Hach Method 8043 – Determination of the Biochemical Oxygen Demand in Water and Wastewater: Updated with Dissolved Oxygen Measurement by Hach Method 10360 - Luminescence Dissolved Oxygen Sensing (LBOD™)

HACH METHOD 8043
Determination of the Biochemical Oxygen Demand in Water and Wastewater

1.0 Scope and Application

1.1 Hach Method 8043 (Measurement of the Biochemical Oxygen Demand in Water and Wastewater) is a United States Environmental Protection Agency (USEPA) Accepted Alternate Test Procedure (July 7, 1994)) to the reference methods (40 CFR 136.3)) of AOAC 973.44, USGS 1578-78, and SM 5210B, for sample preparation and calculation of the biological oxygen demand (BOD) from incubation and measurement of dissolved oxygen (DO) in water and wastewater samples.

1.2 This method may be used for National Pollution Discharge Elimination System (NPDES) compliance reporting in Clean Water Act (CWA) programs.

1.3 The (BOD) is an empirical measurement of the oxygen requirements of municipal and industrial wastewaters and sewage. The test results are used to calculate the effect of waste discharges on the oxygen resources of the receiving waters. The BOD test is of limited value in measuring the actual oxygen demand because temperature change, biological population, water movement, sunlight, oxygen concentration, and other environmental factors cannot be reproduced accurately in the laboratory. The BOD test is of greatest value after patterns of oxygen uptake for a specific effluent and receiving water has been established.

1.4 The BOD is performed by incubating a sealed wastewater sample (or a prepared dilution) for the standard five-day period and then determining the change in DO content. The BOD value can then be calculated from the results of the dissolved oxygen tests.

1.5 Dissolved oxygen is measured using Hach Method 10360 – Measurement of Luminescence Dissolved Oxygen (LDO®) in Water and Water.

1.6 For a thorough understanding of BOD and techniques for its determination, the reader is referred to the Bibliography in section 19 of this method.

2.0 Summary of Method

2.1 A volume of wastewater, or an appropriate volume diluted in ionic buffer is incubated for 5 days at 20°C in the dark. The reduction in DO concentration during the incubation period yields a measure of the biochemical oxygen demand.

3.0 Interferences

3.1 Many chlorinated and industrial effluents require special treatment to ensure reliable BOD results. Residual chlorine, hydrogen ion activity less than a pH of 6.0 and greater than 8.5, and heavy metals and phenol may impact the reliability of the BOD analysis. Sample interference mitigation may be required.

4.0 Safety

4.1 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of any chemicals specified in this

method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses.

5.0 Equipment and Supplies

5.1 Hach HQD40 Meter with IntelliCAL Luminescent Dissolved Oxygen Probe (LDO®) for BOD Measurement (LBOD™) – Hach Part Number HQDBOD01
5.2 Hach IntelliCAL pH Probe for HQD40 Meter – Hach Part Number PHC10103
5.3 BOD bottle 300mL – Hach Part Number 621-00
5.4 Hach 890 Colorimeter or DR2800 Spectrophotometer – Hach Part Number 4847000 (890 Colorimeter) or DR2800-01 (DR2800 Spectrophotometer)
5.5 BOD bottle cap, Hach Part Number 2419-06
5.6 BOD Incubator – Hach Model 26162-00
5.7 Burette, 25 mL – Hach Part Number 26365-40 Pipette, Pasteur 229 mm length – Hach Part Number 21234-01
5.8 Pipette, Mohr 10 and 25 mL capacity – Hach Part Numbers 20934-38 (10 mL) and 20934-40 (25 mL)
5.9 Dilution Water Carboy Container (20 L) – Hach Part Number 1486860
5.10 Graduated Cylinder, 50 mL, 250 ml, 500 mL capacity – Hach Part Numbers 26363-41 (50 mL), 26363-46 (250 mL), 26363-49 (500 mL)
5.11 Bottle cap dispenser for nitrification inhibitor (for 35g bottle) – 459-01
5.12 Aquarium Pump with air-stone and tubing

6.0 Reagents

6.1 Buffers
6.1.1 BOD Buffer Pillows – Hach Part Numbers 14861-66 (3 L, 50/pk), 24364-66 (4 L, 50/pk), 14862-66 (6 L, 50/pk), 14863-98 (19 L, 25/pk).
6.1.2 Prepared Stock Buffer Solutions – Phosphate Buffer (Hach Part Number 43149), Calcium Chloride (Hach Part Number 42853), Magnesium Sulfate (Hach Part Number 43049), Ferric Chloride (Hach Part Number 42953).
6.1.3 DPD Total Chlorine Powder Pillows – Hach Part Number 2105669.
6.2 Nitrification Inhibitor Formula 2533™ – Hach Part Numbers 2533-35 (35 g) or 2533-34 (500 g).
6.3 BOD Seed Inoculum – fresh primary effluent or Hach Poly Seed (Part Number 29187-00).
6.4 Acetic Acid, Glacial ACS Reagent Grade – Hach Part Number 10049.
6.5 Glucose/Glutamic Acid – Hach Part Number 25144-20.
6.6 Potassium Iodide Solution 100 g L^{-1}, NIST – Hach Part Number 343-49 or Hach Powder Pillows (Part Number 1077-99).
6.7 Reagent Water – ASTM Type 1 or 2.
6.8 Sodium Hydroxide Solution, 50% w/w – Hach Part Number 2180-59 or Hach Dissolved Oxygen3 Powder Pillow (Part Number 987-99).
6.9 Sulfuric Acid ACS Grade – Hach Part Number 979-49.
6.10 Sodium Thiosulfate – Hach Part Number 352-53
6.11 Starch Indicator Solution – Hach Part Number 349-53.

7.0 Sample Collection, Preservation, and Storage

7.1 See Title 40 of the Code of Federal Regulations Part 136.3, Table II for information regarding required sample collection containers, preservation techniques and holding times.

8.0 Quality Control

8.1 It is recommended that each laboratory that uses this method be required to operate a formal quality assurance program as referenced in section 12.4 of this method. The minimum requirements of this program consist of an initial demonstration of laboratory capability and ongoing analyses of DO in laboratory prepared water standards as a test of continued performance to assess accuracy and precision.

9.0 Procedure for the Determination of BOD

9.1 Preparation of Dilution Water

9.1.1 Buffer Pillows or Prepared Stock Buffer Solutions - Follow the instruction provided with the buffer pillows or prepared buffer solutions in section 6.1. Prior to use of dilution water, insure DO is at saturation and the dilution water temperature is 20 ± 1 °C.

9.2 Sample Pretreatment

9.2.1 Samples for BOD determination should have a hydrogen ion activity (pH) of 6.0 to 8.5. For samples outside of this range, adjust pH with either sulfuric acid (H_2SO_4) or sodium hydroxide (NaOH) using a solution strength that does not dilute the sample by more than 0.5%. Samples that require pH adjustment also require the addition of biological seed.

9.2.2 Samples that have been chlorinated but no have no measurable chlorine residual (total chlorine) require the addition of biological seed.

9.2.3 Samples that have measurable total chlorine residual require dechlorination with sodium thiosulfate (Na_2SO_3).

9.2.3.1 Measure 100 mL of sample into a 250-mL Erlenmeyer flask. Add 10 mL of 0.020 N sulfuric acid solution and 10 mL of potassium iodide solution to the flask.

9.2.3.2 Add three droppersful of starch indicator solution and swirl to mix.

9.2.3.3 Fill a 25-mL burette with 0.025 N sodium thiosulfate solution and titrate the sample from dark blue to colorless.

9.2.3.4 Calculate the amount of 0.025 N sodium thiosulfate solution to add to the sample:

$$mL\ 0.025\ N\ sodium\ thiosulfate = \frac{mL\ titrant\ used\ x\ volume\ of\ remaining\ sample}{100}$$

9.2.4 Add the required amount of 0.025 N sodium thiosulfate standard solution to the sample. Mix thoroughly. Wait 10 to 20 minutes before running BOD test.

9.2.5 De-chlorinate a sufficient volume of sample required for the BOD test.

9.3 Sample Preparation

9.3.1 Prior to dilution (if required), bring the sample to a temperature of 20 ± 1 °C.

9.3.2 Determine the approximate dilutions required to obtain a minimum residual DO of 1 mgL^{-1} and a minimum DO depletion value of 2 mgL^{-1} after 5-d incubation at 20 ± 1 °C. At least of two sample dilutions must yield a residual DO value of 1 mgL^{-1} and a minimum DO depletion value of 2 mgL^{-1}.

9.3.3 Transfer calculated volume of sample to a 300-mL BOD bottle.
9.3.4 Fill the BOD bottle to an approximate volume of 275 mL with dilution water water.
9.3.5 Add 1 – 15 mL of seed (section 9.3.2).
9.3.6 If BOD determination is for carboneaous BOD (cBOD), add 0.16 g of nitrification inhibitor.
9.3.7 Complete filling the BOD bottle with enough dilution water so that the stopper will displace all air, leaving no bubbles.

9.4 Seed Control Sample Preparation

9.4.1 Using a seed volume of 1 – 15 mL per 300 mL of dilution water, choose a primary effluent that will yield a glucose-glutamic acid BOD of 198 ± 30 mgL^{-1}. If using the Hach Poly Seed, prepare in similar fashion as the primary effluent seed source.
9.4.2 Fill the BOD bottle with enough dilution water so that the stopper will displace all air, leaving no bubbles.

9.5 Dilution Water BOD Blank Preparation

9.5.1 With each set BOD sample preparations, prepare three 300-mL samples of unseeded dilution water.
9.5.2 Measure and record the initial DO following the procedures in Hach Method 10360.
9.5.3 Replace any displaced dilution water sample with unseeded dilution water to fill the bottle. Re-stopper the BOD bottles and add a water seal around the neck of the bottle. Place the water seal cap over the stopper.

9.6 Glucose-Glutamic Acid Seed Sample Preparation

9.6.1 With each set of BOD samples, prepare a glucose-glutamic acid seed check sample.
9.6.2 Fill a BOD bottle with a minimum of 275 mL of dilution water.
9.6.3 Snap off the top of a glucose-glutamic acid ampoule and place the glass top, vial, and its contents into a 300-mL BOD bottle.
9.6.4 Add 1 – 15 mL of seed (section 9.3.2).
9.6.5 Complete filling the BOD bottle with enough dilution water so that the stopper will displace all air, leaving no bubbles.
9.6.6 The BOD for glucose-glutamic acid samples should be 198 ± 30 mgL^{-1}.

9.7 Initial DO Measurement and Sample Processing (Day 0)

9.7.1 Measure and record the initial DO of each dilution water blank, seed control, glucose-glutamic acid seed sample, and water and wastewater sample following the procedures in Hach Method 10360.
9.7.2 Replace any displaced diluted sample with dilution water to fill the bottle. Re-stopper the BOD bottle and add a water seal around the neck of the bottle. Place the water seal cap over the stopper.
9.7.3 Place the sample in the BOD incubator and incubate for 120 h (5-d) at 20 ± 1 °C.

9.8 Final DO Measurement (Day 5)

9.8.1 Measure and record the final DO of each dilution water blank, seed control, glucose-glutamic acid seed sample, and water and wastewater sample following the procedures in Hach Method 10360.

9.9 Calculations

9.9.1 Calculate the DO depletion of the dilution water samples. Depletion must be ≤ 0.2 mgL^{-1} for diluted water and wastewater samples to be valid.

9.9.2 Calculate the 5-d BOD for each sample that meets the 2 mg L^{-1} DO depletion and 1 mg L^{-1} residual DO requirement in section 9.3.2, and the BOD for each dilution blank and glucose-glutamic acid seed check sample using the following equations:

Unseeded BOD Sample Equation

$$5-day\ BOD,\ mgL^{-1} = \frac{D_1 - D_2}{P} f$$

Seeded BOD Sample Equation

$$5-dayBOD,\ mgL^{-1} = \frac{(D_1 - D_2) - (B_1 - B_2)\ f}{P}$$

where:

D_1 = DO of sample immediately after sample preparation, mgL^{-1},
D_2 = DO of sample after 5-d incubation at 20°C, mgL^{-1},
P = Volumetric fraction of sample used, decimal nomenclature,
B_1 = DO of seed control before incubation, mgL^{-1},
B_2 = DO of seed control after incubation at 20°C, mgL^{-1},
f = ratio of seed in sample to seed in seed control (% seed in sample/% seed in control).

10.0 Pollution Prevention

10.1 The reagents used in this method pose little threat to the environment when recycled and managed properly.

10.2 Standards should be prepared in volumes consistent with laboratory use to minimize the volume of expired standards to be disposed.

11.0 Waste Management

11.1 It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect air, water, and land by minimizing and control all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

11.2 For further information on waste management, consult "The Waste Management manual for Laboratory Personnel", and Less is Better: Laboratory Chemical Management for Waste Reduction", both available from the American Society's Department of Government relations and Science Policy, 1155 16th Street N.W., Washington, D.C. 20036.

12.0 References

12.1 USEPA Acceptance Letter for Hach Method 8043, July 7, 1994.

12.2 USEPA Approval Letter for Hach Method 10360 July 26, 2006.

12.3 Hack Method 10360 - Measurement of Luminescence Dissolved Oxygen (LDO®) in Water and Water.

12.4 "Handbook of Analytical Quality Control in Water and Wastewater Laboratories," USEPA, EMSL-Cl, Cincinnati, OH 45268, EPA-600-4-79-019, March 1979.

13.0 Definitions and Purposes

13.1 The definitions and purposes are specified to this method but have been conformed to common usage as much as possible.

13.2 Units of weight and measure and their abbreviations

13.2.1 Symbols

°C degrees Celsius

13.2.2 Alphanumeric characters

mgL^{-1} milligram per liter

d day

h hour(s)

13.3 Definitions, acronyms, and abbreviations Alphabetical characters

13.3.1 LDO® - Luminescence Dissolved Oxygen

13.3.2 LBOD™ - Luminescence Biochemical Oxygen Demand

13.3.3 BOD - Biochemical Oxygen Demand

13.3.4 DO - Dissolved Oxygen

14.0 Bibliography

14.1 Doctor BOD; http//www.boddoctor.com

14.2 R.L.; Gibbs, *Journal of Water Pollution Control Federation*, 1979, 51(9), 2257.

14.3 *Water Analysis Handbook*, 4th ed., pages 697 – 708, Hach Company, Loveland, Colorado, U.S.A.

15.0 Example of Data Collection and Calculations

15.1 Example of a 5-d BOD test on influent and effluent wastewater samples from a typical municipal wastewater treatment plant

Data Collection

Sample Name	Sample Vol. (mL)	Seed Vol. (mL)	Dilution Water Vol. (mL)	Day 0 DO mgL^{-1}	Day 5 DO mgL^{-1}	DO Depletion mgL^{-1}	Pass/Fail Min. DO and Depletion
DB #1	0	0	300	7.33	7.31	0.01	+
DB #2	0	0	300	7.34	7.31	0.03	+
SC #2	0	30	270	6.71	4.29	2.42	+
Inf. #1	3.0	10.0	287	7.02	4.86	2.16	+
Inf. #2	5.0	10	285	6.93	3.53	3.40	+
Inf. #3	7.0	10	283	6.86	2.77	4.09	+
Eff. #1	50	10	240	6.98	5.08	1.90	-
Eff. #2	100	10	190	7.17	4.71	2.46	+
Eff. #3	200	10	90	7.29	4.06	3.23	+
G/GA	6	10	294	7.01	2.17	4.84	+

Calculations

Sample Name	D_1	D_2	B_1	B_2	f	P	BOD mgL^{-1}
Inf. #1	7.02	4.86	6.71	4.29	0.1	0.010	192
Inf. #2	6.93	3.53	6.71	4.29	0.1	0.017	186
Inf. #3	6.86	2.77	6.71	4.29	0.1	0.023	167
Eff. #1	6.98	5.08	6.71	4.29	0.1	0.167	2.8
Eff. #2	7.17	4.71	6.71	4.29	0.1	0.333	1.8
Eff. #3	7.29	4.06	6.71	4.29	0.1	0.667	1.2
G/GA	7.01	2.49	6.71	4.29	0.1	0.020	214

DB – dilution blank
SC – seed control
Inf – influent to treatment
Eff – treated effluent
G/GA – glucose – glutamic acid DO depletion check sample