BIOCHEMICAL OXYGEN DEMAND

(BOD)

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Background

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

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

BOD Sampling and Preservation

Samples for analysis of BOD can be collected either as grab samples (an individual sample collected over a period of time not exceeding 15 minutes) or as a composite sample (a sample composed of a number of discrete samples collected during the composite period) depending on permit requirements.

If a grab sample is collected and the analysis is begun within two hours, refrigeration of the sample is unnecessary. Otherwise, the sample must be kept at 4°C from the time it is collected.

If using an automatic sampler for collection of a composite sample the unit must be maintained at 4°C during the entire composite period. The composite time must be limited to no more than 24 hours from the end of the sampling period.

If manually taking the discrete samples for a composite, each discrete sample must be maintained at 4°C until compositing takes place. The composite must then be kept at 4°C until analysis takes place.

The BOD analysis must begin no more than 24 hours from the time the last discrete sample is collected. It is preferable to perform the analysis within six hours from the time the last sample is collected.

Just before analysis, the sample must be warmed to 20°C.

Equipment

The equipment needed to perform this analysis includes the following:

Air Pump. Used as a means of aerating the dilution water.

Beakers, Assorted Sizes. Always pour reagents into small beakers before pipetting to avoid contamination of the entire reagent bottle.

Carboy. To contain the dilution water. The size needed depends on the number of samples you expect to run. We attach a rubber hose to this for delivery of the dilution water into the cylinders.

Dissolved Oxygen Bottles. This bottles must have a flared mouth with ground glass stoppers and are generally 300 mls in volume.

Dissolved Oxygen Meter. With a stirrer type probe, specially designed to fit into D.O. bottles.

Filter. Used as a means of aerating the dilution water.

Graduated Cylinders, Assorted Sizes. For measuring the sample, etc.

Incubator. The incubator must be capable of maintaining a constant temperature of 20°C (plus or minus just 1°).

Mixer. The Propeller type mixer is used to mix the sample just before siphoning into the D.O. bottles.

One liter Graduated Cylinders. If using the syphoning method, we need a one liter cylinder for each of the dilutions of sample, as well as for the blank and GGA solution.

Overcaps. Placed over the flared mouth of the D.O. bottles to reduce evaporation of the water seal during incubation.

pH Meter. The pH of the sample must be 6.5 to 7.5.

Pipette. For measuring small volumes of sample and reagents.

Pipette Bulb. For drawing reagents or sample into pipette. Never mouth pipette.

Refrigerator. Capable of maintaining 4°C. For storage of reagents and sample.

Siphon (if using cylinder method). For delivery of homogenous samples into D.O. bottles while avoiding the entrainment of air.

Stand. A stand or shelf used to set cylinders on for elevation, which is helpful in the syphoning procedure.

Thermometer. Thermometers must be NIST traceable or annually calibrated against either an NIST or NIST traceable thermometer.

Tubing. Used as a means of aerating the dilution water.

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

Burette and Burette Stand. A 25 ml burette with 0.1 ml graduations is used for titration of the sodium thiosulfate solution.

Dissolved Oxygen Bottles With Auto Pipettes. These are used to store the reagents for D.O. analysis. The auto pipettes can be set to consistently deliver 1 ml. of each of the reagents.

Dropping Bottles. These bottles with dropper caps are handy for storing the starch and back titrant solutions.

Erlenmeyer Flasks. 500 ml wide mouth flasks are used during the titration process.

Reagents

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

PHOSPHATE BUFFER SOLUTION: Dissolve 8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4 \cdot 7H_2O$ and 1.7 g NH_4Cl in about 500 ml distilled water and dilute to 1 L. The pH should be 7.2 without further adjustment. Discard reagent (or any of the following reagents) if there is any sign of biological growth in the stock bottle.

MAGNESIUM SULFATE SOLUTION: Dissolve 22.5 g $MgSO_4 \cdot 7H_2O$ in distilled water and dilute to 1 L.

CALCIUM CHLORIDE SOLUTION: Dissolve 27.5 g $CaCl_2$ in distilled water and dilute to 1 L.

FERRIC CHLORIDE SOLUTION: Dissolve 0.25 g $FeCl_3 \cdot 6H_2O$ in distilled water and dilute to 1 L.

ACID and ALKALI SOLUTIONS, 1 N: for neutralization of caustic or acidic water samples.

1) Acid - Slowly and while stirring, add 28 mL conc sulfuric acid to distilled water. Dilute to 1 L.

2) Alkali - Dissolve 40 g sodium hydroxide in distilled water. Dilute to 1 L.

SODIUM SULFITE SOLUTION, 0.025N: Dissolve 1.575 g Na_2SO_3 in distilled water and dilute to 1 liter (or 0.788 g to 500 ml). This solution is not stable; prepare daily.

NITRIFICATION INHIBITOR, 2-CHLORO-6-(TRICHLORO METHYL) PYRIDINE. This can be purchased from the Hach Chemical Company.

GLUCOSE-GLUTAMIC ACID SOLUTION: Dry reagent-grade glucose and reagent-grade glutamic acid at 103°C for 1 h. Add 150 mg glucose and 150 mg glutamic acid to distilled water and dilute to 1 L. Prepare fresh immediately before use. This can be purchased pre-made.

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

If performing the Winkler Titration Method for determination of initial and final Dissolve Oxygen readings or for standardizing the D.O. meter. All reagents listed under that heading in Section 11 on Pages 3 through 6, will be needed.

DILUTION WATER PREPARATION

Dilution water consists of high quality distilled water to which we add nutrients and a buffering solution (to optimize the conditions for bacteriological growth). The use of deionized water is not recommended as organic compounds can leach through from the resins. It is extremely important that the oxygen depletion of the dilution water is 0.2 mg/l or less. A number of suggestions for producing dilution water of this quality are offered in the 17th and 18th Editions of Standard Methods.

To the carboy containing distilled water add one milliliter each of calcium chloride, magnesium sulfate, ferric chloride, and phosphate buffer solutions per liter of distilled water, then aerate using the air pump, filter, and tubing.

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

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

DISSOLVED OXYGEN METER STANDARDIZATION

The U.S. EPA accepts any meter standardization procedure which is guaranteed by the manufacturer to produce accurate results. The two most commonly used and highly recommended procedures for BOD analysis are the **iodometric** and **barometric** methods. The AZIDE modification of the iodometric method is most useful for wastewater application.

To standardize the meter using the barometric method be sure to follow the meter or probe manufacturer's calibration procedure exactly. Perform the "air calibration"--Fresh Water. Always use the "atmospheric correction factor" (determined from Table II after reading the barometer) as opposed to the altitude factor.

An example of **Barometric Calibration** instructions given below. (This example is taken from the YSI D.O. meter instruction manual).

AIR CALIBRATION--FRESH WATER

1) Place the probe in moist air. BOD probes can be placed in partially filled (50 ml) BOD bottles or the small calibration bottle (the one with the hole in the bottom) along with a few drops of water. The probe can also be wrapped loosely in a damp cloth taking care the cloth does not touch the membrane. Wait approximately 10 minutes for temperature stabilization. This may be done simultaneously while the probe is stabilizing.

2) Switch to TEMPERATURE and read. Refer to Table I--Solubility of Oxygen in Fresh Water, and determine calibration value.

3) Determine atmospheric correction factor using Table II.

4) Multiply the calibration value from Table I by the correction factor from Table II.

   EXAMPLE: Assume temperature = 21 °C and barometric pressure = 730 mmHg. From Table I the calibration value for 21 °C is 8.9 mg/l. From Table II the correction factor for 730 mmHg is about 0.96. Therefore, the corrected calibration value is 8.9 mg/l x 0.96 = 8.54 mg/l.

5) Switch to the appropriate mg/l range and adjust the CALIBRATE knob until the meter reads the corrected calibration volume from Step 4. Wait two minutes to verify calibration stability.

   Readjust if necessary.

Table I shows the amount of oxygen in mg/l that is dissolved in air saturated fresh water at sea level (760 mmHg atmospheric pressure) as temperature varies from 0°C to 45°C.

TABLE I
SOLUBILITY OF OXYGEN IN FRESH WATER

Temperature °C	mg/l Dissolved Oxygen	Temperature °C	mg/l Dissolved Oxygen
0	14.60	23	8.56
1	14.19	24	8.40
2	13.81	25	8.24
3	13.44	26	8.09
4	13.09	27	7.95
5	12.75	28	7.81
6	12.43	29	7.67
7	12.12	30	7.54
8	11.83	31	7.41
9	11.55	32	7.28
10	11.27	33	7.16
11	11.01	34	7.05
12	10.76	35	6.93
13	10.52	36	6.82
14	10.29	37	6.71
15	10.07	38	6.61
16	9.85	39	6.51
17	9.65	40	6.41
18	9.45	41	6.31
19	9.26	42	6.22
20	9.07	43	6.13
21	8.90	44	6.04
22	8.72	45	5.95

Table II shows the correction factor that should be used to correct the calibration value for the effects of atmospheric pressure. Find the true atmospheric pressure in the left hand column and read across to the right hand column and read across to the right hand column to determine the correction factor (NOTE that "true" atmospheric pressure is as read on a barometer. Weather Bureau reporting of atmospheric pressure is corrected to sea level).

TABLE II

Atmospheric Pressure mmHg	Correction Factor
775	1.02
760	1.00
745	.98
730	.96
714	.94
699	.92
684	.90
669	.88
654	.86
638	.84
623	.82
608	.80
593	.78
578	.76
562	.74
547	.72
532	.70
517	.68
502	.66

Reference

Derived from 15th Edition "Standard Methods for the Examination of Water and Wastewater."

IODOMETRIC METHOD FOR METER STANDARDIZATION

The meter must have been "on" for at least 15 minutes before standardization is attempted in order to insure that the probe is polarized. First turn the selector knob to the "red-line" position. The needle should line up perfectly with the red line on the scale. The meter is zeroed by turning the selector knob to the zero position and adjusting the meter so that the red needle lines up perfectly with the zero mark on the scale. When the needle is properly lined up you cannot see its reflection in the mirror. The selector knob is then set to the 0 to 10 range.

Next, fill a one liter graduated cylinder with distilled water. Using the following syphoning method, fill three D.O. bottles from the graduated cylinder.

SYPHONING

* **NOTE**: Syphoning of the sample from the graduated cylinders is not mandatory but this method does offer a good means of delivering a good homogeneous sample with less chance of having air bubbles in the D.O. bottles.

To syphon, fill the syphoning tube with distilled water leaving an air pocket. With the delivery end of the tube pinched, the other end is placed carefully into the graduated cylinder which contains distilled water. Then waste (or drain) the distilled water from the syphon tube stopping the flow after the air pocket has passed through.

Be sure that after wasting, the graduated cylinder contains at least 900 mls. Otherwise you might not have enough sample to fill the third D.O. bottle.

After "wasting" the distilled water, place the syphon into the first of the three D.O. bottles and fill the bottle completely.

Each of the three D.O. bottles is filled in this fashion. One of these bottles is set aside for meter standardization.

To each of the two remaining bottles, perform either the 203 ml or 300 ml (full bottle) Winkler titration procedures described in Section 11 on Page 5 (203 ml) or Page 8 (300 ml).

Repeat the titration process with the second D.O. bottle. The two D.O. concentrations from these bottles should be very close. Average the two concentrations.

Place the probe into the third D.O. bottle containing distilled water, and turn on the stirrer. If your D.O. probe is not equipped with a stirrer, a magnetic type stirrer can be used. The meter reading should be the same as the average D.O. concentration obtained on the first two D.O. bottles by the titration method. If this is not the case, the meter is adjusted by turning the calibration knob until you get the correct reading.

SAMPLE PRETREATMENT

Sample pretreatment is necessary in order to avoid potential toxicity effects on the microorganisms which are needed to metabolize the samples organic content during the incubation period.

pH

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

Temperature

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

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

Dechlorination

Samples should be taken prior to chlorination if conditions and your NPDES permit allow. If this is not possible, the sample must be dechlorinated (if at the time of analysis there is still a chlorine residual) and seeded. The dechlorination procedure consists of adding Sodium Sulfite in sufficient quantity to destroy the residual chlorine.

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

The method of dechlorination is as follows:

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

Review

At this point the following tasks have been performed:

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

Sample Dilution

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

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

SAMPLE TYPE		DILUTION	BOD mg/l
Dilution Water	=	100%	
Good Secondary Effluent	=	10%, 20%, 50%	4 - 75
Poor Secondary Effluent	=	5%, 10%, 20%	10 - 150
Very Poor Secondary or Primary Effluent	=	2%, 5%, 10%	20 - 375
Influents	=	1%, 2%, 5%	40 - 750
GGA	=	2%	200 ± 37
Dairy Wastes	=	.01, .03, .1, .3, 1% (depending on the strength)	200 - 75,000
Meat Packing Wastes	=	.2, .7, 2%	100 - 750
Paper Wastes, Untreated	=	1%, 2%, 5%	40 - 750
Paper Wastes, Treated	=	2%, 5%, 10%	20 - 275

We strongly suggest that you always use three dilutions for each sample. By doing this you reduce the likelihood of not producing the required oxygen **depletion** of at 2 mg/l (the oxygen depletion of the sample after the five day incubation period MUST be at least 2.0 mg/l or the results are considered invalid). Also (the oxygen **residual** at the end of the five day incubation period MUST be at least 1 mg/l). This is referred to as the R1-D2 rule. It is very important that this requirement be met. The terms depletion and residual are defined below.

Depletion

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

Example:	Initial D.O.	= 8.5
	Average Final D.O.	= 5.5
	Depletion	= 8.5 to 5.5
	Depletion	= 3.0

Residual

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

Sample Dilution

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

Using 1 liter as the total volume 200, 600 and 900 mls of sample are required respectively.

The **blank** consists of dilution water only. Simply fill a 1 liter graduated cylinder with dilution water.

Next, measure our the 200 ml sample using a 200 or 250 ml graduated cylinder. Remember to shake the sample first. Pour this into the next 1 liter graduated cylinder.

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

Measure each out the 600 ml and 900 ml samples. **Remember to shake the sample before pouring**. These volumes can be measured directly into the 1 liter graduated cylinders.

SEEDED SAMPLES

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

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

The seed volume is determined by using this formula when using the one liter cylinder dilution method:

0.8 divided by the expected seed BOD multiplied by 1,000 = mls of seed

EXAMPLE: If the influent being used as a seed usually produces a BOD of 250 mg/l we would divide 0.8 by 250 and then multiply this number by 1,000.

$$\frac{0.8}{250} \times 1{,}000 = 3.2$$

We would round this number off to 3 and use 3 mls of seed. If you are diluting in the 300 ml bottles instead of the liter cylinders you would multiply by 300 instead of 1,000:

$$\frac{0.8}{250} \times 300 = 0.96$$ (Round off to 1 ml of seed per bottle.)

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

Next, **prepare the seed sample dilutions.** For 1, 2 and 5% concentrations of seed measure out 10, 20 and 50 mls of seed respectively into the three 1 liter cylinders.

Now add dilution water to each of the cylinders to the one liter mark.

Place the propeller type mixer in the "blank" and carefully mix. Syphon this "blank" water into three D.O. bottles.

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

Replace the ground glass stoppers.

Determination of the Initial D.O.

Before beginning to read initial D.O.'s, we should once again check the meter reading of the distilled water bottle set aside in the Winkler analysis. It should be very close to the original reading.

Now after dumping the excess liquid in the well, we simply remove the cap of the first of the three "blank" bottles. Place the D.O. probe into the bottle, being careful not to entrap air in doing so. Turn on the stirrer.

Check for air bubbles in the sample. If there are no air bubbles record the reading (from the 0 to 10 scale). If there are air bubbles, reinsert the probe carefully eliminating all air.

Place the plastic cover over each of the two remaining bottles and place them into the box to be incubated.

Rinse the propeller mixer and place it in the 20% concentration sample. Carefully mix the sample.

After rinsing the syphon outside and inside, refill with distilled water and waste. Syphon the 20% sample solution into the three D.O. bottles. Rinse the D.O. probe and place into the first bottle. Read the D.O. of the first bottle. Place the plastic cover over each of the remaining two bottles and place them into the box to be incubated.

Repeat this procedure for the remaining dilutions of effluent, seed and GGA.

Always move from the **least concentrated to most concentrated solutions** to reduce the chances for contamination.

Bottles are to be incubated at 20°C plus or minus 1° for five days.

Determination of the Final D.O.

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

Blank Depletion

The D.O. depletion of the blank after the five-day incubation period should not exceed .2 mg/l. There is no "Blank Correction" calculation. If the Blank demonstrates a depletion of greater than .2 mg/l, the cause of the problem must be identified and corrected. Only the highest quality distilled water should be used when making up the dilution water. Sources of contamination must be eliminated.

CALCULATION OF UNSEEDED BODS

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

BOD mg/l = (Initial D.O. - Average Final D.O.) * Dilution Factor

To find the dilution factor:

$$Dilution\ Factor = \frac{100\%}{\%\ concentration\ of\ sample}$$

EXAMPLE: A 10% concentration of sample yields these results: Initial D.O. = 8.5 mg/l, Average Final D.O. = 6.0 mg/l.

BOD = (Initial D.O. - Average Final D.O.) * Dilution Factor
BOD = (8.5 - 6.0) * 10
BOD = 2.5 * 10
BOD = 25 mg/l

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

A BOD sample yields the following results:

% Concentration	Initial D.O. mg/l	Average Final D.O. mg/l	Depletion mg/l
10	8.0	7.0	1.0
20	8.0	6.0	2.0
30	8.0	3.0	5.0

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

The 10% sample is not included in the calculation because the DEPLETION is less than 2.0.

The result of the 20% concentration (8-6) * $\frac{100\%}{20\%}$ = 2 x 5 = 10 mg/l

The result of the 50% concentration (8-3) $\frac{100\%}{50\%}$ = 5 x 2 = 10 mg/l

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

CALCULATION OF SEEDED BODS

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

The actual BOD calculation for a seeded sample is:

Dilution Factor * ((I.D.I. - A.F.D.O.) - (Seed Correction Factor))

Where: Dilution Factor = $\frac{100\%}{\%\text{ Concentration of Sample}}$

I.D.O. = Initial Dissolved Oxygen reading before incubation.

A.F.D.O. = Average Final Dissolved Oxygen. The average of the two Dissolved Oxygen readings taken after the five day incubation period.

Seed Correction Factor = $\frac{\text{Actual Seed BOD x mls of seed used}}{\text{Total volume of sample after dilution}}$

(*) For our purposes the volume of sample after dilution is 1,000 mls. If your dilution is done directly into a 300 ml BOD bottle you would use 300 mls as a total volume.

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

STEP 1 - Calculate the Dilution Factor.

EXAMPLE: If we used a 10% concentration of sample the dilution factor would be 100% divided by 10% = 10.

STEP 2 - Calculate the Initial minus the average final D.O.'s.

EXAMPLE: If we had an initial D.O. reading of 8.6 mg/l and final D.O. readings of 4.7 and 4.5 our answer would be 4 mg/l. We get this answer by subtracting 4.6, the average of the two final D.O. readings 4.7 and 4.5, from the initial D.O. reading of 8.6.

$$8.6 - \frac{(4.7 + 4.5)}{(2)} = 8.6 - 4.6 = 4.0\ mg/l$$

STEP 3 - Calculate the Seed BOD. Since the seed is essentially an unchlorinated sample we use the calculation for unseeded BOD's:

BOD = Dilution Factor * (Initial D.O. - Average Final D.O.)

EXAMPLE: Using a 1% concentration of influent sample (this influent will serve as our seed material) if our Initial D.O. reading was 8.5 and our final D.O. readings were 4.4 and 4.6 our Seed BOD would be 400. We arrive at this number by multiplying the Dilution Factor 100%/1% = 100 times the Initial D.O. minus the average final D.O.

$$D.O.\ depletion = 8.5 - \frac{(4.4 + 4.6)}{(2)} = 8.5 - 4.5 = 4.0\ mg/l$$

Seed BOD =
100 * 4.0 = 400 mg/l

STEP 4

Perform the calculation for the Seed Correction. For our example assume that we used 2 mls of seed in each dilution of sample and that we used the liter cylinder dilution method (samples were diluted to 1,000 mls).

$$\text{Seed correction factor} = \frac{(\text{seed BOD, mg/l}) * (\text{mls of seed used})}{\text{Total volume of sample dilution mls}}$$

$$\text{Seed correction factor} = \frac{(400\ \text{mg/l}) \times (2\ \text{mls})}{1{,}000\ \text{mls}} = 0.8\ \text{mg/l}$$

STEP 5

Plug these numbers into the equation.

Following our example above, we have a 10% concentration of sample, with an initial D.O. of 8.6 and an average final D.O. of 4.6. Our seed correction factor as calculated in Step 4 above is 0.8.

Seeded BOD, mg/l = Sample Dilution Factor * {(I.D.O. - A.F.D.O.) - (Seed Correction)}

$= \frac{100}{10} [(8.6 - 4.6) - 0.8] = 10 [4.0 - 0.8] = 10*3.2 = 32$ mg/l

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

Finally:

Calculate GGA

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

This 2% glucose-glutamic acid solution should yield a BOD of 200 ± 37 mg/l. If the BOD does not fall within this range, find the possible source of errors, correct the problems, and try the test again. It should deplete at least 2 mg/l of dissolved oxygen and leave at least 1 mg/l dissolved oxygen residual after five day incubation. Remember to take the seed depletion into account when calculating the glucose-glutamic acid BOD.

Remember to R1-D2 rule!!

Good Luck.

FACILITY: Maple City **SAMPLE BENCH SHEET #1**

SAMPLING: **DATE:** 02/15/94 **COLLECTED BY:** AB

CHLORINATED: No **Other Disinfection:** None

Composite: 1/hour **Start Time:** 7 a.m. **Duration:** 8 hours **Grab Sample**_____ **Time**_____

In to incubator: Date: 02/16/94 **Time** 11 a.m. **Temp.** 20.5°C **Analyst** CD **Seed Used** None

Out of incubator: Date: 02/21/94 **Time** 1 p.m. **Temp.** 20°C **Analyst** AB

D.O. VALUES

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

(INCLUDE ACTUAL CALCULATIONS ON REVERSE SIDE)

CALCULATIONS: (To accompany bench sheet)

Influent 1% $\frac{100\%}{1\%} \times 2.50 = 100 \times 2.50 = 250$

$\frac{250 + 300}{2} = 275$ mg/l BOD, Influent

Influent 2% $\frac{100\%}{2\%} \times 6.00 = 50 \times 6.00 = 300$

Influent 5% ---> Average residual less than 1

Effluent 10% ---> Depletion less than 2

Effluent 20% ---> Depletion less than 2

Effluent 50% ---> $\frac{100\%}{50\%} \times 3.05 = 2 \times 3.05 = 6.1$

Effluent Duplicate 10% ---> Depletion less than 2

3 Effluent Duplicate 20% ---> Depletion less than 2

Effluent Duplicate 50% ---> $\frac{100\%}{50\%} \times 3.95 = 2 \times 3.95 = 7.9$

$\frac{6.1 + 7.9}{2} = 7.0$ mg/l BOD, Effluent

FACILITY: Maple City

SAMPLE BENCH SHEET #2

SAMPLING: **DATE:** 02/15/94 **COLLECTED BY:** AB

CHLORINATED: No **Other Disinfection:** Ultra Violet

Composite: 1/hour **Start Time:** 7 a.m. **Duration:** 8 hours **Grab Sample**_____ **Time**_____

In to incubator: Date: 02/16/94 **Time** 11 a.m. **Temp.** 20.5°C **Analyst** CD **Seed Used** 3 mls Primary Effluent

Out of incubator: Date: 02/21/94 **Time** 1 p.m. **Temp.** 20°C **Analyst** AB

D.O. VALUES

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

(INCLUDE ACTUAL CALCULATIONS ON REVERSE SIDE)

CALCULATIONS FOR SEEDED BOD

Seed 1% $\frac{100\%}{1\%} \times 2.50 = 100 \times 2.50 = 250$

Seed BOD = $\frac{250 + 300}{2} = 275$ mg/l

Seed 2% $\frac{100\%}{2\%} \times 6.00 = 50 \times 6.00 = 300$

Seed Correction = $\frac{275 \times 3}{1000} = .825$

Seed 5% ---> Average residual less than 1

Effluent 10% ---> Depletion less than 2

Effluent 20% ---> Depletion less than 2

Effluent 50% ---> $\frac{100\%}{50\%} \times (3.05 - .825) = 4.45$

Effluent Duplicate 10% ---> Depletion less than 2

Effluent Duplicate 20% ---> Depletion less than 2

Effluent Duplicate 50% ---> $\frac{100\%}{50\%} \times (3.95 \cdot .825) = 6.25$

$\frac{4.45 + 6.25}{2} = 5.35$ mg/l BOD

BIOCHEMICAL OXYGEN DEMAND
TROUBLESHOOTING GUIDE

PROBLEM	LIKELY CAUSE	SOLUTION
BLANK DEPLETION (unseeded dilution water) is greater than the allowable .2 mg/l.	Contamination of BOD bottles, carboy, or syphon.	Clean bottles, carboy and syphon thoroughly with hot 2% Micro solution. Rinse thoroughly with hot tap water followed by distilled water rinses. If the problem persists use chromic acid to clean the bottles, followed by several rinses of hot tap water and finally several distilled water rinses. Use an appropriate sized brush to aid in thorough cleaning.
	Contaminated distilled water.	Thoroughly clean the distilled water storage tank. Place a .4 micron organic filter at the end of the system, especially if deionizing resins are used. Check the conductivity of the distilled water. If >1.0. Find another source of distilled water until the problem can be eliminated or as a last resort an attempt can be made to oxidize the contaminating materials by adding a small amount of seed material (.5 mls) to the distilled water 5-7 days before the dilution water is prepared.
	Contaminated nutrients used to make up the dilution water.	If the nutrients, especially the phosphate buffer, are over 1 year old or show any sign of contamination discard and prepare new solutions.

BIOCHEMICAL OXYGEN DEMAND
TROUBLESHOOTING GUIDE (continued)

PROBLEM	LIKELY CAUSE	SOLUTION
	Initial D.O. of dilution water as >9.2 mg/l.	Never begin the BOD analysis if the D.O. of the dilution water or samples is greater than 9.2 mg/l. The ideal range if 7.5-8.5 mg/l. The D.O. can be reduced by shaking or aerating the dilution water then letting it set for 1/2 hour before use. Recheck the D.O. at this point. Be sure the dilution water is 20°C before beginning the analysis.
	D.O. meter improperly calibrated.	Check calibration method. If calibrated against sodium thiosulfate or PAO, check the standardization of the titrant. Check D.O. probe. Make sure membrane is in good shape--no bubbles under membrane and fresh filling solution.
All dilution of sample produce less than the required D.O. depletion of 2 mg/l.	Sample concentrations too low. Toxicity.	Increase sample concentration. Check sample for chlorine residual and pH extreme. Remove chlorine and/or adjust pH if necessary and properly seed the sample.
All dilution of sample product less than the required residual D.O. of 1 mg/l.	Sample concentration too high.	Decrease sample concentration.
Bubbles appear in BOD bottles after incubation.	Supersaturated samples -- Initial D.O. was >9.2 mg/l. Temperature of sample was less than 20°C.	D.O. not begin analysis until D.O. sample is <9.2. Ideal range = 7.5-8.5 mg/l.
Reverse depletion. The depletion of the more concentrated sample is less than the depletion of the least concentrated sample.	Toxicity.	Check sample or seed for pH (should be between 6-8) adjust if necessary. Check sample or seed for chlorine residual and dechlorinate as necessary using sodium sulfite solution.

Quality Control for
BIOCHEMICAL OXYGEN DEMAND
Standard Method #5210-B

Documentation

Sample Collection

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

HOLD TIME: **Preferably 6 hours (at 4°C) - max 24 hours after last discrete sample is collected**

Sample line and container **clean** - no residual organics

*Mix sample thoroughly before pouring off for composite **BUT** don't agitate excessively.

Glassware Preparation (and sampling equipment)

*Must be scrupulously clean

No residue
No dust
Well rinsed (5-10 times) with distilled H_2O.

BOD Bottles - air tight, ground glass stoppers.

Reagents

Distilled water for dilution water preparation:
No copper
Conductivity <1
Preparation and expiration dates for all reagents.

Reagents (continued..)

NIST Traceability
GGA - MUST be made the day the analysis is performed
Sodium Sulfite solution for neutralization of chlorine must be made the day the analysis is performed.

Equipment

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

Sample Preparation - **Before beginning analysis!**

Sample and Dilution water must be 20°C or slightly higher
Sample and Dilution water DO must be ≤9 mg/l

Validity of Results

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

Duplication Schedule

Minimum 5% duplication or
Minimum 5% replication 10% replication
GGA - minimum 10%
Seeded Sample - GGA minimum 100%

Recommended Control Limits

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

References

Simplified Laboratory Procedures for Wastewater Examination, Third Edition 1985. Published by the Water Pollution Control Federation, 2626 Pennsylvania Avenue NW, Washington, DC 20037

Standard Methods for the Examination of Water and Wastewater, 18th Edition, 5210-B, pages 5-2 through 5-6. Published by the America Public Health Association, 1015 Fifteenth Street NW, Washington DC 20005.

Methods for Chemical Analysis of Water and Wastes, (1983) EPA - 600/4-79-020. Published by the United States Environmental Protection Agency, Office of Research and Development, U.S. Government Printing Office, Washington, DC 20005.

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

Water Chemistry Manual for Water and Spentwater Personnel. Copyright 1990 by the Chemists Group, Inc. c/o of Dr. Gaines Bradford Jackson, 1044 SW, 26th Street, Oklahoma City, Oklahoma 73109.

Seeded Dilution Water. (see appendix) An article written by Susan Romatzick, Maine Department of Environmental Conservation.

APPENDIX I

SEEDED DILUTION WATER

One option not yet discussed in the BOD analysis is that of seeding the dilution water. This is a perfectly acceptable method and will be briefly described here.

The setup of the analysis for a single chlorinated sample would be as follows: (using either the 1 liter dilution method or direct bottle dilution method)

Blank

3 bottles are filled with dilution water only.

Seed Control

Three (3) bottles are half filled with dilution water then a known volume of seed is added to each. the bottles are then topped off with dilution water.

Seeded Dilution Water

After preparing the Blank and Seed Control Bottles, Seed is added to the carboy containing the dilution water. The amount of seed required is calculated in the same manner as described earlier in the text.

Volume of seed to use per liter of seeded dilution water required =

$$\frac{.8}{\textit{expected seed BOD}} \; x \; 1000 \; mls$$

Three bottles are filled with the seeded dilution water.

Sample Dilutions

Three bottles are prepared for each dilution of sample, by adding the required volume of sample and then topping off each bottle with the seeded dilution water.

Initial DOs of blank, seed control, seeded dilution water and each dilution of sample are read.

The remaining bottles are incubated at 20°C.

Final DOs are read after five days incubation.

The results can be calculated using this equation obtained from Standard Methods 18th Edition, 5210B Page 5-5.

$$BOD\ mg/l = \frac{(D_1 - D_2) - (B_1 - B_2)f}{P}$$

where:

D_1 = D.O. of diluted sample immediately after preparation, mg/l,
D_2 = D.O. of diluted sample after five day incubation at 20°C, mg/l,
P = Decimal volumetric fraction of sample used,
B_1 = D.O. of seed control before incubation, mg/l,
B_2 = D.O. of seed control after incubation, mg/l, and
f = Ratio of seed in diluted sample to seed in seed control
= (% seed in diluted sample)/(% seed in seed control).

OR

Using an equation found in Simplified Laboratory Procedures for Wastewater Examination, Third Edition.

$$mg/l\ BOD\ of\ the\ seed + \frac{(D_1 - D_2)\ (300)}{S}$$

where:

D_1 = Initial D.O. of seeded dilution water immediately after preparation, mg/l,
D_2 = Final D.O. of seeded dilution water after 5 day incubation at 20°C, mg/l,
300 = Volume of BOD bottle in ml, and
S = ml of seed in BOD bottle.

NOTE: In the following examples 300 mls is assumed for the sample volume.

EXAMPLE: mg/l BOD seed = $\frac{(8.9 - 4.1)(300)}{12}$

= 120 mg/l

where:

D_1 = 8.9 mg/l
D_2 = 4.1 mg/l, and
S = 12 ml.

Then mg/l BOD_5 of seeded sample =

$$[D_1 - D_2 - \frac{(L)(BOD\ seed)}{300}][\frac{300}{S}]$$

where:

D_1 = Initial D.O. of sample diluted with seeded dilution water immediately after preparation, mg/l,
D_2 = Final D.O. of sample diluted with seeded dilution water after five days incubation at 20°C,
300 = Volume of BOD bottle in ml,
S = ml of sample used in dilution, and
L = Volume of seed in bottle that contained sample diluted with seeded dilution water.

"L" can be calculated for each sample dilution by using the equation:

L = (300 - volume of sample seed)(seed concentration in dilution water)

NOTE: Seed concentration in dilution water is simply the volume of seed used per liter of dilution water prepared.

EXAMPLE #1: A 2% solution of sample is prepared by diluting 6 mls of sample to 300 mls with seeded dilution water. The BOD of the seed has been established to be 120 mg/l. The initial D.O. of this dilution of sample is 8.7 mg/l. The final D.O. after 5 day incubation is 2.4 mg/l. Using the equation:

$$BOD\ mg/l = [D_1 - D_2 - \frac{(L)(BOD\ of\ seed)}{300}][\frac{300}{S}]$$

$$BOD\ mg/l = [[8.7 - 2.4 - [\frac{(300 - 6)(3/1000)(120)}{300}][\frac{300}{6}]$$

$$= [[8.7 - 2.4 - [\frac{(294)(.003)(120)}{300}][50]$$

$$= [8.7 - -2.4 - (.3528)][50] \rightarrow = (6.3 - .3528)(50)$$

$$= (5.947)(5) \rightarrow BOD\ mg/l = 297$$

where: as stated at the beginning of this example.

D_1 = 8.7 mg/l
D_2 = 2.4 mg/l
Seed BOD = 120 mg/l
S = 6 mls for this dilution = (300 - 6)(3/1000)

EXAMPLE #2 : Three concentrations of sample 1%, 2% and 5%, are analyzed using the seeded dilution water method.

The BOD of the seed has been established to be 120 mg/l. The seeded dilution water contains 3 mls of seed per liter. The results of the analysis are as follows:

SAMPLE CONC	MLS OF SAMPLE USED	INITIAL D.O.	FINAL D.O.
1%	3	8.8	6.8
2%	6	8.7	4.7
5%	15	8.6	0.6

Note that the 5% concentration has a residual of <1 mg/l. Therefore it is not necessary to perform calculations for that concentration.

For the two usable concentrations the results would be:

$$For\ 1\%\ Conc \rightarrow BOD\ mg/l = [(8.8 - 6.8) - \frac{(300-3)(3/1000)(120)}{300})][\frac{300}{3}]$$

$$= [(8.8 - 6.8) - \frac{(297)(.003)(120)}{300})](100)$$

$$= [8.8 - 6.8) - (.3564)](100)$$

$$= (1.64)(100)$$

$$BOD\ mg/l = 164$$

$$For\ 2\%\ Conc \rightarrow BOD\ mg/l = [(8.7 - 4.7) - \frac{(300 - 6)(3/1000)(120)}{300}][\frac{300}{6}]$$

$$= [(8.7 - 4.7) - (\frac{(294)(.003)(120)}{300})](50)$$

$$= [8.7 - 4.7 - (.3528)](50)$$

$$= (3.65)(50)$$

$$BOD\ mg/l = 182$$

The reported BOD would be the average of these two results 173 mg/l.