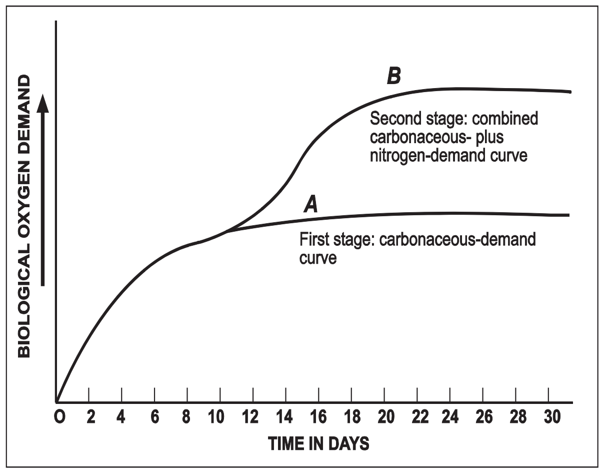
Biological Oxygen Demand

What is biological oxygen demand (BOD)?

Biological oxygen demand (BOD), also known as *biochemical oxygen demand*, is a bioassay procedure that measures the dissolved oxygen (DO) consumed by bacteria from the decomposition of organic matter. The BOD analysis is an attempt to simulate by a laboratory test the effect that organic material in a water body will have on the DO in that water body.

Biochemical oxygen demand values are a measure of food for naturally occurring microorganisms or, in other words, a measure of the concentration of biodegradable organic material. When nutrients are introduced, naturally occurring microorganisms begin to multiply at an exponential rate, resulting in the reduction of DO in the water. The test does not determine the total amount of oxygen demand present, since many compounds are not oxidized by microorganisms under conditions of the test.

There are two stages of decomposition involved in biological oxygen demand (BOD): a carbonaceous stage and a nitrogenous stage (refer to the figure below). The typical carbonaceous-demand curve (A) shows the oxidation of organic matter. The typical carbonaceous-plus-nitrogenous-demand curve (B) shows the additional oxidation of ammonia and nitrite.[1]



The carbonaceous stage, or first stage, represents that portion of oxygen demand involved in the bacterial conversion of organic carbon to carbon dioxide. The nitrogenous stage, or second stage, represents a combined carbonaceous plus nitrogenous demand, when organic nitrogen, ammonia, and nitrite are converted to nitrate by bacteria, a process that also consumes DO.

The most common method for measuring BOD is the 5-day BOD method (BOD_5), which is a method approved by the U. S. Environmental Protection Agency (EPA) for use during regulatory monitoring. In the BOD_5 test, which uses naturally occurring microorganisms to oxidize the carbonaceous organic matter, the change in DO concentration is measured before and after a 5-day period in water samples that are incubated at a specified temperature (20 °C ± 1 °C) in darkness. The BOD test results are reported as mg/L DO.[1]

The BOD_5 method measures most of the carbonaceous stage of the BOD (typically about 60% to 70%; refer to the figure showing Biological Oxygen Demand). This method has been widely adopted as a standard, based on historical use and convenience, since it is usually impractical to wait 20 or more days for the outcome of the test.

Why measure BOD?

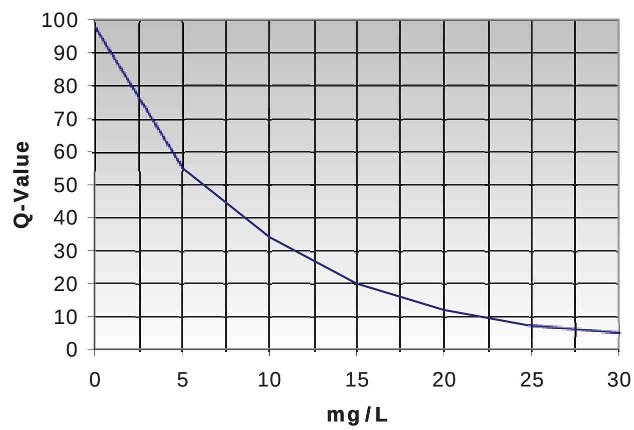
Biological oxygen demand is an important water quality parameter because it greatly influences the concentration of DO that will be in the water. The Q-value curve for BOD used in calculating a water quality index[2] illustrates the relationship between BOD and water quality—the higher the BOD, the lower the water quality, with 0 to 2 mg/L being associated with high water quality and values greater than 10 mg/L being associated with low water quality (refer to the BOD Test Results chart).

The BOD_5 test is used to measure the potential of wastewater and other waters to deplete the oxygen level of receiving waters. The test is also used to examine influents and effluents from wastewater processing facilities to compute the efficiency of operation of the treatment units.

The Q-curve for BOD used in calculating a water quality index[2] illustrates the relationship between BOD and water quality.

Chart 1

BOD Test Results



(Note: If BOD > 30, Q=2.0)

What factors affect BOD levels?

Human and animal waste in sewage is a significant contributor to elevated BOD levels. Runoff containing fertilizer from farms and other sources contributes to accelerated eutrophication, in which water bodies become choked with excessive plant growth, which contributes to the carbonaceous (organic) content of water bodies as these plants die and decompose.

Levels of BOD can be reduced by the introduction of low-BOD water from rain or snow melt. Additionally, when animals and plants die and settle on the bottom of the water body under conditions that permanently remove them from the water column, BOD levels can drop. Ultimately, river waters with high BOD discharge into the oceans where nutrients become highly diluted. The high BOD levels of untreated sewage are greatly reduced by wastewater treatment procedures.

What are ideal BOD values?

As shown on the BOD Q-curve, the less the BOD value, the better. High BOD levels will result in anoxic conditions, with the resulting growth of anaerobic microorganisms that produce noxious gases and cause the death of aerobic aquatic organisms. In the case of wastewater, the larger the drop in BOD following treatment procedures, the better. The regulated level of wastewater effluents depends on local conditions.

Overview of the two procedures for the 5-day BOD (BOD_5) test

About the official procedure

The procedure specified by the United States Geological Survey for the BOD_5 test is done in the laboratory and is quite complex to perform and difficult to control for reliable results. Among the considerations for a reliable test are the following:[1]

- ❖ The water sample must be obtained in a fashion such that it is representative of the larger water body.
- ❖ The test must be initiated within 2 hours of collecting the water sample, or the sample must be refrigerated (4 °C) for up to 48 hours before initiating the BOD_5 test.
- ❖ Special BOD bottles that minimize the accidental introduction of oxygen or nutrients must be used.
- ❖ Glassware and pipets must be scrupulously cleaned with a non-phosphate detergent and with multiple rinses with distilled or deionized water to avoid introducing nutrients or toxins.

- An aquarium pump, tubing, and air diffusion stone are needed, and they must be scrupulously cleaned as above.
- The pH must be maintained between 6.5 and 7.5 using 1 M NaOH and H_2SO_4, if necessary.
- A nutrient dilution solution containing $CaCl_2$, $FeCL_3$, $MgSO_4$, and phosphate buffer must be prepared using very high quality water that contains no nutrients or toxins. This solution supports the growth of the bacteria used in the assay. It should be discarded in the event that bacterial growth occurs in it.
- The DO sensor must be calibrated at the current atmospheric pressure and the temperature of the water sample before each day's measurements.
- The test samples must be incubated in the dark at 20 °C ± 1 °C.
- Any residual chlorine, if present, must be removed before the test.
- A sample that contains any toxic metals, arsenic, or cyanide must be specially treated before the test.
- A sample that has been treated by disinfectants may need to be "seeded" with bacteria.
- A negative (blank) and positive (glucose/glutamic acid standard solution) control must be run with the test, and the results of these must meet standards.

About the modified 5-day BOD (BOD_5) test for use in the classroom

The BOD_5 procedure presented in this field guide is a modification of the standard procedure that will produce results that are acceptable in a classroom environment and that support learning of the basic concepts of BOD_5 testing. It should not be used in a regulatory environment or when meaningful comparisons to test values obtained using the EPA-approved BOD_5 procedures[1] are needed. It is intended for use with natural water bodies, not treated sewage effluent. The following assumptions, which are reasonable for most natural water bodies and typical classroom lab conditions, apply to this procedure:

- there is an adequate population of aerobic bacteria in the test water;
- there are no toxins in the test water, test equipment, or dilution water that would prevent these bacteria from growing and multiplying;
- the pH of the test water is between 6.5 and 7.5;
- there is a negligible amount of nitrogenous material dissolved in the test water; and
- the ambient room temperature is about 20 °C and does not vary appreciably.

Modified BOD_5 procedure for the classroom

Materials List

Item	Quantity
Dissolved Oxygen Sensor*	1
Temperature Sensor* or Thermometer	1
Plastic lab bottle with screw top, 2-L	2
Plastic lab bottle with screw top, 1-L	1
Dark plastic or glass lab (“BOD”) bottle with air-tight top, 300-mL	3
Graduated cylinder, 100-mL	2
Graduated pipet*	1
Wash bottle containing deionized water	1
Waste container	1
*The Water Quality MultiSensor may also be used. **Note:** If you are making your own nutrient dilution solution from scratch, you will also need the chemicals listed in the table below, a mass scale, and weighing supplies.	

Preparation for the BOD_5 test

1. **Prior to the procedure, ensure that all equipment has been thoroughly cleaned and rinsed, including final rinses with deionized water, and is dry.**
2. **The day before beginning the BOD_5 test, aerate 1 L of laboratory-quality distilled water and bring it to 20 °C.**

   Suggestion: To aerate, pour the deionized water into the 2-L bottle, cap, and shake vigorously. Then loosen the cap to allow contact with the atmospheric air.

   Note: It is very important that the distilled water used for the dilution water be of high grade and free from contaminants (such as copper and chlorine) that could inhibit the growth of bacteria. For this reason, it is recommended that ordinary commercial distilled water (for example, for use in car batteries) not be used.
3. **Collect at least 1 L of a representative sample of the natural body of water.**

   Note: If the BOD_5 test will be delayed by more than 2 hours, store the sample at 4 °C (not frozen). The BOD_5 test must be started within 48 hours.
4. **Approximately 1 hour before the BOD bottles are to be set up, prepare about 1 L of the BOD_5 dilution medium, using the distilled water prepared in Step 2 and prepackaged reagents (from retailers that sell water quality testing supplies; see the Recommended Reading and Resources section for suggestions) according to the manufacturers instructions, or using the procedure shown in the table below.**
5. **Store at room temperature in a container that does not have an air-tight lid (to allow the air dissolved in the medium to equilibrate with the atmospheric air).**

Preparing the nutrient buffer dilution solution

Solution	Procedure
Calcium chloride ($CaCl_2$) solution	Dissolve 27.5 g of $CaCl_2$ in deionized water and dilute to 1 L.
Ferric chloride ($FeCl_3$) solution	Dissolve 0.25 g of $FeCl_3 \cdot 6H_2O$ in deionized water and dilute to 1 L.
Magnesium sulfate ($MgSO_4$) solution	Dissolve 22.5 g of $MgSO_4 \cdot 7H_2O$ in deionized water and dilute to 1 L.
Phosphate buffer solution	Dissolve 8.5 g of KH_2PO_4, 21.8 g of $KHPO_4$, 33.4 g of $Na_2HPO_4 \cdot 7H_2O$, and 1.7 g of NH_4Cl in about 500 mL of deionized water. Dilute to 1 L.
Nutrient buffer dilution solution	To 1 L of the aerated deionized water prepared in Step 2, add 1 mL of each of the 4 solutions above and mix well.

*maximum contamination level (MCL) of either chloramines or free chlorine

Conducting the BOD_5 test

1. **Saturate the test water with air by pouring 1 L of it into a 2-L bottle, capping, and shaking vigorously.**
2. **Allow this water to sit undisturbed with the cap off until the air bubbles dissipate and the water temperature is the same as the room temperature (about 20 °C). If necessary, tap the container gently to dislodge air bubbles from the sides and bottom.**
3. **Using a Dissolved Oxygen Sensor that has just been calibrated �(2.6) and a data collection system, measure the DO in the test sample and record this value.**

   Note: This value should be approximately that of 100% DO saturation.
4. **Pour about 50 mL of the nutrient buffer dilution solution into each of the BOD bottles that will contain diluted test samples.**

   Note: Use a pouring method that minimizes the introduction of air bubbles, such as pouring slowly down the side of the bottle.
5. **From the table below, select a range of 3 dilutions that bracket the anticipated BOD of the test sample.**

   Suggestion: If you do not have an idea of this range, use the first 3 dilutions on the table. Using a graduated cylinder, measure each specified volume of test water and pour it into the BOD bottle as noted in Step 4.
6. **Fill the BOD bottles to the brim with the dilution water, and cap the bottle so it is air-tight, being careful to avoid trapping an air bubble.**
7. **Incubate the BOD bottles at room temperature for 5 days.**

   Note: If the BOD bottles are not completely opaque, incubate in a dark room or box.
8. **After 5 days, measure the DO level in each BOD bottle as in Step 3, and record the result.**

Example dilutions for the 5-day BOD test

Anticipated Range of the BOD_5 Value (mg/L)	Milliliters of Sample	Milliliters of Dilution Water
0–7	300	0
4–12	150	150
6–21	100	200
12–42	50	250
30–105	20	280
60–210	10	290
120–420	5	295
300–1,050	2	298
600–2,100*	1	299

* It is necessary to dilute the original test sample if it has a BOD that is greater than this range.

Calculating the 5-day BOD

1. **Select the dilution for which the 5-day DO reading dropped at least 2 mg/L from the first reading but remained above 1 mg/L.**

   Note: If more than 1 dilution fell into this category, calculate the 5-day BOD for each one, and then average the results for the final BOD_5 value.

2. **Use the following formula to calculate the final BOD_5 value:**

   BOD_5 (mg/L) = $(D_1 - D_2)/P$

   where

   D_1 is the initial DO of the sample

   D_2 is the final DO of the sample after 5 days, and

   P is the decimal volumetric fraction of sample used.

   For example, if a sample of 100 mL is diluted to 300 mL, then $P = 100/300 = 0.33$

   Note: If no dilution was necessary, then $P = 1.0$ and the BOD_5 is determined by $D_1 - D_2$.

More background information

More information about biological oxygen demand and its role in water quality may be found in the references listed below, with availability information listed in the Recommended Reading and Resources section.

References

1. G.C. Delzer, McKenzie SW. *Five-Day Biochemical Oxygen Demand.* In: DN Myers; FD Wilde, editors, translator and editor *National Field Manual for the Collection of Water-Quality Data*. Vol. 7.2, Techniques of Water-Resources Investigations Reports: U.S. Geological Survey; 1999.
2. National Science Foundation. *Water Quality Index.* 2004.