Chapter 12
Biological Oxygen Demand

Application

The BOD (Biochemical Oxygen Demand) test represents the amount of biochemically digestible organics (food). The test is performed on wastewater and other contaminated samples for various reasons:

1. To satisfy the requirements of an NPDES permit.
The monthly average for BOD in the effluent of a WWTP with a NPDES permit usually cannot exceed 30 mg/l.

2. To evaluate the effectiveness of a treatment unit or process.
Performing the BOD test on the influent and effluent of a primary clarifier, the activated sludge process, or the full wastewater treatment plant can indicate removal efficiency.

3. To calculate the organic loadings (amount of food) on a treatment plant or specific treatment unit to determine if the plant or unit is under loaded or over loaded.
Various organic loadings calculations would be:

a. pounds/day BOD/1000 cubic feet (Trickling Filters)
b. pounds/day BOD /1000 sq feet (RBCs),
c. pounds/day/acre (ponds)
d. F:M ratio (Activated Sludge)

4. To determine industrial surcharges.
Industrial wastewaters such as from food processing plants (Coca Cola, chile processors, meat processors) often contain high levels of concentrated organics. Many cities require either pretreatment to lower the BOD or charge an additional amount (surcharge) to compensate for the extra treatment capacity required.

Oxygen Demand

Measuring the digestible organics directly is difficult, so the amount of oxygen used by the bacteria to digest these organics is measured instead. This digestion process creates an ***oxygen demand***. The BOD test measures the amount of free oxygen (O_2) consumed by polluted waters such as wastewater treatment plants, rivers, lakes, industries, etc. The following reactions indicate the types of reactions that create the oxygen demand.

1. Oxygen is consumed by microorganisms to biochemically degrade (oxidize) organic materials (***carbonaceous demand***)

Reaction 1

$$\text{Organics (food)} + O_2 + \text{microorganisms (heterotrophic bacteria)} \rightarrow CO_2 + H_2O$$

2. Oxygen is consumed by microorganisms to oxidize reduced forms of nitrogen such as ammonium or nitrite. (***nitrogenous demand***)

Reaction 2

$$NH_4^+ + O_2 + \text{microorganisms (nitrosomonas bacteria)} \rightarrow NO_2^- + H_2O$$

$$NO_2^- + O_2 + \text{microorganisms (nitrobacter bacteria)} \rightarrow NO_3^- + H_2O$$

3. Oxygen is consumed when reduced inorganic material such as sulfides and ferrous iron are present. These are often created in septic sewer lines.

$$Fe^{+2} + O_2 \text{ ----------> } Fe^{+3}$$

$$S^{-2} + O_2 \text{ ----------> } SO_4^{-2}$$

These reactions can occur spontaneously or can be caused by microorganisms as well.

Problems created by lack of oxygen

If an organic overload occurs in a wastewater biological treatment unit, the dissolved oxygen (DO) may drop too much because the aeration equipment may not be able to keep up with the rate of oxygen depletion. In other words, the microorganisms use more oxygen than can be supplied. This would result in incomplete stabilization (digestion) of the organics. Low dissolved oxygen (in activated sludge systems) may cause the growth of undesirable filamentous bacteria, which in turn might cause a bulking sludge problem.

If wastewater effluent has a high BOD and is discharged to a receiving stream (river, stream, lake, etc), the bacteria in the receiving stream may deplete the oxygen in the stream (using Reaction 1 again). While the microorganisms are digesting the discharged organics in the wastewater, fish kills or other undesirable conditions may occur if the oxygen in the receiving stream is not quickly replaced. This problem was the primary reason the NPDES permit system was established.

BOD Test Theory

The BOD test is one of the most difficult tests to run because there are so many variables that effect the outcome. In order for the test outcome to be acceptable the following variables must be controlled.

1. BOD dilution water must be aerated and non-contaminated.
2. The pH environment must be acceptable (6.5 - 7.5)
3. The incubation temperature must be controlled and consistent.(20 $\pm$ 1°C)
4. The microorganisms must be viable, must be acclimated to the waste material must be active and must be at acceptable concentration.
5. The sample strength must be sufficient to cause a DO depletion of at least 2 mg/L but leave at least 1 mg/L residual.
6. Toxic materials must be absent. (Dechlorinate)

In summary, the BOD test must create ideal growing conditions which will encourage microorganisms to effectively and efficiently utilize the digestible organic materials (waste).

Running a BOD analysis on a sample consists of placing a portion of a sample (along with prepared dilution water) into an air-tight bottle (300 mls volume) and incubating the bottle at 20 +/- 1 deg C for (usually) 5 days. The dissolved oxygen is measured initially and again after the 5 day incubation. The bottle size, incubation temperature, and incubation period are all specified. Most wastewater and other samples (such as industrial waste waters) contain more organics (food) than the amount of dissolved oxygen available. Therefore, it is necessary to dilute the sample before incubation to bring the oxygen demand and oxygen supply into appropriate balance. The nutrients, magnesium sulfate, calcium chloride, and ferric chloride, are added to the dilution water to maintain ionic strength and to promote bacterial growth. A phosphate buffer is added to help maintain a pH of 6.5 - 7.5, which is desirable for good bacterial growth.

In other words, (Reaction 1) the amount of organics can be measured by measuring the amount of oxygen used up by microorganisms over a 5 day period. In order for the test to work properly, the microorganisms must be healthy and happy. To be happy, they need food

(organics), oxygen, and an acceptable growth environment including normal pH, temperature, nutrients and no toxics).

In the BOD test, microorganisms are charged with eating all the organics (food). In a BOD bottle, organics from a sample are added to dilution water containing nutrients, oxygen, and microorganisms, then capped and incubated at 20°C for 5 days. Initially the microorganism level is fairly low, but the environmental growing conditions are excellent, so the microorganisms quickly enter the log growth reproduction phase and begin to consume the organics.

Growth curve

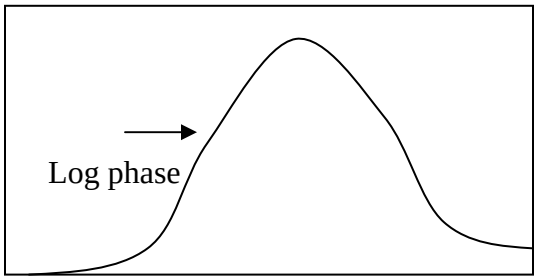


Figure 1: Logarithmic growth

The large number of microorganisms consume the organics and as a result also consume the oxygen.

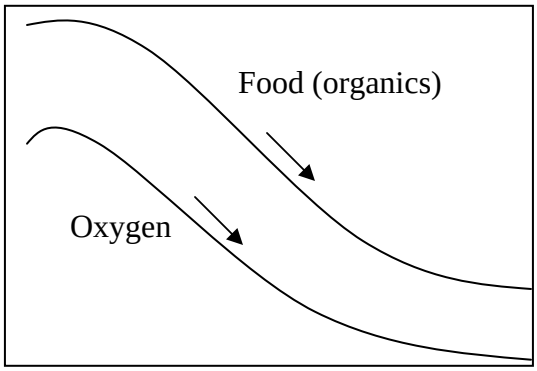


Figure 2: Organics (food) is consumed

If the BOD bottle is capped tightly, no external oxygen can enter. The microorganisms can only utilize the oxygen initially available. Over the next couple of days the microorganisms continue to digest the organics, reproduce logarithmically, and utilize oxygen rapidly

By the 4th day of the BOD test, the organics have begun to become limiting, just barely able to maintain the current microorganism population (stationary growth phase) Figure 3.

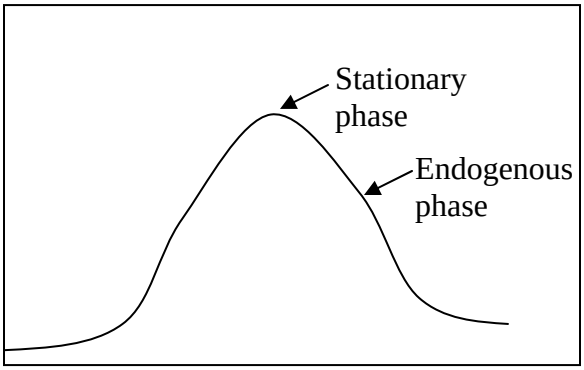


Figure 3: Stationary and endogenous phase

The lack of food causes a slowing in the reproduction rate as well as a decrease in the amount of oxygen used. With the high microorganism population, the remaining organics are quickly consumed and the microorganism enter the endogenous phase. During the endogenous phase, the microorganisms utilize internal food reserves, and many die (endogenous phase). If everything is OK, most of the microorganisms are hungry, but alive and there is still sufficient oxygen left in the bottle to be measured. The amount of oxygen that has been consumed over the 5 days is proportional to the amount of organics (BOD) consumed. The BOD curve in Figure 4 represents the total amount of food used up over the 5 days.

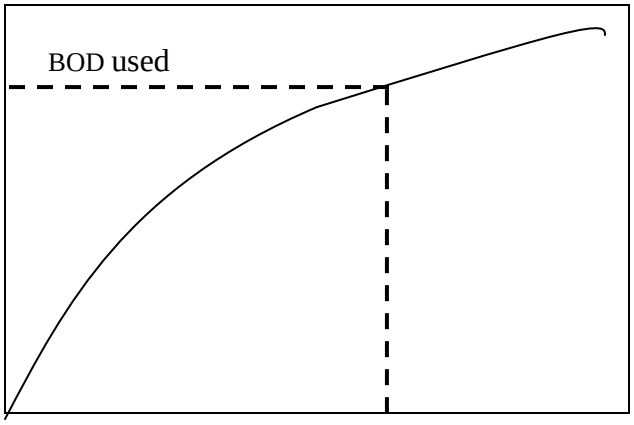


Figure 4: BOD used

BOD_5 vs CBOD

Complete stabilization of a sample may require a period of incubation too long for practical purposes, therefore, five days has been determined as the standard incubation period.

BOD measures food that create a carbonaceous oxygen demand (Reaction 1) and nitrogenous oxygen demand (Reaction 2). In New Mexico, the NPDES permit generally requires BOD and does not distinguish between carbonaceous and nitrogenous BOD. If the nitrogenous oxygen demand is not desired, an inhibiting chemical may be use to prevent ammonia oxidation. The resulting BOD is called CBOD. With this technique carbonaceous and nitrogenous demands can be measured separately.

$$BOD_5 = CBOD + NBOD$$

Before using inhibitors, the permittee must first apply for a BOD variance from the EPA. The extent of oxidation of nitrogenous compounds during the 5-day incubation period depends on the presence of microorganisms capable of carrying out this oxidation (Reaction 2). Such organisms (nitrosomonas and nitrobacter) are not usually present in raw sewage or primary effluent in sufficient numbers to oxidize significant quantities of nitrogen in the 5-day BOD test.

Newer treatment processes involve nitrification and denitrification. These processes may have a NBOD component.

BOD Procedure

Dilution Water Preparation

Dilution water is used to provide oxygen for the bacteria to breath during the 5 day incubation period. It is important to have the dilution water saturated but not super-saturated. There are 3 commonly accepted ways to aerate the water. The water can be:

1. Shaken vigorously for a few minutes.
2. Aerated overnight using an oil-less air source.
3. Allowed to sit in the incubator at 20°C for several days.

For example:
10 liters of deionized water is aerated using air from a clean oil-less air supply. A ***CLEAN*** large bore serological pipet is attached to a clean section of tygon tubing. The tubing should not touch the water as it is often left lying around on lab benches and can easily become contaminated. This may easily be a source of error that contributes to bad dilution water. The pipet is inserted into the BOD carboy and allowed to bubble gently overnight. The next morning the pipet and hose are removed and the air turned off. The aerated water is now allowed to set for a minimum of 1 hour to allow any supersaturated air to dissipate.

Passing air through the dilution water may cool the water several degrees. If the air temperature is below 20°C the aerated water will become too cold to be acceptable. Cold water holds more oxygen than warm water and will release oxygen as it warms

up. This may create problems with the dilution water blank. Allow any aerated water to stabilize for a minimum of 1 hour before use or longer if the water temperature is still below 20°C. Likewise, warm air may raise the temperature of the dilution water and make it difficult to hold sufficient amounts of oxygen.

Nutrients are also added to the water. The four nutrients are Magnesium Sulfate, Calcium Chloride, Ferric Chloride, and Phosphate Buffer. One milliliter of each solution should be added for each liter of dilution water. In this example, if 10 liters of dilution water is being prepared, 10 ml of each nutrient solution should be added. A serological pipet is acceptable for this process. Always add the nutrient to the water. Do not add the nutrients first then the water, since this may cause the nutrient to precipitate out.

* These reagents can be purchased already prepared and can be purchased in individual packets.

The BOD water could also be aerated by allowing the dilution water to equilibrate in a cotton-plugged bottle for several days. The dilution water is usually stored in the BOD incubator, thus avoiding any dissolved oxygen shifts caused by temperature shifts. The potential problem here is the growth of microorganisms in the water during the equilibration period. These microorganisms will become BOD and cause the blanks to be bad.

The least effective method of aerating the BOD water is to vigorously shake a partially filled bottle for a few minutes.
This will provide an aerated water but is not usually saturated. DO meter calibration can be inaccurate when using this aeration method.

Dilution Water Blank Preparation
Now that the dilution water containing the nutrients has been allowed to equilibrate to room temperature, the dilution water must be checked for contamination. Checking the dilution water will require the water to be taken through the entire BOD incubation procedure.

1. Fill two or more clean BOD bottles with dilution water being careful to avoid bubbling and supersaturation.

Fill the bottle slowly to avoid excessive agitation and supersaturation of DO

Carefully allow the dilution water to slowly fill the BOD bottle. If the BOD bottle is filled too rapidly, excessive agitation and bubbling may result in a supersaturated water. Avoid using hoses to fill the BOD bottles. Hoses help avoid supersaturation but can easily add contamination.

2. Once the BOD bottle is filled to the neck, measure the initial dissolved oxygen level.
3. Place the glass stopper in the BOD bottle and check to make sure there are no bubbles beneath the stopper.
4. Place a small amount of water above the stopper. This will provide a water seal to prevent air from entering the bottle over the 5 day incubation period. Cap the

bottle to prevent evaporation of the water seal during incubation at 20°C for 5 days in the dark.

5. After 5 days, once again measure the dissolved oxygen.
6. Record data on the BOD worksheet.

Run Blanks in duplicate. Remember the blanks have only nutrients, oxygen and water. There should be <0.2 mg/L drop in dissolved oxygen after 5 days. If the DO drops more than 0.2 mg/L, the technician needs to investigate the cause of the excessive drop. Running duplicates helps identify problems.

Seed Control

Many wastewater effluent samples may be partially or completely sterile as a result of chlorination, ozonation, UV sterilization, exposure to toxic chemicals, heat, unfavorable pH, or other factors detrimental to biological activity. In the BOD test, microorganisms are an essential part of the procedure.

$$\text{Organics} + O_2 + \text{microorganisms} \rightarrow CO_2 + H_2O$$
(food) (heterotrophic bacteria)

Validity of the BOD results depends upon the presence of organisms capable of prompt and effective biodegradation. Since harsh environmental conditions may have killed the original bacteria, the sample must be re-inoculated with "seed" bacteria from receiving waters (rivers, ponds), soil suspensions, raw wastewater or lypholyzed commercial seed to provide enough microorganisms to oxidize the waste properly. Raw wastewater has the advantage because it usually has a high number of bacteria that are indigenous to the waste itself. By using bacteria that are already common to the wastewater, there is no delay while other bacteria get acclimated to the food. Using raw wastewater provides numerous organisms but also contain excess organics which must be corrected for.

How many bacteria are needed?

The amount of bacteria needed is determined by trial. The bacteria added should be "active" and initiate biochemical activity promptly. If the seed is either low in concentration or unacclimated (doesn't like the type of waste), the BOD reaction is slowed. In other words, if there are not enough microorganisms, the amount of food (organics) eaten is slow and the amount of oxygen consumed is low. On the other hand, if there is sufficient microorganisms but they don't have the correct enzymes needed to digest the food, the amount of food eaten will also be low and the amount of oxygen consumed will again be low.

Figure 5.

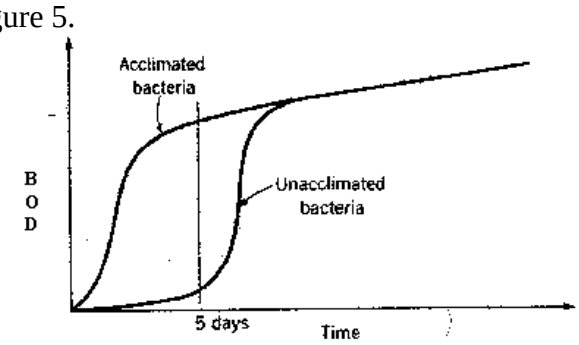


Figure 5: Acclimated seed

Seeding is always an uncertain procedure but very necessary at times. A seed containing viable organisms at a lower energy state (hungry bugs) because of limited food (organics) theoretically is the best available seed source. A microorganism population created from the waste itself should be most effective for initiating biochemical activity as soon as the nutrient situation favors more activity. (In other words, if you are a vegetarian and like eating carrots, it would not take you long to begin eating (log growth) if they brought you a plate of carrots. On the other hand, if you are a vegetarian and they brought you a plate of barbeque ribs, you would have to think about eating for awhile before eating and you may decide not to eat at all.) The microorganism population should not be stored too long because organism redistribution and die-out become limiting. (If the seed organisms are already hungry, they will begin to die and must be used quickly). This type of seed would most likely be found in a surface water or a treatment plant effluent with a history of receiving the particular waste material under consideration. Different seed sources and locations should be checked out to determine the best available material from a standpoint of rapid initiation of activity, low correction, and predictable high oxygen depletion under test.

Seed Collection

Collect the seed in a 500 ml bottle and let settle at least 1 hour and up to 36 hours. This will allow settleable solids to settle and help assure the seed is homogeneous.

It is very important to allow the settleable solids to settle and the supernatant to be poured off slowly. The more consistent the supernatant (homogeneous) the more consistent and reliable the BOD test results will be. If there are a lot of large suspended solids (organics) in the supernatant, the seed correction and test results will be very erratic.

Figure 6: Settleable solids allowed to settle at least 1 and up to 36 hours

Figure 7: Supernatant carefully poured off the top without disturbing solids.

<u>Seed Correction Bottle Preparation</u>

1. Set up 3 or more clean BOD bottles.
2. Add varying amounts of seed to each BOD bottle. For example: add 6, 9, and 12 ml of seed. Use a large bore 10 ml serological pipet.
3. Carefully fill with BOD dilution water. Be careful not to overfill the BOD bottle. Overfilling will cause loss of seed.
4. Record initial dissolved oxygen level for each bottle.

5. Stopper, place a water seal, cap, and incubate at 20°C in the dark for 5 days.
6. Determine the 5 day dissolved oxygen level.
7. Calculate the dissolved oxygen depletion caused by the added seed.

Seed Correction Calculation
When seed is added to a BOD sample, it usually contains some amount of "extra" organics. Since the BOD test measures all digestible organics, the extra organics that come with the seed will give a BOD answer that is higher than it should be. This extra organic must be corrected for (subtracted out). Since this test is often used for the NPDES permit, an answer that is too high may cause a violation of the permit.
To make this correction, seed correction bottles are used. The seed correction bottles determine how much of the oxygen used over 5 days was due to the seed itself.

sample organics + O_2 + seed → BOD_1

seed organics + O_2 + seed → BOD_2

The BOD test will measure $BOD_1 + BOD_2$

The BOD caused by the seed organics must be subtracted or "corrected".

For example, if 10 ml of seed is added to a BOD bottle, filled with dilution water, then incubated for 5 days.

Initial Dissolved Oxygen = 7.7 mg/L
Final Dissolved Oxygen = 5.4 mg/L
Dissolved oxygen used = 2.3 mg/L

$$\frac{2.3 \text{ mg/L}}{10 \text{ ml}} = \frac{0.23 \text{ mg/L}}{1 \text{ ml of seed}}$$

Each milliliter of seed used 0.23 mg/L of oxygen over the 5 days. Two milliliters of seed would have used 0.46 mg/L of oxygen. Three milliliters of seed would use 0.69 mg/L of oxygen, etc. See if you can calculate the seed correction for the following example?

25 ml of settled seed is added to the BOD bottle, filled with dilution water, then incubated for 5 days. If the initial DO is 8.5 mg/L and the final DO after 5 days is 2.7, what is the seed correction?
(answer: 0.23 mg/L/ml seed.)

How many milliliters of seed would be needed to give a depletion between 0.6 - 1.0 mg/L? (Answer: 3 or 4 ml)

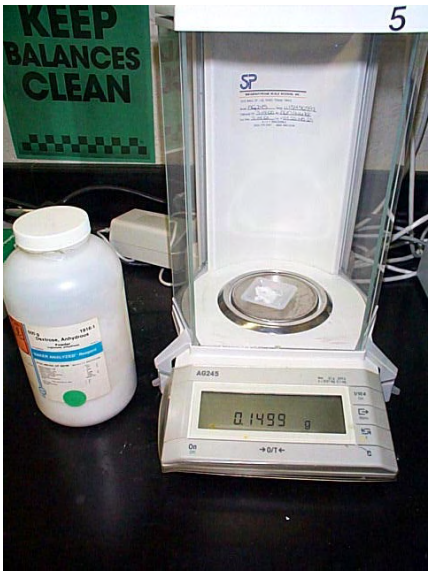
The BOD test suggest the analyst add enough seed so that each standard or sample bottle have a seed correction of 0.6-1.0 mg/L. By targeting this range, there should be ample microorganisms to digest the sample organics (BOD)

Glucose - Glutamic Acid Standard
In order to validate the BOD test conditions, reagents, and procedure, it is necessary to measure a solution with a known amount of organics. A solution that has a known value is called a standard. In the BOD test, a solution containing a carbohydrate and a protein is used as the source of organics. The carbohydrate is glucose and the protein is the amino acid glutamic acid. This standard provides a source of food for a wide variety of microorganisms (seed). If everything in the test is acceptable, the BOD

should be 198 ± 30.5 mg/L. ***Results outside this range indicate a problem that requires immediate remediation and invalidates all samples that were also run.***

Glucose-Glutamic acid preparation
The glucose and glutamic acid are both powders that must be dried in an oven for an hour at 103°C before being used. Once dried, the glucose and glutamic acid can be cooled and stored in a desiccator.

Each of the powders should be weighed on a calibrated analytical balance.



0.1500 ± 0.0005 gm of each powder is weighed out, then carefully transferred to a 1000 ml volumetric flask.

Be sure to rinse the weighing boats to recover all the powder. Once the powder has been transferred, fill the volumetric flask to the mark with deionized or distilled water. Add a magnet and mix for at least 1 hour to allow the glucose and glutamic acid to completely dissolve.

The glucose - glutamic acid standard should be prepared fresh, on the day of the test. Commercial preparations can be purchased. Be sure to check for growth and contamination before using. Do not exceed the expiration date.

Glucose-Glutamic Acid Standard Procedure
1. Set up 2 or more clean BOD bottles.
2. Use a large bore serological pipet to measure the appropriate amount of seed to each BOD bottle. (Remember, the ideal seed should show a drop between 0.6-1.0 mg/L). If the influent is weak, a higher amount of seed may be required.

The GG requires seed to supply the microorganisms needed. Here 3 ml of seed is being added.

3. Use a 6 ml Class "A" volumetric pipet to pipet 6.0 ml of the glucose glutamic acid standard into each bottle. Remember volumetric pipets are not blown out.

6.0 ml of glucose-glutamic acid standard are added to duplicate bottles as a BOD control

4. Carefully fill the remainder of the bottle with BOD dilution water. Be careful not to overfill the BOD bottle. Overfilling will cause loss of seed and standard.
5. Measure the initial BOD.

6. Insert the stopper and place a water seal and cap, and incubate at 20°C for 5 days.

Once the BOD bottle has been filled to the neck with dilution water, the initial dissolved oxygen is measured, the stopper twisted in place and covered with a cap to prevent water loss and prevent air from entering for the next 5 days

7. Measure the final DO after 5 days.

Glucose-Glutamic Acid Calculations
The calculations for the GG standard will be discussed later. Remember, if the GG standard is not correct, all sample data is invalid.

BOD Sample Collection
The BOD sample can come from several sources. It may come from industry, from the influent, the effluent, river, etc. The sample may be a composite or grab sample. The sample can be collected in glass or plastic bottles. Because the BOD test measures organics, preservation is required to minimize degradation of organics during collection and transport. Analysis should begin as soon as possible after collection. If the sample can be analyzed within 2 hours, preservation is not required. If the sample is to be transported or stored for more than 2 hours, it must be transported on ice or refrigerated.

Grab samples should be run within 24 hours and must be run within 48 hours. Store in a refrigerator below 6°C.

Composite samples must be stored in a refrigerator or on ice during the entire compositing period. The analysis should begin within 24 hours after the last composite sample has been collected. Samples collected for NPDES permits should be analyzed within 6 hours after compositing.

Sample Pretreatment
As discussed earlier, the BOD is a measure of the organic strength of a waste. In order to measure organics, oxygen consumed by the microorganisms is measured at the beginning and at the end of 5 days. In order for microorganisms to grow and metabolize the organics, their environment must be controlled. The factors which need to be controlled are:

1. pH
2. Temperature
3. Toxic disinfectants
4. Supersaturation

pH Adjustment
In the BOD test, a **sample** with a pH between 6.0 and 8.5 does not need to be adjusted. If the sample pH is below <6.0 or above pH >8.5, the sample pH must be adjusted by adding either acid or base to bring the sample pH to 6.5-7.5. Be sure to use a calibrated pH meter. The concentration of the acid or base used to adjust the pH should be high enough to avoid significant dilution of the sample. No more than 5 ml of acid or base can be added to one liter of sample (0.5%) for pH adjustment. Samples with extreme pH may not have any viable microorganisms present and must always be seeded.

Sample Temperature Adjustment
Temperature affects the metabolism rate of microorganisms as well as the dissolved oxygen concentration. In order to minimize these effects the sample temperature is adjusted to 20 $\pm$ 1°C. Often the sample temperature is <6°C because it has been composited and refrigerated. It is undesirable and time consuming to let the sample warm to room temperature so it can quickly and efficiently be warmed in a waterbath.

Sample can be quickly warmed to 20oC using a waterbath. Use a thermometer, the sample warms quickly.

Sample Dechlorination
As discussed earlier, if this is an effluent sample, it has been subjected to disinfection. If the sample has been chlorinated, there will be few if any microorganisms still present. If the effluent has been disinfected properly, there will be a chlorine residual. If this chlorinated sample is added to the BOD bottle, the residual chlorine will continue to kill the microorganisms and the BOD will be invalid. (If there are no microorganisms present in the BOD bottle, no oxygen will be used and no organics will be consumed).

You get a great answer for your permit but its WRONG.

To prevent the chlorine from killing the microorganisms, the sample must be checked for the presence of a chlorine residual. A 100 ml aliquot of sample is added to an Erlenmeyer flask, 1 ml of the acetic acid , potassium iodide reagents are added. If a blue color is present, chlorine is present and must be destroyed.

The blue color is titrated by a sodium sulfite solution until the color disappears.

$Cl_2 + Na_2SO_3 \text{ ----> } Cl^- + Na_2SO_4$

Once the 100 ml sample has been titrated, record the volume of sodium sulfite used. The 100 ml sample is **DISCARDED.**

Next, determine how much sodium sulfite should be added to dechlorinate the volume of sample for the BOD test. The volume of sulfite added needs to be proportional to the 100 ml aliquot dechlorinated.

For example, if the volume of sodium sulfite titrated was 0.2 ml for 100 ml, how many milliliters of sodium sulfite would be added to dechlorinate 1000 ml of sample.

$$\frac{0.2 \text{ ml}}{100 \text{ ml}} \times 1000 \text{ ml} = 2.0 \text{ ml}$$

This volume is then added to 1 liter of sample. Like pH, the volume of sodium sulfite added can not exceed the 5 ml/1000 ml sample additional volume limit. In this example, 2 ml is acceptable as long as the volume used to adjust the pH was below 3.0 ml. Mix the sample and allow to sit for 20 minutes to allow time for the sodium sulfite to dechlorinate the l liter of sample.

Once the sample has been dechlorinated, it must be checked to verify that dechlorination was accomplished. Repeat the sodium sulfite titration to verify the absence of a blue color.

Sample Supersaturation

In New Mexico, it is very possible to have samples with dissolved oxygen levels above 9.0 mg/L. Cold water and photosynthesis contribute to high levels of DO. This excess DO may interfere with the BOD test and should be removed prior to analysis. Warm the sample to 20°C while vigorously shaking the sample or by aerating with clean filtered air.

The sample environment has now been adjusted for pH, temperature, and chlorine.

Effluent BOD Sample

The effluent sample is usually chlorinated and must have gone through the pre-treatment procedures discussed earlier. Since the water has been chlorinated, then dechlorinated, there are few viable microorganisms. This will require that

"seed" be added to the effluent sample. This should be the same seed as discussed earlier.

Procedure

1. Determine the approximate sample size needed from the table below. Notice that the lower the expected BOD, the more sample volume needed.

Volume added	Expected BOD
3	200 - 560 mg/L
6	105 - 280
9	70 - 187
12	53 - 140
15	42 - 112
30	21 - 56
45	14 - 37
60	11 - 28
100	5 - 15
300	0 - 5

After gently mixing the effluent sample, measure out at least three dilutions (using a large bore serological pipet or a graduate cylinder) and add to three clean BOD bottles.

In most cases, samples with consistent BOD's only need 3 BOD dilutions set up. However, if the BOD is truly unknown, a wide range of dilutions should be selected. It is recommended that at least 2 of the BOD dilutions meet the **DO depletion rule.**

DO Depletion Rule

The final day 5 DO must drop at least 2 mg/L from the initial DO and be greater than 1 mg/L

2. Add the appropriate amount of seed to each BOD bottle. Carefully fill the BOD bottles with dilution water.
3. Measure the initial DO.
4. Be careful not to entrap air beneath the stopper, place a stopper and water seal on the bottle and incubate for 5 days at 20°C in the dark.
5. Measure the 5 day DO.
6. Calculate the BOD in mg/L.

For example: What volumes of sample should be added to the BOD bottles if the BOD is less than 30 mg/L?

Look at the table! Any sample volume between 30 and 60 milliliters should measure a BOD of 30. The analyst selects 45 ml. What if your BOD was higher than 30 mg/L? By using 30 ml for a second bottle, you would extend your range to as high as 56 mg/L. By using 60 ml, you would extent your low range to as low as 11 mg/L. Therefore, by running at least 3 dilutions of 30, 45, and 60 ml, the analyst would be sure to measure any BOD between 11 and 56 mg/L. More dilutions could be prepared to extend the range.

What sample volumes would you use if the expected BOD was 12 mg/L? Would 30, 60 and 100 ml work?
Yes, the three dilutions would give at least 2 valid results for a sample with a BOD

between 5-56 mg/L. In this example, the 60 ml and 100 ml bottles would both show good DO depletion for a sample with a BOD of 12 mg/L. The 30 ml sample would have no DO left and would not meet the depletion rule.

DO Meter Calibration

Most DO meters have specific calibration procedures set by the manufacturer. Because oxygen is a gas, it is pressure and temperature dependent. The DO meter must be calibrated against these two variables. As a rule the warmer the water, the less DO the water can hold. Likewise, the colder the water, the more DO the water can hold. This is one of the reasons sample are pre-warmed prior to placing in the BOD bottles. A BOD bottle filled with cold sample will have a different DO than a bottle filled with warm sample. Bottles having different volumes of sample will also potentially have different DO readings, that is why the DO on each bottle must be read initially.

Altitude also affects the amount of DO present. The higher the altitude, the less DO the sample can contain. A laboratory at an elevation of 5000 feet will have less oxygen in the saturated dilution water than a laboratory at sea level. For instance, at sea level, saturated water might have a DO of 8.5 mg/L while the same water at 4000 feet will only have a saturated DO value of 7.5 mg/L.

Problems with DO meters almost always center around the membrane. Whenever problems arise and the DO doesn't "look right", change the membrane. Follow the manufacturers troubleshooting and maintenance procedures. Record the maintenance in the log book.

BOD Calculations

BOD calculations follow the general formula:

$$\text{BOD} = \frac{\text{DO used by sample x 300}}{\text{sample volume}}$$

Unfortunately, it's not going to appear that easy. The dissolved oxygen used by the sample represents the amount of oxygen consumed by the microorganisms as they digest the organics over the 5 day incubation period at 20°C. The organics can come from the sample and from the seed source.

Lets check the Unseeded blanks first! The blanks were filled with aerated BOD dilution water.
How much organics were in the BOD dilution water? No organics should be present in the BOD dilution water.

How many microorganisms were in the BOD dilution water? No microorganisms would be expected to be present in BOD dilution water either.

If there are no microorganisms and no food, there will be very little metabolic activity. If there are no organics and microorganisms present in the BOD dilution water, how much oxygen would you expect to be consumed?

Thus the DO used in the unseeded blanks would be expected to be zero. Because oxygen is temperature and pressure sensitive, a drop of less than 0.2 mg/L is acceptable.

If the unseeded blanks are not acceptable, the test is still valid but an effort to correct the problem must be made.

Seed Correction Calculations

The seed correction subtracts the amount of oxygen used metabolizing the organics from the seed source. Calculate the seed correction from the following data.

Example 1:
First select all bottles which meet the DO depletion rules. Remember the dilution rule requires the bottle to use at least 2 mg/L of DO but have a least 1 mg/L of oxygen remaining at the end of 5 days. Which bottles meet the DO depletion rule?

Volume seed added	3 ml	5 ml	8 ml
Initial DO	7.4	7.4	7.4
Final DO	5.9	4.1	2.5
Difference	**1.6**	**3.3**	**4.9**
Correction	**BAD**	**0.66**	**0.61**

The 5 ml and 8 ml bottle are acceptable because they dropped at least 2.0 mg/L and the final DO is above 1.0 mg/L. The 3 ml bottle dropped only 1.6 mg/L and does not meet the depletion rule and cannot be used.

What is the seed correction for each acceptable bottle?

$$\frac{3.3 \text{ mg/L}}{5 \text{ ml seed}} = \frac{0.66 \text{ mg/L}}{1 \text{ ml seed}}$$

$$\frac{4.9 \text{ mg/L}}{8 \text{ ml seed}} = \frac{0.61 \text{ mg/L}}{1 \text{ ml seed}}$$

Calculate the seed correction by averaging all bottles that meet the depletion rule. What is the average seed correction?

$$\frac{0.66 + 0.61}{2} = \mathbf{\underline{0.64 \text{ mg/L}}}$$

Example 2: Calculate the seed correction from the following data. Volume seed added	5 ml	10 ml	15 ml
Initial DO	8.3	8.4	8.3
Final DO	6.1	4.1	2.5
Difference	**2.2**	**4.3**	**5.8**
Correction	**0.44**	**0.43**	**0.39**

Which bottles meet the DO depletion rule?
All the bottles meet the depletion rules.

What is the seed correction for each acceptable bottle?

$$\frac{2.2 \text{ mg/L}}{5 \text{ ml seed}} = \frac{0.44 \text{ mg/L}}{1 \text{ ml seed}}$$

$$\frac{4.3 \text{ mg/L}}{10 \text{ ml seed}} = \frac{0.43 \text{mg/L}}{1 \text{ ml seed}}$$

$$\frac{5.8 \text{ mg/L}}{15 \text{ ml seed}} = \frac{0.39 \text{ mg/L}}{1 \text{ ml seed}}$$

What is the average seed correction?

0.42 mg/L

GG Standard Calculations

It's time to find out if all your test conditions, procedures, and lab techniques are acceptable. To do this, a known BOD standard solution containing glucose-glutamic acid is measured. If the glucose-glutamic acid standard results are not within 198 ± 30.5 mg/L, the test is invalid and all data **must be rejected.**

To calculate the G-G concentration, you need to know:

Volume of GG added	**6.0 ml**
Volume of seed added	**1 ml**
Initial DO	**7.8**
Final DO	**3.2**
DO Difference	**4.6**
Seed correction	**0.6**
Corrected DO	**4.0**

In this example, the microorganisms used up 4.6 mg/L of oxygen digesting 6 ml of glucose-glutamic acid. However, because seed was used, 0.6 mg/L must be subtracted to correct for the organics that were added with the seed. The final oxygen used up digesting the glucose-glutamic acid is 4.0 mg/L.
Does this bottle meet the oxygen depletion rules?

$$\text{BOD} = \frac{\text{DO used by sample x 300}}{\text{sample volume}}$$

$$\text{BOD} = \frac{(4.6 - 0.6) \times 300}{6.0} = \frac{4.0 \times 300}{6.0}$$

$$= 200 \text{ mg/L}$$

Because the GG result is within 198 $\pm$ 30.5 mg/L, the test conditions validate the sample data. If the result was not within 198 $\pm$ 30.5 mg/L, the test is **INVALID** and all data must be discarded. Determine the GG results from the data in example1.

Example 1:
Six ml of GG and 2 ml of seed were added to a BOD bottle. The seed correction value was 0.44 mg/L per ml of seed. If the initial DO was 7.8 and the final DO was 2.2, what is the BOD for the GG.

Volume of GG added	**6.0 ml**
Volume of seed added	**2 ml**
Initial DO	**7.8**
Final DO	**2.2**
DO Difference	
Seed correction	
Corrected DO	

Calculate the seed correction. **(0.88 mg/L)**
Calculate the DO used by just the GG. **(4.72 mg/L)**
Calculate the BOD in mg/L. **(236 mg/L)**
Determine if this is this an acceptable BOD. **(No, the BOD is above 228.5 mg/L)**

Example 2:
Six ml of GG and 5 ml of seed were added to a BOD bottle. The seed correction value was 0.14 mg/L per ml of seed. If the initial DO was 6.8 and the final DO was 2.7, what is the BOD for the GG.

Volume of GG added	**6.0 ml**
Volume of seed added	**5 ml**
Initial DO	**6.8**
Final DO	**2.7**
DO Difference	
Seed correction	
Corrected DO	

Calculate the seed correction.
0.14 mg/L x 5 ml seed added = 0.70 mg/L

Calculate the corrected DO.
(6.8 - 2.7) = 4.1 mg/L This represents the total oxygen used.
4.1 - 0.70 = 3.4 mg/L This represents the oxygen used up by just the GG.

Calculate the BOD in mg/L.

$$\textbf{BOD} = \frac{3.4 \times 300}{6.0} = 170 \text{ mg/L}$$

Determine if this is this an acceptable BOD. **Yes**. This value (170 mg/L) does fall within 198 $\pm$ 30.5 mg/L. The results are satisfactory and sample data may be accepted. The BOD is just barely within the acceptable range. If this continues to be a borderline problem, the analyst should troubleshoot the procedure to bring the BOD closer to the mean.

Seeded Sample Calculations

This is the final step in the BOD test-sample analysis. There are two types of samples typically run.

1. Those with sufficient microorganisms
2. Those with few or no microorganisms

Samples with abundant populations of microorganisms will not require extra "seed". A raw influent sample would be an obvious sample that would have its own population of bacteria often as high as 10,000,000/100 ml.

On the other hand, samples with few microorganisms will require an outside source of microorganisms or "seed". A disinfected secondary effluent is a common sample that requires "seed". A disinfected effluent sample may have no higher than 10 bacteria/100 ml.

Example 1: Calculate the sample BOD concentration for a chlorinated WWTP effluent sample with an expected BOD of 20 mg/L. The seed correction was 0.3 mg/L per ml of seed.

BOD bottle number	6	3	51
Volume of sample added	30	60	100
Volume of seed added	2	2	2
Initial DO	7.5	7.5	7.4
Final DO	3.9	1.1	0.0
DO Difference	3.6	6.4	
Seed Correction	0.6	0.6	
Corrected Difference	3.0	5.8	
BOD	30.0	29.0	

In this example, three different volumes of sample were placed in the BOD bottles, 30, 60, and 100 ml. The sample volumes selected were based on the anticipated answer. Standard Methods recommends preparing enough bottles so that at least 2 of the bottles will meet the DO Depletion Rule.

Volume added	Expected BOD
3	200 - 560 mg/L
6	105 - 280
9	70 - 187
12	53 - 140
15	42 - 112
30	21 - 56
45	14 - 37
60	11 - 28
100	5 - 15
300	0 - 5

In the example above, which bottles meet the DO Depletion Rule? Bottles 6 and 3 meet the DO Depletion Rule. Bottle 51

dropped below 1 mg/L, and should not be used.

To calculate the sample BOD, the same formula is used. Since seed was added, a seed correction is necessary.

$$\text{BOD} = \frac{\text{DO used by sample-seed correction} \times 300}{\text{sample volume}}$$

$$\text{BOD} = \frac{(7.5 - 3.9 - 0.6) \times 300}{30} = \frac{3.0 \times 300}{30} = 30.0$$

$$\text{BOD} = \frac{(7.5 - 1{,}1 - 0.6) \times 300}{60} = \frac{5.8 \times 300}{60} = 29.0$$

To calculate the BOD, average the two valid bottles.

$$30.0 + 29.0 = \frac{59}{2} = 29.5 \text{ mg/L}$$

Example 2

The lab expects an effluent BOD between 5-30 mg/L. Using the table, select 3 sample volumes to use.

In this example, the technician selects 50, 100, and 300 ml. Complete the table and calculate the sample BOD. The seed correction factor is 0.21 mg/L per ml of seed.

BOD bottle number	26	33	89
Volume of sample added	50	100	300
Volume of seed added	4	4	4
Initial DO	7.7	7.5	7.4
Final DO	5.2	3.0	0.8
DO Difference			
Seed Correction	0.84	0.84	0.84
Corrected DO			
BOD			

Is the seed correction OK? Yes, the correction falls between 0.6 and 1.0 mg/L

Is the DO depletion on all the bottles OK? No. Bottle 89 has dropped below 1.0 mg/L and should not be used in the calculations. The BOD answer is 10.5.

Example 3

Calculate the BOD from the data below. The seed correction is 0.25 mg/L per ml of seed.

BOD bottle number	22	29	55
Volume of sample added	10	20	40
Volume of seed added	2	2	2
Initial DO	8.5	8.7	9.0
Final DO	6.6	4.9	2.1
DO Difference			
Seed Correction			
Corrected difference			
BOD			

Is the seed correction OK? Yes, however, if the seed correction is consistently around 0.5, the volume of seed should be increased from 2 ml to 3 ml in order to bring the seed correction between 0.6-1.0 mg/L

Is the DO depletion on all the bottles OK? No. Bottle 22 has not dropped at least 2.0 mg/L and should not be used in the calculations The answer is 48.8 mg/L.

Example 4
Calculate the BOD from the data below. This is a chlorinated effluent. The seed correction is 0.12 mg/L per ml of seed.

BOD bottle number	12	39	58
Volume of sample added	10	20	40
Volume of seed added	4	4	4
Initial DO	7.5	7.7	8.0
Final DO	4.9	3.9	3.4
DO Difference			
Seed Correction			
Corrected difference			
BOD			

You should have calculated BODs of 63.6, 49.8, and 30.9.
Are all the bottles acceptable? Yes
What is the correct BOD to report?
Normally, all the acceptable BOD values are averaged. The average calculated BOD is 48.1 mg/L. However in this example, notice the erratic nature of the data. Notice that as more sample was used in the BOD bottles, the BOD became lower. This is a common picture when dechlorination of the sample has not been performed properly or other toxic materials are present. As more sample is added, more chlorine is added to the BOD bottle. The higher amount of chlorine then kills the seed and results in low answers. The BOD should be repeated if possible. If that is not possible, the highest BOD (63.6 mg/L) should be reported.

BOD Troubleshooting

The BOD test is only invalidated when

1. BOD incubator temperature is outside $20 \pm 1^{o}C$ or
2. The GG standard is outside 198 ± 30.5

Anything else is problematic and must be evaluated.

Troubleshooting BOD Dilution Water:
If the dilution water blanks drop more than 0.2 mg/L, the dilution water needs to be evaluated. The BOD test is not invalid. However, the analyst must begin to seek a remedy for the problem. Sources of error include.

1. Poor aeration procedure. (Common error)
2. The dissolved oxygen meter improperly calibrated. (Common error)
3. Bottle filling was too vigorous
4. Dilution water temperature was too cold.
5. Dirty BOD bottles and **stoppers**. It's easy to forget to scrub the stoppers (Common error)
6. The nutrient reagents were contaminated.
7. Deionized water source was contaminated.

The lab technician needs to think about every step taken to prepare the dilution water. Contaminated tubing, dirty pipets, dirty air, dirty bottles could all contribute to a dissolved oxygen change. Be sure the pipet is clean and the hose does not touch the water. Dirty air is not a likely source if an oil-less compressor is used.

The DO meter calibration can be verified by titrating a separate sample using a second method called the Winker Method, Standard Methods 4500-O-C
If the final DO reading is not acceptable, the DO meter should be recalibrated. Perhaps the battery is old, weak. Perhaps the membrane is contaminated. Perhaps the

probe storage container is contaminated.

Filling the BOD bottles too rapidly can cause supersaturation. This "extra" oxygen may come out of solution after 5 days in the incubator. Fill the bottles slowly.

Remember gases are temperature and pressure dependent. The colder the water the more oxygen that can be held. If the dilution water is colder than the BOD incubator temperature (20°C), the extra oxygen contained in the cold water will be released during the incubation period. This may create an error. An elevated dilution water temperature, such as room temperature, does not usually create a significant error.

Proper glassware washing must be observed. Be sure to wash with a non-toxic detergent, scrub the bottle thoroughly with a brush, then rinse at least 10 times with tap water to remove the detergent, then rinse 5 times with small quantities of deionized water. The detergent may have an organic component.

Inspect the reagent stock solutions for cloudiness, growth, or precipitation. If present, discard and replace with fresh reagents. Cloudiness is usually indicative of bacterial growth.

The deionized water source should be checked monthly for conductivity, heterotrophic plate count, and chlorine residual. A large heterotroph (bacteria) count in the DI water is undesirable. DI is not sterile but should have only trace microorganism. Perhaps the DI system is contaminated. Is there an activated carbon canister on the system? Is there an RO? All equipment can be a possible source of contamination that will make the dilution water blanks unacceptable.

Seed Correction Troubleshooting

The seed correction should have a value between 0.6 - 1.0 mg/L in the glucose-glutamic acid standard and all seeded sample bottles. Selecting the volume of seed to use is basically an educated guess (voo doo for you). When a seed sample is collected, the activity is unknown which makes it difficult to decide how much seed to use. If the results do not fall within the expected range, all is not lost. However, the analyst must now try to adjust the seed to correct the situation. Remember the purpose of the seed is to provide a source of active microorganisms in log phase. Possible causes for bad seed corrections could be:

1. Weak seed activity. Usually indicates a dilute influent strength (low numbers of bacteria). For instance if the seed correction used for the GG standard is less than 0.6 mg/L, the first remedy is simply to add a larger volume of seed. If 3 ml of seed had been previously used, try adding 4 or 5 ml the next time. As an example, if the seed correction is 0.1 mg/L/ 1 ml of seed and 3 ml of seed were added, the seed correction for the GG would have been 0.3 mg/L. Since this is below the desired 0.6 - 1.0 mg/l range, the analyst might add 6 ml next time. This would potentially give a seed correction of 0.6 mg/L. Unchlorinated secondary effluent and receiving streams usually have weak seed activity.

Glucose-Glutamic Acid Standard

Volume GG used	6 ml	6 ml
Volume seed used	3 ml	3 ml
Initial DO	7.0	7.0
Final DO	3.0	3.1
DO used	4.0	3.9
Seed Correction	0.3	0.3
Corrected DO	3.7	3.6
BOD mg/L	185	180

Notice the GG value is acceptable, even though the seed activity is low. The test results would be valid but the seed problems should be remedied.

2. Strong seed activity. Usually indicates a high waste strength (lots of bacteria). Same as in #1, except this time the remedy is to lower the seed volume. If 3 ml of seed was giving a GG seed correction of 1.5 mg/L, then reducing the seed volume to 1 or 2 ml would give a seed correction between 0.5-1.0 mg/L. A fresh, normal influent (BOD of 200 mg/L) will usually provide a good seed source and a good seed activity, which is why seed is often collected from the manhole.

Glucose-Glutamic Acid Standard

Volume GG used	6 ml	6 ml
Volume seed used	3 ml	3 ml
Initial DO	7.0	7.0
Final DO	3.0	3.1
DO used	5.0	4.9
Seed Correction	1.5	1.5
Corrected DO	3.5	3.4
BOD mg/L	175	170

As before, the GG value is acceptable, even though the seed activity is high. The test results would be valid but the seed problems should be remedied.

3. Erratic seed corrections. This problem usually presents as seed corrections that are sometimes high, sometimes low, and sometimes OK. Collecting the seed at the same time and place often helps eliminate this problem. Since both flow and waste concentration change throughout the day, collecting seed at 8:00 am, then 1:15 pm most likely will not have the same waste strength and therefore, the same bacterial activity. Seed activities as low as 0.05 or as high as 1.5 mg/L per milliliter of seed can be found throughout the day. However, the seed activity may be between 0.2 - 0.4 mg/L if always collected between 8:00 and 9:00 am. Using 3 ml of seed then gives a depletion of typically 0.6 - 1.2 mg/L.

Seed Correction - Wed. 8:00 am

Volume seed used	3 ml	5 ml	8 ml
Initial DO	7.0	7.0	7.0
Final DO	5.0	3.8	1.6
DO used	2.0	3.2	5.4
Seed Correction	0.67	0.64	0.67
Average Seed Correction/ ml	0.66		

Seed Correction - Thurs. 1:15 pm

Volume seed used	3 ml	5 ml	8 ml
Initial DO	7.0	7.0	7.0
Final DO	6.5	5.8	4.6
DO used	0.5	1.2	2.4
Seed Correction	invalid	invalid	0.3
Average Seed Correction	0.3		

In the example, both seed corrections are OK but only 1 ml of seed would be needed in the first case, while 2 or 3 ml would be acceptable in the second case.

4. Erratic seed correction bottles. For instance, the analyst has run 3 seed correction bottles and ends up with a seed correction of 0.3 mg/L in bottle #1, 0.5 mg/L in bottle #2, and 0.14 mg/L in bottle #3. Averaging the three values would give a value of 0.31 mg/L. However, the spread is very large. This could be due to the way the seed is handled. The seed collected needs to settle for a minimum of an hour to allow the settleable solids to get out of the way. If the seed is used too soon or the seed is shaken before being poured off, the settleable solids will give erratic results. Let seed settle for a minimum of an hour and DO NOT SHAKE. Filtering the seed sample through a filter might also help reduce settleable solid interferences. A Whatman #1 filter might work.--remember your not trying to filter out the bacteria, only the solids.

5. Erratic seed source, similar to #3. If the seed source is very inconsistent or continually weak, then a reasonable alternative remedy could be commercial seed. Commercial seed is lypholyzed bacteria, that "come alive" when they are rehydrated in dilution water. The activity is pretty constant but often low (0.1 mg/L/1 ml seed). The directions call for 4 ml of seed to be added to the GG bottle, which gives a seed correction of 0.4 mg/L so more commercial seed may need to be added. Trial and ERROR!

Glucose-Glutamic Acid Troubleshooting

Here's the big one. All is not well if the GG is bad. The glucose-glutamic acid standard should have a 5 day BOD of 198 $\pm$ 30.5 mg/L. If the results do not fall within this range, all sample data are invalid. The analyst must now determine the cause of the failure and remedy the problem. Possible causes could be:

1. Inactive/low seed. Inactive/low seed means low levels of living microorganisms. Without a living population, the glucose and glutamic acid cannot be metabolized. The GG results are normally low. Remedy: Find another reliable seed source, use commercial seed.

2. Old glucose/glutamic acid. Glucose and glutamic acid normally do not "age" but if the reagents are improperly stored, it is possible that these organic materials can break down. This is not a common problem. Remedy: Keep reagents current and store the stock bottles in a cool, dry area away from light. Discard outdated reagents. Purchase a commercial standard.

3. Improperly dried glucose/glutamic acid. If the GG was not dried, the GG may contain absorbed water (not visible, but may be noticable if crystals stick together). When weighed on the analytical balance, the 0.1500 gm will now contain a small amount of water weight. This will result in less than 0.1500 gm of GG being weighed. A lower amount of GG will most likely result

in a lower GG BOD. Remedy: Dry for an hour in the drying oven at 103°C, then store in a properly operating desiccator.

4. Improperly calibrated analytical balance. If the analytical balance is not zeroed or is not calibrated, the actual weight of the GG may be incorrect. Remedy: Verify the analytical balance is calibrated by comparing to Class 1 weights monthly.

5. Dirty BOD bottles. Dirty BOD bottles may cause either high or low GG results. If the "dirt" is toxic detergent residue, the microorganisms added may very well be killed. If the "dirt" is residual organics from a previous sample that was not washed out completely, the GG result will be higher. Remedy: Wash the bottles with hot water and detergent. Use a brush that is capable of getting the corners and the shoulders of the bottle. For samples that are extremely greasy, the bottles may need to be acid washed. Autoclaving the bottles is not really necessary, although it will denature the organics. Rinse the detergent with tap water 10x. It is best to use small volumes of rinse water, multiple times and allow time for the water to drain completely. What often happens is the rinsing is done so fast, that the water in the BOD bottle does not have enough time to drain out before the next rinse water is added. After rinsing with tap water, rinse with DI water at least 5 times. After drying, examine the bottle corners and shoulders. They should look clean. Don't allow the dirty BOD bottles to dry before washing. Leave them full until washing can be done. Its harder to clean your glass after leaving it in the sink for a week isn't it?

6. Contaminated dilution water. If the unseeded dilution water blanks drop more than 0.2 mg/L, the dilution water may be contaminated. Remedy: Correct the contamination problem by cleaning the bottle, replacing any hoses, inspecting reagents, etc.

7. Improperly calibrated DO meter. An improperly calibrated DO meter can give either high or low GG results. Before invalidating the test results, **always** recheck the calibration. While it is unlikely that meter calibration by itself will cause GG failure, it may be part of a number of problems which when added together cause the GG standard to fail. Remedy: Maintain the meter in good working condition by periodically verifying the DO using the Winkler Method and replacing the membrane, battery, and electrolyte periodically.

8. Deionized water. While unlikely, the deionized water could potentially be contaminated with heavy metals. If other samples indicate toxic conditions, repair or replace the DI water source.

BOD Quality Control

The lab technician should document the following information to support the BOD data. Use the following checklist to determine if sufficient information has been recorded to support the data in court.

Sample QC

☐Sample holding time cannot exceed 48 hours. *Document time of collection, arrival, and analysis.*

☐Samples must be preserved on ice or refrigeration until time of analysis. *Document refrigerator temperature*

☐Samples that have been chlorinated must be dechlorinated prior to analysis. *Document volume used to dechlorinate.*

□After samples have been dechlorinated in the lab, the chlorine residual must be rechecked prior to BOD analysis. *Document dechlorination check results.*

□Sample pH must be adjusted to between 6.5-7.5 if the initial pH is <6.0 or >8.5 *Document initial pH, volume of acid/base added.*

□After samples have been adjusted for pH, the adjusted pH must be documented. *Document final adjusted pH.*

□Samples that have been chlorinated must have seed added at the time of BOD analysis. *Document volume of seed added to each bottle.*

□Samples that have had pH adjustment must have seed added at the time of BOD analysis.

□Samples must be warmed to room temperature (20 $\pm$ 3 ^{o}C) prior to BOD analysis. *Document adjusted temperature.*

□Mix all samples thoroughly just prior to analysis

□Homogenize samples with large amounts of chunky suspended solids

□Effluent samples should be run in duplicate a minimum of 10% of the time.

Equipment QC

□BOD incubator must be 20°C $\pm$ 1.0^{o}C and should be checked and recorded daily when in operation. *Document daily adjusted temperature.*

Corrective Action: Incubator outside the control limits must be adjusted. ***Invalidate*** the BOD sample results

□The incubator thermometer must be immersed in water or glycerol.

□The incubator thermometer must be calibrated at least annually against a NIST certified thermometer. The calibration must include date, thermometer correction factor, serial number, and initials of the person performing the calibration. *Document thermometer calibration.*

□The DO meter must be calibrated at the time of use. *Document the calibration value.* Typical DO should be around 7.0-8.0 if calibration is OK.

Corrective Action: DO meter that does not calibrate, requires maintenance. *Document battery and membrane cap replacement.*

□The analytical balance must be calibrated annually by a certified balance technician. *Document date, balance condition, and name of technician and company.*

□The analytical balance should be checked monthly using Class 1 weights. A series of weights should be selected to cover the range of balance operation. Usually 1, 2, 5,10, 50, and 100 gm weights are used. *Document weight values.*

Corrective Action: If the weights deviate more than 0.0003 grams, the balance needs service. Request balance service.

□pH meters must be calibrated daily, prior to use.

□A minimum of 2 buffers should be used, with a 3rd buffer typically used. *Document calibration.*

Corrective Action: If the pH meter cannot be calibrated or the slope outside acceptable

levels, replace the electrode. *Document the electrode replacement.*

□Validate the DO meter calibration quarterly using the Winkler Azide Method. Check DO meter operation if readings are not within 0.2 mg/L. *Document calibration check*

BOD Test

□Dilution water should be checked annually for heavy metals. *Document values for lead, nickel, chromium, copper, zinc, and cadmium.*

□Collect seed on day of test, preferably around 8-9 am. *Document seed collection location and time.*

□Run a seed control set (minimum of 3) with each run of samples. *Document seed correction data.*

□Seed correction factor should be 0.6-1.0 mg/L. Do not invalidate BOD results if the seed correction is outside the range.

Corrective Action: If the seed correction factor is not within 0.6-1.0, adjust the volume of seed added to obtain the desired depletion for the next series of samples.

□Run unseeded blanks in duplicate. *Document test results*.

□Use large bore pipets for pipetting samples. Preferably, use graduated cylinders for large sample volumes

□Unseeded blank DO depletion should be <0.2 mg/L, preferably <0.1 mg/L. Do not invalidate the BOD results if the DO depletion is >0.2 mg/L.

Corrective Action: Check the bottles for cleanliness, recalibrate the DO meter, check the dilution water carboy for cleanliness, replace the aeration hose and pipet, clean the DO electrode in a separate bottle prior to use.

□There should be not air bubbles below the stopper in the BOD bottles at either the beginning or the end of the test. Do not invalidate the BOD results if there are air bubbles in the bottle at the end of the test.

Corrective Action: Fill the BOD bottles before placing the stopper. Check the water seal to be sure water is in the flared part of the bottle above the stopper and no bubbles are below the stopper. Place the plastic cap over the stopper.

□check the sample and dilution water temperature (should be close to 20°C), equilibrate the BOD dilution water longer.

□Glucose-glutamic acid standards should be run in duplicate. *Document test results.*

□Glucose-glutamic acid standards should be within 198 $\pm$ 30.5 mg/L after 5 days. If both standards are outside the control limits, the BOD data should be invalidated. However, the analyst should use his judgment and historical data prior to invalidating the sample results if one of the glucose-glutamic acid standards is within the acceptable range.

Corrective Action: Check the other controls such as unseeded blanks and seed control. If these controls are OK, then redry the glucose-glutamic acid. Check the analytical balance operation. Check the preparation procedure. Check DO meter calibration. Check the incubator temperature.

□DO depletion should be >2.0 mg/L and have a final DO >1.0 mg/L

Corrective Action: BOD bottles outside this range should be disregarded and any calculated values should not be used in the answer. Exception: If the sample is very clean and 300 ml of sample is used, calculate and report the results even if the DO depletion is not >2.0 mg/L.

Performance evaluation samples should be run at least annually. *Document results*.