

c. Biochemical Oxygen Demand

i. Why is this test important/What does it measure?

This test measures the oxygen usage by living matter in the sample in the absence of photosynthesis. During the five days (in the dark), living “critters” use oxygen to survive. By subtracting the BOD(5) value from the D.O. value, oxygen usage by life in the water can be calculated. When little oxygen is removed during the five days that indicates there are very few organisms using the oxygen. The main contributor to BOD is organic waste. Organic waste can come from manure (animal or human), decomposing plant matter (such as leaves or grass clippings dumped near a stream), discharge from food processing plants, or agricultural runoff. High BOD can also come from natural sources, such as runoff from swamps.

ii. Water Quality Standards

Although there are no Michigan Water Quality Standards pertaining directly to BOD, effluent limitations for BOD must be restrictive enough to insure that the receiving water will meet Michigan Water Quality Standards for dissolved oxygen. Also, facilities that discharge waste to Michigan Rivers and streams might have certain requirements as part of their permit. The City of Flint Wastewater Treatment Plant has a BOD permit for 9-24mg/L BOD depending on the season. Good BOD levels are under 3mg/L BOD.

iii. How to conduct the test

The BOD test is essentially the same as the Dissolved Oxygen test, done 5 days later from a dark bottle.

1. Pick your location to sample. You want an area of the stream where you can completely submerge the bottle. Try to select a sampling location that is representative of the stream.
2. Rinse the sample bottle with the stream water.



3. After you have rinsed the bottle with stream water, tightly cap the bottle.
4. Submerge the bottle underneath the water completely, and remove the cap with the bottle under the water, and allow the bottle to fill.



5. While the bottle is still underneath the surface of the water, tap the sides of the bottle to get out all the air bubbles.



6. While the bottle is still underneath the surface of the water, put the cap back on the bottle.



7. Retrieve the bottle and make sure there are no trapped bubbles, if you have trapped bubbles, go back to step 3.



8. Wrap the bottle carefully in electrical tape or foil to keep any light from reaching the sample.
9. Place the BOD sample bottle in a dark drawer or cupboard and incubate for 5 days at room temperature (about 68 degrees Fahrenheit).

NOTE: When adding chemicals, be sure to not add air to the sample.

10. Carefully remove the cap from the bottle.
11. Add 8 drops of Manganous Sulfate Solution *NOTE: Bottle may overflow, that is ok; that is why you are wearing gloves.*
12. Add 8 drops of Alkaline Potassium Iodide Azide *NOTE: Bottle may overflow, that is ok; that is why you are wearing gloves.*
13. Cap the bottle and wipe off any chemical that overflowed the bottle.

14. Mix by inverting several times. Put your thumb over the cap and turn the bottle upside-down, then right-side-up. DO NOT shake it like a bottle of ketchup, it is more likely to go flying out of your hand that way. "Chunky stuff" will form, this is called precipitate.
15. Set the bottle down and allow the precipitate to settle down below the "shoulders" or curved part of the bottle. This should take about 5 minutes.
16. Immediately use the 1.0 g spoon (0697) to add one level measure of Sulfamic Acid Powder (6286). NOTE: Bottle may overflow, that is ok; that is why you are wearing gloves. ☺
17. Cap the bottle and wipe off any chemical that overflowed the bottle.
18. Mix by inverting several times. Put your thumb over the cap and turn the bottle upside-down, then right-side-up. DO NOT shake it like a bottle of ketchup, it is more likely to go flying out of your hand that way. Keep inverting the bottle until all the precipitate is dissolved. If oxygen is present, the sample will turn a yellow or orange color

NOTE: At this point, the oxygen is "fixed" and the rest of the procedure can be done back at the school, or even on another day.

19. Fill the titration tube (0608) to the 20 mL line with the fixed sample. Cap the tube. There is a small hole in the cap to insert the titration tube.
20. Depress plunger of the Titrator (0377). The titrator looks like a blunt syringe.
21. Insert the Titrator into the plug in the top of the Sodium Thiosulfate, 0.025N (4169) titrating solution.
22. Invert the bottle and slowly withdraw the plunger until the large ring on the plunger is opposite the zero (0) line on the scale. NOTE: If small air bubbles appear in the Titrator barrel, expel them by partially filling the barrel and pumping the titration solution back into the reagent container. Repeat until bubble disappears.
23. Turn the bottle upright and remove the Titrator.
24. Add 8 drops of Starch Indicator Solution (4170WT). The sample should turn blue.
25. Insert the tip of the Titrator into the opening of the titration tube cap.
26. Slowly depress the plunger to dispense the titrating solution (Sodium Thiosulfate). As you add the titrating solution, the sample will become more pale. Gently swirl the sample as you add the titrating solution.
27. If you go through a complete titrator-full of Sodium Thiosulfate, repeat steps 19-22 to refill your titrator.



28. Continue titrating until the blue color disappears and the solution becomes colorless. Holding the titration tube over a white piece of paper helps to determine if the solution is colorless.
29. Read the test result directly from the scale where the large ring on the Titrator meets the Titrator barrel. Record as ppm Dissolved Oxygen. Each minor division on the Titrator scale equals 0.2 ppm.

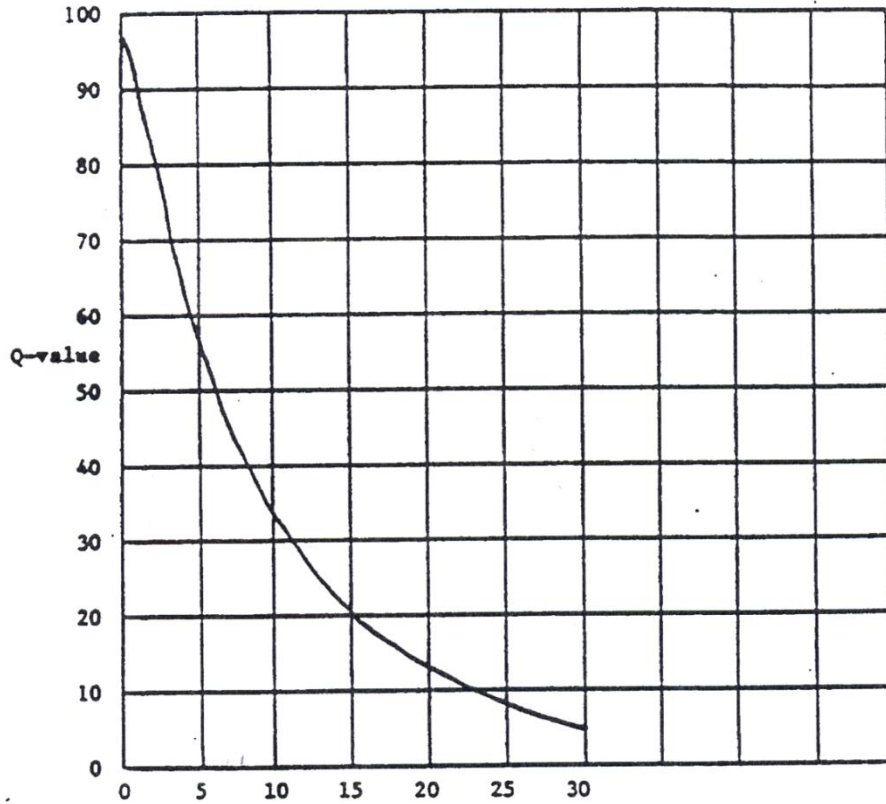
NOTE: If you filled the Titrator more than once, each full Titrator counts as 10ppm

30. Determine the BOD level by subtracting the DO level from this sample (the 5 day) from the original DO level: $\text{DO (original)} - \text{DO (5 day)} = \text{BOD5 level}$

iv. Determining the Q – Value

CALCULATING THE RESULTS

Chart 4: 5-Day Biochemical Oxygen Demand(BOD₅) Tests



BOD₅: mg/l

Note: if BOD₅>30.0, Q=2.0

v. Common Mistakes

1. A very common mistake is having a student collect a sample in a bucket, and bringing it up to the shore of the stream, and then pouring the water from the bucket into the sample bottle. This introduces lots of extra oxygen into the sample leading to an inaccurate result. The DO sample bottle **MUST** be submerged below the surface of the stream and bubbles removed underneath the surface of the water. A student in waders will probably have to get the sample for this test.
2. Be careful not to introduce air bubbles when adding chemicals.
3. Make sure you get all air bubbles out of the bottle.
4. Make sure you remove air bubble from the titrator when adding the Sodium Thiosulfate.
5. Make sure you have temperature in Celsius when calculation percent saturation.
6. Make sure you use percent saturation to determine the q-value, not ppm oxygen.
7. Students who go through multiple titrations of Sodium Thiosulfate, and only get the reading from the last one.
8. Try to get your temperature reading as close to the time of collecting and “fixing” your dissolved oxygen sample as possible.
9. Remember to put your sample in a dark bottle and a dark place.

vi. Analysis

If you have a high BOD reading, try to determine what might be using up the oxygen in the water. Was there a high amount of organic matter or “muck” at the bottom of the stream? If there was, where did that “muck” come from? Leaves from nearby trees? Runoff from a field? Grass clippings being dumped in the stream?