing resins are used. Check the conductivity of the distilled water. If >1.0. Find another source of distilled water until the problem can be eliminated or as a last resort an attempt can be made to oxidize the contaminating materials by adding a small amount of seed material (.5 mL) to the distilled water 5-7 days before the dilution water is prepared.
	Contaminated nutrients used to make up the dilution water.	If the nutrients, especially the phosphate buffer, are over 1 year old or show any sign of contamination discard and prepare new solutions.

PROBLEM	**LIKELY CAUSE**	**SOLUTION**
BLANK DEPLETION (unseeded dilution water) is greater than the allowable .2 mg/L.	Initial D.O. of dilution water as >9.2 mg/L.	Never begin the BOD analysis if the D.O. of the dilution water or samples is greater than 9.2 mg/L. The ideal range if 7.5-8.5 mg/L. The D.O. can be reduced by shaking or aerating the dilution water then letting it set for 1/2 hour before use. Recheck the D.O. at this point. Be sure the dilution water is 20 °C before beginning the analysis.
	D.O. meter improperly calibrated.	Check calibration method. Check D.O. probe. Make sure membrane is in good shape--no bubbles under membrane and fresh filling solution.
All dilution of sample produce less than the required D.O. depletion of 2 mg/L.	Sample concentrations too low.	Increase sample concentration.
	Toxicity.	Check sample for chlorine residual and pH extreme. Remove chlorine and/or adjust pH if necessary and properly seed the sample.
All dilution of sample product less than the required residual D.O. of 1 mg/L.	Sample concentration too high.	Decrease sample concentration.
Bubbles appear in BOD bottles after incubation.	Supersaturated samples -- Initial D.O. was >9.2 mg/L. Temperature of sample was less than 20 °C.	D.O. not begin analysis until D.O. sample is <9.2. Ideal range = 7.5-8.5 mg/L.

PROBLEM	LIKELY CAUSE	SOLUTION
Reverse depletion. The depletion of the more concentrated sample is less than the depletion of the least concentrated sample.	Toxicity.	Check sample or seed for pH (should be between 6-8) adjust if necessary. Check sample or seed for chlorine residual and dechlorinate as necessary using sodium sulfite solution.

Quality Control for Biochemical Oxygen Demand: Standard Method #5210-B

Document the Following (Including by not limited to):

Sample Collection

- Grab - Exact Time
- Composite - Duration - sampling start and stop times
- Flow Proportioning -
  - Include:
    - Proportioning Factor
      - Example: 100 mL/1000 gallons
    - Flow Measuring Device
    - Calibration
- Collection Device
- Refrigeration of Sample - during and after sampling
- Location
- Representative Nature - Volume required

Hold Time

- Preferably 6 hours (at 4 °C) - max 48 hours after last discrete sample is collected
  - Sample line and container **clean** - no residual organics
  - Mix sample thoroughly before pouring off for composite **BUT** don't agitate excessively.

Glassware Preparation/Sampling Equipment

- Must be scrupulously clean
  - No residue
  - No dust
  - Well rinsed (5-10 times) with distilled H_2O.
- BOD Bottles - airtight, ground glass stoppers.

Reagents

- Distilled water for dilution water preparation: **Deionized water is not recommended**
  - No copper
  - Conductivity <1
- Preparation and expiration dates for all reagents.
- NIST Traceability
- GGA - MUST be made the day the analysis is performed
- Sodium Sulfite solution for neutralization of chlorine must be made the day the analysis is performed.

Equipment

- Properly calibrated D.O. meter with stirrer equipped probe (or magnetic stirrer) Incubator 20 °C ±1 °C - **DARK!** - checked every 4 hours

Sample Preparation - Before beginning analysis!

- Sample and Dilution water must be 20 °C or slightly higher
- Sample and Dilution water DO must be ≤ 9.2 mg/L (preferably less than 9.0 mg/L)

Validity of Results

- Blank - Depletion ≤0.2 mg/L
- Sample - Residual D.O. ≥ 1 mg/L
- Sample - D.O. Depletion ≥ 2 mg/L
- GGA - = 200 ± 37 mg/L
- Seed BOD - must be analyzed not calculated
- Seed correction factor - calculation must be recorded

Duplication Schedule

- Minimum 5% duplication 10% replication
- GGA - minimum 10%
- Seeded Sample - GGA minimum 10%

Recommended Control Limits

- Perform minimum of 20 GGA checks over 6 to 12 month period.
- Calculate mean and standard deviation
- Perform minimum of 10% GGA checks after that with results remaining within one standard deviation of mean.

References

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Annual Book of ASTM Standards, Part 31 Water. Published by the American Society of Testing and Materials, 1916 Race Street, Philadelphia, Pennsylvania 19103.

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