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Biochemical Oxygen Demand (BOD)

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BIOCHEMICAL OXYGEN DEMAND (BOD)

Section 1: GENERAL

In the presence of free oxygen, aerobic bacteria use the organic matter found in wastewater as "food". The BOD test is an estimate of the "food" available in the sample. The more "food" present in the waste, the more Dissolved Oxygen (DO) will be required. The BOD test measures the strength of the wastewater by measuring the amount of oxygen used by the bacteria as they stabilize the organic matter under controlled conditions of time and temperature.

Section 2: BOD INTRODUCTION

The BOD test is used to measure waste loads to treatment plants, determine plant efficiency (in terms of BOD removal), and control plant processes. It is also used to determine the effects of discharges on receiving waters. A major disadvantage of the BOD test is the amount of time (5 days) required to obtain the results.

When a measurement is made of all oxygen consuming materials in a sample, the result is termed "Total Biochemical Oxygen Demand" (TBOD), or often just simply "Biochemical Oxygen Demand" (BOD). Because the test is performed over a five day period, it is often referred to as a "Five Day BOD", or a BOD_5.

In many biological treatment plants, the facility effluent contains large numbers of nitrifying organisms which are developed during the treatment process. These organisms can exert an oxygen demand as they convert nitrogenous compounds (ammonia and organic nitrogen) to more stable forms (nitrites and nitrates). At least part of this oxygen demand is normally measured in a five day BOD.

Sometimes it is advantageous to measure just the oxygen demand exerted by organic (carbonaceous) compounds, excluding the oxygen demand exerted by the nitrogenous compounds. To accomplish this, the nitrifying organisms can be inhibited from using oxygen by the addition of a nitrification inhibitor to the samples. The result is termed "Carbonaceous Biochemical Oxygen Demand", or CBOD.

Section 3: GLOSSARY

Aerobic: A condition in which "free" or dissolved oxygen is present in an aquatic environment.

Anaerobic: A condition in which "free" or dissolved oxygen is not present in an aquatic environment.

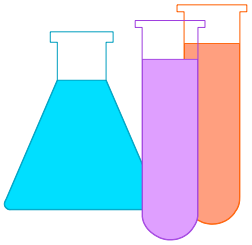
Blank: A preliminary analysis omitting only the sample to provide an unbiased reference point or baseline for comparison.

Nitrification: An aerobic process in which bacteria change ammonia and organic nitrogen in wastewater into oxidized nitrogen (usually nitrate). The second-stage BOD is sometimes referred to as the "nitrification stage". (The first stage is called the "carbonaceous stage".)

Nutrient: Any substance used by living things that promotes growth.

Respiration: The process in which an organism uses oxygen for its life processes and gives off carbon dioxide.

Seeding: The process of adding live bacteria to a sample.



Section 4: APPROVED METHODS

Always refer to your facility MPDES Permit and 40 CFR Part 136 for the approved sampling and test procedure. The approved methods for determining the initial and final dissolved oxygen levels in the five day BOD test are the modified Winkler and membrane probe methods.

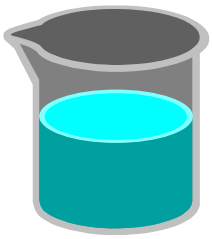
Section 5: SAFETY AND HYGIENE

When testing for BOD, the concerns for safety involve wastewater hazards and exposure to chemicals. Precautions to follow include the following:

1. Cover all abrasions and wear good quality latex gloves when in direct contact with raw wastewater.
2. Wash hands frequently, and always wash hands prior to eating or smoking.
3. Clean up all spills of wastewater or buffers immediately.
4. Wear a protective smock, apron, or lab coat, and surgical or rubber gloves when working in the laboratory to protect clothes and skin.
5. Read all labels carefully and know what to do in case of a spill.
6. Always use a pipette bulb. NEVER pipette anything by mouth.

7. Always pour acids or bases into water, never add water to the acid or base. Mixing concentrated acids or bases with water can create a significant amount of heat.

8. Use care when handling sharps (broken glass etc.).



Section 6: SAMPLING

Good sampling is necessary if laboratory testing is to be accurate. The sample must be representative, collected properly, handled carefully and preserved correctly. No matter how accurate the actual testing is, if the sample is not representative, the results of the test will be misleading and can lead to poor plant performance.

TYPES OF SAMPLES

Samples used for the BOD test can be either grab or composite. A composite sample is usually specified in most NPDES Permits and will be more representative of the wastestream over a period of time than will a grab sample. The type and location of sample taken will depend on your facility NPDES Permit. Samples should be the type which best fits the capabilities of and requirements for each individual plant. Samples should be taken at a point where they will be well-mixed and proportional to the amount of the flow.

SAMPLE PRESERVATION

Samples for BOD analysis may change greatly during handling and storage. Testing should be started as quickly as possible. To reduce the changes in those samples which must be held, keep the samples at or below 4°C. Do not allow samples to freeze. Samples may be kept for no more than 48 hours before beginning the BOD test.

NOTE: The 48 hours starts when the very first aliquot of a composite sample is collected (i.e. when the composite sampler starts collecting a composite sample).

SAMPLE CONTAINERS

Special sampling devices and storage containers are not necessary for collecting samples for BOD determinations. Sampling devices should be capable of collecting samples from well-mixed areas of tanks and/or pipes, made of resistant materials that will not rust or corrode, capable of taking samples that are proportional to the plant's flow and easily cleaned (including acid cleaning).

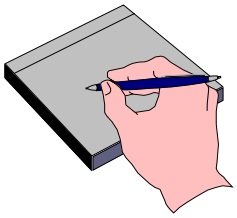
NOTE: A long-handled aluminum dipper attached to a wooden handle, or an equivalent device, is acceptable for collecting samples. Do not use containers such as coffee cans.

Storage containers should be made of corrosion-resistant material (such as plastic), which can stand repeated refrigeration. These containers should have leak-proof caps or lids.

SAMPLE CONTAINER PREPARATION

All collection containers should be cleaned thoroughly on a regular basis (preferably at the end of each day's sampling) with soap and water, and rinsed well with distilled water. This will prevent buildup (such as grease and scum) from contaminating samples. Between sample collections, the sampling containers should be rinsed thoroughly and allowed to dry. This is especially important for containers used for samples which are high in solid and/or grease content. It is recommended that each collection point have its own sampling container. One sampling container should not be used throughout the plant. If separate containers are not possible, be sure to clean sampling containers thoroughly between collections.

Sample storage containers should also be cleaned thoroughly between samples. It is recommended that they be acid cleaned on a regular basis to prevent residue buildup which occurs over time. It is also recommended that each sampling point have its own storage container. (For example, if influent and effluent samples are taken, always use the same containers for influent and do not use a container for influent one day and then effluent the next.) The containers should be clean and dry before a new set of grab or composite samples are stored in them.



Quiz 5.1

1. What does a BOD test measure?
2. What can the BOD test be used for?
3. What are the types of samples and maximum sample holding time?

Section 7: BOD - DESCRIPTION OF METHOD

A sample is pipetted into a BOD bottle containing aerated dilution water. The DO content is determined and recorded and the bottle is incubated in the dark for five days at 20°C. At the end of five days, the final DO content is determined and the difference between the final DO reading and the initial DO reading is calculated. The decrease in DO is corrected for sample dilution, and represents the biochemical oxygen demand of the sample.

Section 8: EQUIPMENT AND REAGENTS

REAGENTS

See Appendix B for the procedures for preparation of the reagents used in this method. Test reagents are as follows:

1. Phosphate buffer solution
2. Magnesium sulfate solution ($MgSO_4$ $7H_2O$) *

3. Calcium chloride solution ($CaCl_2$) *

4. Ferric chloride solution ($FeCl_3$) *

5. Sodium hydroxide (NaOH), 1 N *

6. Sulfuric acid (H_2SO_4), 1 N *

7. Sodium sulfite (Na_2SO_3), 0.025 N

8. Potassium iodide solution (KI), 10%

9. Acetic acid solution (CH_3CO_2H), (1+1) *

10. Sulfuric acid solution (H_2SO_4), (1+50) *

11. Starch indicator solution

12. Glucose-glutamic acid solution

13. Nitrification inhibitor (2-chloro-6-(trichloromethyl) pyridine) *

14. Distilled water.

NOTE: Use only high-grade distilled or deionized water. The water must contain less than 0.01 mg/L copper, and be free of chlorine, chloramines, caustic alkalinity, organic material, or acids.

***** These reagents are poisonous or corrosive and should be handled **with extreme caution.**

EQUIPMENT

1. BOD meter with probe for measurement of dissolved oxygen in 300 mL BOD bottles

2. 300 mL BOD bottles

3. Incubator, capable of maintaining 20 +/- 1°C

4. 250 mL graduated cylinders

5. 100 mL graduated cylinders

6. 25 mL measuring pipettes (wide-mouth)

7. 10 mL measuring pipettes (wide-mouth)

8. 100 mL beaker

9. 1000 mL beaker

10. 250 mL Erlenmeyer flask

11. Burette graduated to 0.1 mL

12. Dilution water bottle of suitable volume for the number of tests to be performed

13. Pipette bulb

14. Equipment for pH measurements **

15. Magnetic stirrer and stirring bars **

** Optional equipment

Section 9: DETERMINATION OF SAMPLE SIZE

The BOD test relies on a measurable depletion of DO over a specified period of time. Because most samples of wastewater will have a BOD higher that the amount of oxygen available in the BOD bottle during the incubation period, the samples must be diluted. This dilution is done by adding dilution water to the sample in the BOD bottle. If the sample is not diluted, the biological activity of the microorganisms will use up the DO in the BOD bottle before the five day incubation time is up. If the final DO is too low, the BOD cannot be determined. There is no way of knowing at what point during the five days the DO reached zero.

One of the most difficult steps in the BOD procedure is deciding how much sample to place in the BOD bottles for incubation. Some plants have influent and effluent BOD's that do not vary greatly over time, while others fluctuate greatly from day to day. In all cases, several different dilutions of each sample should be prepared to obtain the desired DO depletions.

Once a general range for the BOD of a sample has been determined, the dilutions can be established which will ensure that at least one dilution will meet the criteria for valid BOD results. The following procedure can be used to calculate volumes for sample dilution from the estimated BOD.

For example, suppose the estimated BOD of an influent sample is 400 mg/L and assume the DO of saturated dilution water is 8.0 mg/L. Since the criteria for most valid results states that the DO depletion at the end of five days incubation should be at least 2.0 mg/L and the residual DO at least 1.0 mg/L, the formulas to calculate the minimum and maximum estimated dilution are as follows:

A. **mL sample added to BOD bottle = (minimum allowable** depletion, mg/L x Volume of BOD bottle, mL)/estimated BOD, mg/L

Example:

minimum mL sample = [(8 mg/L - 6 mg/L) x 300 mL]/400 mg/L

minimum mL sample = (2 x 300)/400 = 600/400 = 1.5 mL

B. **mL sample added to BOD bottle = (maximum allowable** depletion, mg/L x Volume of BOD bottle, mL)/estimated

BOD, mg/L

Example:

maximum mL sample = [(8 mg/L - 1 mg/L) x 300 mL]/400 mg/L

maximum mL sample = (7 x 300)/400 = 2100/400 = 5.25 mL

Since the BOD value used is only an estimate, and BOD bottles do not always have a volume of exactly 300 mL, several bottles with different volumes of sample are set up to ensure that test requirements are

met. For the examples above, four bottles would be used with 1 mL, 3 mL, 4 mL, and 6 mL, and the results averaged for the final BOD.

NOTE: Those sample dilutions which deplete less than 2 mg/L, or have a final DO of less than 1 mg/L would not be used in the calculation of the average sample BOD.

Section 10: PREPARATION OF DILUTION WATER

It is very important that the distilled water used for dilution water be of high grade and free from contaminants (such as copper and chlorine) which could inhibit the growth of bacteria. For this reason, it is recommended that ordinary commercial distilled water (i.e. for use in car batteries) not be used. Prepare dilution water as follows:

1. If necessary, aerate a bottle of distilled water long enough to allow the water to become saturated with dissolved oxygen (approximately 8 mg/L at room temperature). This can be accomplished by aerating with clean compressed air. If 5-gallon water bottles are used, aerate for 24 hours with a small aquarium pump.

2. If less than the entire bottle will be used in a single day, siphon into a separate container an amount slightly greater than will be needed for the sample dilutions.

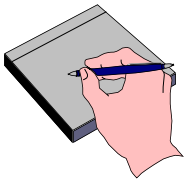
3. Add 1 mg/L each of phosphate buffer, magnesium sulfate solution, calcium chloride solution and ferric chloride solution.

NOTE: The Hach Company manufactures slurry pillows which can be used as a substitute for the nutrient reagents. Use the appropriate slurry pillow for the volume of dilution water being prepared.

4. For those samples which require seeding, the analyst may add a sufficient volume of seed directly to the dilution water, or a small amount of seed directly to the sample dilutions.

5. If nitrification inhibition is used, store seeded dilution water at 20°C long enough for the dilution water depletion to meet the quality criteria (depletion of no more that 0.2 mg/L DO). Storage is not recommended when nitrification inhibition is not going to be used because nitrifying bacteria can develop in the dilution water during storage.

6. If nitrification inhibition is to be used, add enough nitrification inhibitor to the dilution water to produce a final concentration of 10 mg/L. As an alternative, 3.33 mg of nitrification inhibitor can be added to each BOD bottle for inhibition.



Quiz 5.2

1. What reagents are required for the BOD_5 and/or $CBOD_5$ test?

2. What equipment, apparatus, or instrumentation is required for the BOD_5 and/or for $CBOD_5$ test?

3. If the expected BOD of a sample is in the range 25 mg/L to 75 mg/L, what would be the minimum and maximum sample volumes to use for the sample dilution?

Section 11: PRETREATMENT OF SAMPLE

Samples with extreme pH values and samples containing disinfectants such as residual chlorine must be treated prior to testing.

PRETREATING SAMPLES

THAT CONTAIN CAUSTIC ALKALINITY OR ACIDITY

Caustic alkalinity or acidity can prevent bacteria from growing during the course of the BOD test. To prevent this, samples which have pH values higher than pH 8.0 or lower than pH 6.0 must be neutralized to pH 7.0 before the test is performed.

NOTE: Neutralized samples must be seeded for the BOD test.

Procedure for neutralizing samples

1. Pour 50 mL of sample into a 100 mL beaker.

2. Measure the pH of the sample using a pH meter. If the pH is out of the range of pH 6.0 to pH 8.0 continue with steps 3-6, otherwise perform the BOD test on the untreated sample.

3. Add 1 N sulfuric acid if the sample is alkaline, or 1 N sodium hydroxide if the sample is acidic, until the pH reaches 7.0.

4. Calculate the amount of sulfuric acid or sodium hydroxide needed to neutralize 1000 mL of the sample.

5. Add the calculated amount of acid or base to the sample.

6. Repeat steps 1-5 until the pH test shows pH 7.0.

Calculation

7. Calculate the amount of 1 N sodium hydroxide or 1 N sulfuric acid needed to neutralize the sample to pH 7.0 using the following formula:

mL needed = (mL acid or base used x mL total test sample)/mL sample portion used for neutralization.

For example, suppose 1.3 mL of 1 N NaOH are used to neutralize 50 mL of sample to pH 7.0. Calculate the volume of NaOH to be added to neutralize the sample as follows:

mL 1 N NaOH needed = (1.3 mL x 1000 mL)/50 mL = 1300/50 = 26 mL

TO PREVENT INTERFERENCE FROM CHLORINE

Because chlorine is such a strong oxidizing agent, it will inhibit the growth of living bacteria in the BOD test. Any samples containing residual chlorine must be pretreated to remove chlorine before the test is run. This is done by adding sodium sulfite to the sample.

NOTE: Those samples which are dechlorinated must be seeded for the BOD test.

Procedure for dechlorinating samples:

1. To a 250 mL Erlenmeyer flask, add 100 mL of a well-mixed portion of the sample to be dechlorinated.

2. Add 10 mL of either 1+1 acetic acid solution or 1+50 sulfuric acid solution to the flask and swirl to mix.

3. Add 10 mL of potassium iodide (KI) solution, and 1 mL of starch indicator solution. Swirl to mix and let stand for 15 minutes.

4. If a blue color does not appear, there is no chlorine in the sample and it does not require further treatment prior to the BOD test.

NOTE: Do not assume that the sample was not chlorinated simply because there is no reaction. Chlorine can disappear from the sample while it sits in the sample container. The only way to be sure a sample is not chlorinated is to know exactly where the sample was collected.

5. If a blue color appears, titrate the treated portion of sample with 0.025 N sodium sulfite (Na_2SO_3) until the blue color first disappears. Record this amount on a lab sheet.

6. Calculate the amount of sodium sulfite solution needed to dechlorinate the selected BOD sample volume.

7. Add the calculated volume of sodium sulfite to the BOD sample and mix thoroughly.

8. Allow the sample to stand for 10 to 20 minutes, then repeat steps 1-3.

9. If no chlorine is detected, continue with the BOD test procedure, otherwise continue with steps 5-8 until the sample is dechlorinated.

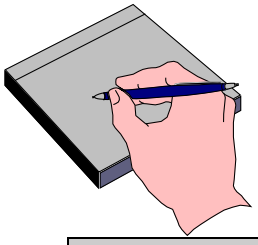
Calculation

10. Calculate the amount of sodium sulfite needed to dechlorinate the BOD sample using the following formula:

    mL Na_2SO_3 needed = (mL Na_2SO_3 used x mL total test sample)/mL sample portion used for dechlorination.

For example, suppose 1.0 mL of Na_2SO_3 is needed to titrate 100 mL of sample for dechlorination. Calculate the volume of Na_2SO_3 needed to dechlorinate 1500 mL of the BOD sample as follows:

mL Na_2SO_3 needed = (1.0 mL x 1500 mL)/100 mL = 1500/100 = 15 mL



Quiz 5.3

1. Why must samples containing caustic alkalinity or acidity be adjusted before preparing BOD dilutions?

2. What reagents and equipment are required to adjust the pH of BOD samples?

3. Why must samples containing residual chlorine be dechlorinated before preparation of BOD dilutions?

4. What reagents are required to chemically dechlorinate a BOD sample?

5. What must be done to samples which have been dechlorinated or adjusted for pH variations?

Section 12: LABORATORY PROCEDURE

1. Completely fill two BOD bottles with dilution water.

2. Into additional BOD bottles, partially filled with dilution water, carefully measure out the proper volume of sample. Add dilution water until the bottles are completely filled.

NOTE: If the modified Winkler procedure is to be used for DO measurements, two BOD bottles should be prepared for each dilution; one for determination of the initial DO and one for incubation and final DO measurement. If the meter method is used for DO measurements the initial and final DO determinations can be performed on the same bottle.

ADDITIONAL NOTE: If the nitrification inhibition is to be used to determine the carbonaceous BOD fraction (CBOD) of the sample, a separate dilution series of uninhibited sample can be prepared to determine the combined nitrogenous and carbonaceous BOD for the sample. To inhibit the nitrifying bacteria in the sample, add 3.33 mg of nitrification inhibitor to one set of sample dilutions, while the second set of dilutions remains untreated. Continue with the remaining procedural steps with both sets of dilutions.

3. Stopper each bottle taking care to avoid trapping air bubbles inside the bottles as the bottle stoppers are inserted.

4. Fill the top of each bottle neck around the stopper with dilution water.

5. Determine the initial DO content on one of each set of duplicate bottles, including the dilution water blank by one of the approved methods and record data on the lab sheet.

6. Place the remaining bottles in the incubator at 20°C and incubate for five days.

7. At the end of exactly five days (+/-3 hours), test the DO content of the incubated bottles.

8. Calculate the BOD for each dilution. The most accurate BOD will be obtained from those dilutions that have a depletion of at least 2 mg/L DO and at least 1.0 mg/L DO residual. If there is more than one dilution that meets these criteria, the BOD results should be averaged to obtain a final BOD value.

9. The dilution water blanks are used only to check the quality of the dilution water. If the quality of the water is good and free from impurities, the depletion of DO should be less than 0.2 mg/L. In any event, do not use the depletion obtained as a blank correction.

10. If nitrification inhibition is used, the BOD test must also be performed on a series of sample dilutions which have not been inhibited.

11. Report the results of the nitrification inhibited samples as $CBOD_5$ and uninhibited samples as BOD_5.

Section 13: CALCULATIONS

To determine the value of the BOD in mg/L, use the following formula:

BOD, mg/L = [(Initial DO - Final DO) x 300]/mL sample

For example:

Initial DO = 8.2 mg/L

Final DO = 4.4 mg/L

Sample size = 5 mL

BOD mg/L = [(8.2 - 4.4) x 300]/5 = (3.8 x 300)/5 = 1140/5 = 228 mg/L

Whenever a sample is dechlorinated, it must be seeded. If the sample is seeded, a correction factor must be calculated to determine the effects that the seed material has on the DO depletion. A number of BOD's must be run on the seed material to determine the seed correction factor.

Section 14: INTERFERENCES

Since the BOD test is dependent on biological activity, the major interferences will be those substances which inhibit the growth of the microorganisms. These will include chlorine, caustic alkalinity or acidity, mineral acids, and heavy metals (such as copper, zinc, chromium, and lead).

Excessive nitrites can interfere with the BOD determination. Growth of algae in the presence of light can cause problems by actually increasing the DO of the sample before testing, which must be removed by deaeration.

A common problem encountered in BOD testing results from residues building up in the BOD and dilution water bottles. To prevent this, all glassware should be acid cleaned on a regular basis.

Section 15: PRECISION AND ACCURACY

While precission is sometimes tough with the BOD test, a check of dilution water quality, seed effectiveness, and analytical technique can be made using a glucose-glutamic acid solution. A 2% dilution (6 mL per 300 mL BOD bottle) should yield 200 +/-37 mg/L BOD, after five days incubation at 20°C. To ensure valid results for this "standard" check, the glucose-glutamic acid dilutions must be seeded since the solution is essentially sterile and does not contain any microorganisms.

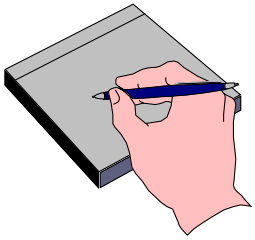
The BOD test is a biological test, dependent on the actions of the microorganisms found in the wastewater and, as such, is subject to a number of variations. These variations can be caused by a number of factors, including changes in temperature, weather, composition of incoming sewage, in-plant operations, and sampling points. Results can vary widely from day to day, or even hour to hour. One of the major disadvantages of the BOD test is the time lag between the collection of samples and the final calculation of results. This makes the BOD test a poor test for determining whether or not operational changes are needed.

In addition, the rate and degree that organic matter in wastewater is decomposed (or oxidized) by the normal bacteria present in a sample is largely dependent on the characteristics of the organic matter. For example, some organic matter (like sugars or starches) are oxidized very easily and rapidly, and will almost always result in measurable "BOD". Other organic matter, however, is sometimes resistant to biological oxidation, and may require special "acclimated" bacteria to oxidize the material and to show a "BOD". Although this is what actually happens in nature, it causes significant variation in BOD results from sample to sample.

Some common ranges of BOD results are as follows, in mg/L:

Influent	150-400
Primary Effluent	60-160
Secondary Effluent	10-60
Digester Supernatant	1000-4000+
Industrial Wastes	100-3000+

CBOD results are almost always lower than TBOD results. For a highly nitrified effluent sample, the difference can be as great as 50%.



Quiz 5.4

1. Why must a dilution water blank be included with each series of BOD tests?
2. What is the accepted temperature range and time of incubation for the BOD test?
3. What is being measured when nutrification inhibition is used in the BOD test?
4. What are the criteria for most valid results of the BOD test?

Section 16: SEED CORRECTION PROCEDURE

The BOD test relies on the presence of healthy organisms. If the samples tested contain materials which could kill or injure the microorganisms (such as chlorine, high or low pH, toxic materials), the condition must be corrected and healthy active organisms added. This process is known as seeding. The following step-by-step description is one technique for seeding samples and the seed correction procedure. There are other techniques which can be used.

NOTE: The appropriate pretreatment step (dechlorination, pH adjustment, acclimation, etc.) should be performed prior to preparation of the sample dilutions for this test.

PREPARATION OF SEED MATERIAL

Select a material to be used for seeding which will have a BOD of at least 180 mg/L. This will help ensure that the seed correction meets the 0.6 mg/L minimum specified in “Standard Methods”, current Edition. Place the material in a suitable container and incubate at 20°C for 24-36 hours. Usually, settled raw domestic sewage prepared in the manner above will have sufficient BOD for use as a seed material. If not, small quantities of digester supernatant, return activated sludge, or an acclimated seed material can be used to increase the potency of the seed material used for the test. As an alternative, commercially available seed material may be used. The seed correction should not exceed 1.0 mg/L BOD, therefore care should be taken not to use too strong a seed material for the test. The key to a good seed correction is a relatively stable seed material which produces a good seed correction in every test situation.

SEED BOD DETERMINATION

This step requires preparation of a dilution series using the seed material and unseeded dilution water. Prepare two bottles of each dilution for the seed control series. If the meter method is used for DO measurements, only one bottle for each dilution needs to be prepared. The percentage of seed used in each dilution and the number of dilutions is optional, but sufficient dilutions should be used to ensure that at least one dilution gives a depletion of 2.0 mg/L with at least 1.0 mg/L DO residual.

Determine the initial DO of each dilution, then incubate the dilutions for five days at 20°C. At the end of the incubation period, determine the final DO of the dilutions. Calculate the depletion of each seed dilution using formula #1 below.

#1 DO depletion = Initial DO - Final DO

Select the seed dilution(s) which meet the required criteria and calculate the BOD of the seed material using formula #2 below. (If more than one dilution meets the criteria, calculate the BOD of each such dilution and average the results for the seed material BOD.)

#2 Seed BOD = (DO depletion x 300)/Seed dilution, mL

The calculated seed BOD represents the BOD exerted by 300 mL of undiluted seed material. The ratio of the seed BOD to 300 mL will be used to calculate the seed correction for seeded samples.

DETERMINATION OF SEED VOLUME

The most common methods for introducing the seed material into the sample dilutions are (a) addition of the seed to the dilution water and (b) addition of the seed directly to the sample BOD bottles. Method (a) requires a calculation to determine the volume of seed for each dilution since the amount of seed will vary with the volume dilution water used for each sample dilution. Method (b) is somewhat easier to use as the volume of seed for each dilution is constant.

When Method (a) is used to introduce the seed material, calculate the volume of seed in each sample dilution using formula #3 below.

#3 Volume of seed in sample dilution = (Volume of seed in dilution water x dilution water in sample, mL)/Total volume of dilution water.

SEED CORRECTION

The calculation of the seed correction is based on the BOD of the seed material and the volume of seed used in each dilution. The seed correction is actually the oxygen demand exerted by the oxidation of the small amount of organic matter in the seed material in the sample dilutions. If the BOD exerted by 300 mL of seed material and the volume of seed material in each sample dilution are known, the seed correction can be calculated using formula #4 below.

#4 Seed Correction = (Seed BOD x mL seed in sample dilution)/300

It should be noted that the seed correction for each sample dilution must be calculated when the seed is added to the dilution water. When the seed is added directly to each BOD bottle, the seed correction is the same for all seeded dilutions using the same seed volume and material.

DETERMINATION OF SAMPLE BOD

The calculated seed correction is subtracted from the DO depletion in the determination of the BOD for each valid sample dilution. It should be noted that there are two criteria specified in “Standard Methods” which should be checked before the seed correction is used to determine the sample BOD. Those sample dilutions meeting these criteria should yield the most valid results. These criteria are as follows:

1. The sample dilutions should deplete at least 2.0 mg/L DO after five days incubation at 20°C.

2. The sample dilutions should have a final DO of at least 1.0 mg/L after five days incubation at 20°C.

   The BOD, using the seed correction, should be calculated for the sample dilutions which meet both criteria. If more than one sample dilution meets the criteria, the final BOD should be an average of

the individual BOD results for the sample dilutions. If none of the sample dilutions meet both of the criteria, the one dilution which comes closest should be used to calculate the final BOD of the sample.

NOTE: If this is the case, a notation should be made on the sample bench sheet that potentially invalid data has been used to determine the noted value. Sample dilution volumes should be carefully selected to ensure that at least one dilution meets both criteria.

Calculate the seeded sample BOD using formula #5 below.

#5 BOD mg/L = (DO depletion - Seed correction) x 300/mL of sample

EXAMPLE BOD DETERMINATION

Seed Material Data:

A series of dilutions were prepared in 300 mL BOD bottles using settled raw sewage and unseeded dilution water. The dilution range, initial DO, final DO, and depletions (using formula #1) are given in Table 2.

Table 2

Bottle #	mL Seed	Initial DO	Final DO	Depletion
1	3	7.95	5.20	2.75
2	6	7.95	3.85	4.10
3	9	7.90	2.40	5.50
4	12	7.85	1.35	6.50

Since all of the dilutions meet the desired criteria, the seed BOD should be the average of all the calculated BOD values for the dilutions. Use formula #2 to determine the BOD of each seed dilution, then calculate the average seed BOD.

Bottle #1 BOD = [(7.95 - 5.20) x 300]/3 = (2.75 x 300)/3 = 275

Bottle #2 BOD = [(7.95 - 3.85) x 300]/6 = (4.10 x 300)/6 = 205

Bottle #3 BOD = [(7.90 - 2.40) x 300]/9 = (5.50 x 300)/9 = 183

Bottle #4 BOD = [(7.85 - 1.35) x 300]/12 = (6.50 x 300)/12 = 162

Average seed BOD = (275 + 205 + 183 + 162)/4 = 825/4 = 206 mg/L

This value represents 206 mg/L BOD exerted by 300 mL of the seed material. In other words, a 300 mL sample of the undiluted seed material would use 206 mg/L DO if incubated at 20°C for five days (assuming that oxygen was available to the sample).

Sample Data

Two series of sample dilutions were prepared at the same time as the seed control series. Sample series "A" was prepared by adding 1 mL of the seed material directly to each 300 mL sample BOD bottle. Series "B" was prepared by adding 4 mL of the seed material to each liter of dilution water. An unseeded dilution water blank was also run with the series (depletion = 0.2 mg/L DO).

Series "A"

Bottle #	mL Seed	Initial DO	Final DO	Depletion
5	10	8.00	6.30	1.70
6	50	7.95	4.60	3.35
7	75	7.70	3.90	3.80
8	100	7.55	0.90	6.65

Examination of the data reveals that bottle #5 can be discarded because it does not meet the 2.0 mg/L depletion criteria, and bottle #8 can be discarded because it does not meet the minimum final DO criteria. The seed correction then will only be applied to bottles #6 and #7.

Using formula #4, the seed correction (S.C.) is determined:

S.C. = (Seed BOD x mL seed in sample dilutions)/300

S.C. = (206 x 1)/300 = 0.69 mg/L

Using formula #5, the BOD for each valid sample dilution can be calculated:

BOD for bottle #6 = [(3.35 - 0.69) x 300]/50 = (2.66 x 300)/50 = 15.96 mg/L

BOD for bottle #7 = [(3.80 - 0.69) x 300]/75 = (3.11 x 300)/75 = 12.44 mg/L

The average BOD for two valid dilutions representing the sample BOD is 14.2 mg/L BOD.

Series "B"

Bottle #	mL Seed	Initial DO	Final DO	Depletion
9	10	8.10	6.30	1.80
10	50	7.95	4.50	3.45
11	75	7.65	3.85	3.80
12	100	7.15	0.70	6.45

As in series "A", examination of the data reveals that bottles #9 and #12 can be discarded because they do not meet both of the criteria for most valid results. Since the dilution water for these samples was seeded, individual seed corrections must be determined for bottles #10 and #11.

Using formula #3, the volume (mL) of seed material in each valid dilution is determined:

mL seed in bottle #10 = (4 x 250)/1000 = 1.0 mL

mL seed in bottle #11 = (4 x 225)/1000 = 0.9 mL

Using formula #4, the seed correction (S.C.) for each valid dilution is determined:

S.C. for bottle #10 = (206 x 1)/300 = 0.69

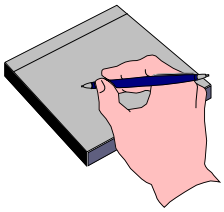
S.C. for bottle #11 = (206 x 0.9)/300 = 0.62

Using formula #5, the BOD for each valid dilution can be determined:

BOD for bottle #10 = [(3.45 - 0.69) x 300]/50 = (2.76 x 300)/50 = 16.56 mg/L

BOD for bottle #11 = [(3.80 - 0.62) x 300]/75 = (3.18 x 300)/75 = 12.72 mg/L

The average BOD for the two valid dilutions representing the sample BOD is 14.64 mg/L.



Quiz 5.5

1. Why must samples which have been dechlorinated or adjusted for pH variations be seeded?

2. What materials can be used to seed a BOD sample?

3. What is the acceptable range for seed correction factors in the BOD test?

Section 17: QA/QC

A Quality Assurance/Quality Control program is required by the NPDES permit. Quality Assurance (QA) is a set of operating principles that must be followed during sample collection and analysis. Lab bench sheets must be maintained that document when the sample was collected, how it was preserved and what results were obtained.

Quality Control (QC) includes any testing which is done to prove that the results are reliable. One of every ten samples analyzed should be a Quality Control check. This may include duplicate samples, spike samples, reagent blank analyses and known QC samples obtained from outside sources.

Duplicate sample analysis involves analyzing the same sample twice and comparing the results. The closer the results, the more accurate the analysis. Results should not differ by more than 10%. Spike sample analysis involves adding known amounts of analyte to a sample and calculating the percent recovery. These are discussed further in Chapter 10.

In BOD testing, dilution water blanks must be run with each group of samples and should not show a depletion of more than 0.2 mg/L DO. Duplicate samples should be analyzed to test for variability. A glucose-glutamic acid solution should be made by dissolving 150 mg each of oven dried glucose and glutamic acid in 1 L of distilled water. Six mL of this solution in a 300 mL BOD bottle should yield 200 +/- 37 mg/L BOD after five days incubation at 20°C. The glucose-glutamic acid dilutions must be seeded since the solution does not contain any microorganisms. A sample bench sheet is included in Appendix C.

Answers To Quizzes

Quiz 5.1

1. What does a BOD test measure?

   A BOD test measures the strength of the wastewater based on the amount of oxygen needed to stabilize the organic material in the wastewater.

2. What can the BOD test be used for?

   A BOD test can be used to measure waste loadings to treatment plants, plant efficiency and the effects of a discharge on a receiving stream, and to control the plant process.

3. What are the types of samples and maximum sample holding time used for the BOD test?

   Grab or composite samples can be used depending on permit requirements. The maximum holding time is 48 hours at 4°C.

Quiz 5.2

1. What reagents are required for the BOD_5 and/or $CBOD_5$ test?

   a. Chemicals for the Winkler DO test if used;
   b. phosphate buffer, pH 7.2;
   c. magnesium sulfate solution;
   d. calcium chloride solution;
   e. ferric chloride solution;
   f. distilled water;
   g. glucose-glutamic acid solution; and,
   h. nitrification inhibitor for CBOD.

2. What equipment, apparatus, or instrumentation is required for the BOD_5 and/or $CBOD_5$ test?

   a. Equipment for DO measurements;
   b. 300 mL BOD bottles;
   c. 10 and 25 mL measuring pipettes;
   d. 100 and 250 mL graduated cylinders;
   e. BOD incubator; and
   f. dilution water.

3. If the expected BOD of a sample is in the range 25 mg/L to 75 mg/L, what would be the minimum and maximum sample volumes to use for the sample dilutions?

   8 mL and 84 mL

Quiz 5.3

1. Why must samples containing caustic alkalinity or acidity be adjusted before preparing BOD dilutions?

   Caustic alkalinity and acidity can prevent the growth of bacteria during the test which prevents the use of oxygen.

2. What reagents and equipment are required to adjust the pH of BOD samples?

   a. Sodium hydroxide, 1 N;
   b. sulfuric acid, 1 N;
   c. pH meter;
   d. 100 and 1000 mL beakers; and,
   e. 10 mL measuring pipettes.

3. Why must samples containing residual chlorine be dechlorinated before preparation of BOD dilutions?

   The presence of chlorine in a sample will inhibit the growth of bacteria during the BOD test.

4. What reagents are required to chemically dechlorinate a BOD sample?

   Sodium sulfite solution, 0.0250 N; Potassium iodide solution, 10%; acetic acid (1+1) or sulfuric acid (1+50); Starch indicator.

5. What must be done to samples which have been dechlorinated or adjusted for pH variations?

   They must be seeded and a seed correction used in the calculation of the BOD.

Quiz 5.4

1. Why must a dilution water blank be included with each series of BOD tests?

   To check the quality of the dilution water.

2. What is the accepted temperature range and time of incubation for the BOD test?

   20 +/-1°C and 5 days

3. What is being measured when nitrification inhibition is used in the BOD test?

   Carbonaceous BOD (CBOD)

4. What are the criteria for most valid results of the BOD test?

   Use dilutions which deplete at least 2.0 mg/L after 5 days and have at least 1.0 mg/L DO remaining in the dilution.

Quiz 5.5

1. Why must samples which have been dechlorinated or adjusted for pH variations be seeded?

   Samples must be seeded to add healthy organisms to a sample which has been stressed by toxic conditions (chlorine, high or low pH, etc.)

2. What materials can be used to seed a BOD sample?

   Any material which can provide a suitable population of organisms can be used, however, settled raw sewage or commercially prepared seed material are the most common sources.

3. What is the acceptable range for seed correction factors in the BOD test?

   0.6 to 1.4 mg/L seed correction

APPENDIX A

References

Standard Methods for the Examination of Water and Wastewater, APHA-AWWA-WEF, 18™ Edition, 1992, Method 5210.

Methods for Chemical Analysis of Water and Wastes, U.S. EPA 600/4-79-020, March 1979, Method 405.1.

NOTES:

APPENDIX B

Preparation of Chemicals

SAFETY NOTE: At a minimum, hand and eye protection should be used when handling any of the chemicals mentioned in this section. Before working with any chemical, consult the appropriate Material Safety Data Sheet (MSDS) to determine if other safety precautions are necessary.

BIOCHEMICAL OXYGEN DEMAND REAGENTS

Phosphate buffer solution

Dissolve 8.5 g potassium dihydrogen phosphate (KH_2PO_4), 21.75 g dipotassium hydrogen phosphate (K_2HPO_4), 33.4 g disodium hydrogen phosphate heptahydrate (Na_2HPO_4 $7H_2O$), and 1.7 g ammonium chloride (NH_4Cl) in about 500 mL of distilled water and dilute to 1 liter. The pH of this buffer should be 7.2 and should be checked with a pH meter. Discard this reagent if there is any sign of biological growth in the storage bottle.

Magnesium sulfate solution

Dissolve 22.5 g magnesium sulfate heptahydrate ($MgSO_4$ $7H_2O$) in distilled water and dilute to 1 liter. Discard this reagent if there is any sign of biological growth in the storage bottle.

Calcium chloride solution

Dissolve 27.5 g anhydrous calcium chloride ($CaCl_2$) is distilled water and dilute to 1 liter. Discard this reagent if there is any sign of biological growth in the storage bottle.

Ferric chloride solution

Dissolve 0.25 g ferric chloride hexahydrate ($FeCl_3$ $6H_2O$) in distilled water and dilute to 1 liter. Discard this reagent if there is any sign of biological growth in the storage bottle.

Sodium hydroxide solution, 1 N

Dissolve 40 g solid sodium hydroxide (NaOH) in approximately 800 mL of carbon dioxide (CO_2) free distilled water. Cool and dilute to 1 liter.

SAFETY NOTE: This reagent is corrosive and can burn hands and clothing. Rinse affected areas with large quantities of tap water to prevent injury and remove contaminated clothing, as residual may still damage skin.

Sulfuric acid solution, 1 N

Cautiously add 28 mL of concentrated sulfuric acid (H_2SO_4), with mixing, to 800 mL of distilled water. Allow to cool and dilute to 1 liter.

SAFETY NOTE: This reagent is corrosive and can burn hands and clothing. Rinse affected areas with large quantities of tap water to prevent injury and remove contaminated clothing, as residual may still damage skin.

Sodium sulfite solution, 0.0250 N

Dissolve 1.575 g anhydrous sodium sulfite (Na_2SO_3) in distilled water and dilute to 1 liter.

NOTE: This solution is not stable and must be prepared daily.

Potassium iodide solution, 10%

Dissolve 10 g potassium iodide (KI) in 100 mL of distilled water. Discard if solution turns yellow.

Acetic acid solution, 1+1

Carefully pour 50 mL of glacial acetic acid (CH_3COOH) into 50 mL distilled water with mixing.

SAFETY NOTE: This reagent is corrosive and can burn hands and clothing. Rinse affected areas with large quantities of tap water to prevent injury and remove contaminated clothing, as residual may still damage skin.

Sulfuric acid solution, 1+50

Cautiously add 5 mL of concentrated sulfuric acid (H_2SO_4) with mixing to 250 mL of distilled water.

SAFETY NOTE: This reagent is corrosive and can burn hands and clothing. Rinse affected areas with large quantities of tap water to prevent injury and remove contaminated clothing, as residual may still damage skin.

Glucose-glutamic acid solution

Dry reagent grade glucose and reagent grade glutamic acid at 103°C for 1 hour. Add 150 mg glucose and 150 mg glutamic acid to distilled water and dilute to 1 liter. Prepare this solution fresh immediately before use.

Nitrification inhibitor

The Hach Chemical Company's Nitrification inhibitor 2533(2-chloro-6-(trichloro methyl) pyridine) or equivalent can be used for inhibition during carbonaceous BOD testing.

APPENDIX C

Sample Bench Sheet (may be duplicated for use)

BOD Benchsheet $CBOD_5$ BOD_5

Facility Name: _____

Lab Tech: _____ **Lab Tech:** _____

Start Date: _____ **End Date:** _____

Time: _____ **Time:** _____

Method: _____ **Method:** _____

Sample Location _____

Bottle # _____

Initial DO _____

- Final DO _____

Depletion _____

- Seed Correction _____

= **Corrected Depletion** _____

X 300 mL _____

Div by Sample _____

Volume (mls) _____

BOD Result _____