

This test determines the amount of organic material in wastewater by measuring the oxygen consumed by microorganisms in biodegrading organic constituents of the waste. The test consists of measuring dissolved oxygen before and after incubating the sample for 5 days at 20°C.

QUANTITY	CONTENTS	CODE
2 x 60 mL	*Manganous Sulfate Solution	*4167-H
2 x 60 mL	*Alkaline Potassium Iodide Azide Reagent	*7166-H
2 x 60 mL	*Sulfuric Acid, Conc.	*5172-H
2 x 500 mL	*Sodium Thiosulfate Solution, 0.025N	*4169-L
100 mL	Starch Indicator Solution	4170-J
250 mL	Phosphate Buffer, pH 7.2	2843-K
250 mL	Magnesium Sulfate, 1.10%	3761-K
250 mL	Ferric Chloride, 0.015%	3760-K
250 mL	Calcium Chloride, 2.75%	3756-K
50	Polyseed BOD Seed Capsules	3-0002
1	Automatic Buret, 10 mL, glass, Teflon stopcock	0997
4	Pipets, Transfer, 1 mL, glass	2-2170
1	Flask, Erlenmeyer, 500 mL	0435
6	BOD Bottles, 300 mL	2-2033
1	Graduated Cylinder, 100 mL, glass	0419
1	Pipet, 2 mL, glass	2-2166
1	Pipet, 10 mL, glass	2-2168
1	Pipet, 25 mL, glass	2-2169
1	Pipet, 100 mL, glass	2-2179
1	Buret Stand, single	1090
1	Pipet Filler Bulb	2-2164

<sup>\*</sup>WARNING: Reagents marked with an \* are considered to be potential health hazards. To view or print a Material Safety Data Sheet (MSDS) for these reagents see MSDS CD or our web site. To obtain a printed copy, contact us by e-mail, phone or fax.

To order individual reagents or test kit components, use the specified code number.

Carefully read the instruction manual for the LaMotte Automatic Buret before performing the test procedure. The buret is calibrated in parts per million (ppm) Dissolved Oxygen, accuracy to within 0.1 ppm.

# **INTERFERENCES**

Caustic alkalinity, mineral acid, free chlorine, and heavy metals are among the factors that may influence test accuracy. BOD bottles must be extremely clean to maintain consistent test results.

## WASTEWATER PRETREATMENT

NOTE: Pretreatment reagents are not supplied with this kit.

The pH of the sample should be in the range of 6.5 to 7.5. Adjust samples with either 1N Sodium Hydroxide or 1N Sulfuric Acid to bring within this range. Samples with free chlorine should be treated with 0.025N Sodium Sulfite solution.

#### DILUTION WATER MAKEUP

Add 1 mL each of Phosphate Buffer, pH 7.2 (2843), Magnesium Sulfate, 1.10% (3761), Ferric Chloride, 0.015% (3760) and Calcium Chloride, 2.75% (3756) solutions for each liter of distilled water. Store at temperature as close to 20°C as possible. This water should not show a reduction in dissolved oxygen of more than 0.2 mg/L on incubation for 5 days.

Follow instruction accompanying "Polyseed" capsule when using seed to determine  $BOD_5$ .

# PROCEDURE FOR BOD DETERMINATION

Determine the amount of sample(s) to be added to a 300 mL BOD bottle. Table I can be used to estimate the amount of sample to add to the BOD bottle. Raw municipal wastewater usually contains about 100 to 300 mg/L BOD<sub>5</sub>, so 3-6 mL of sample water is used. For primary and secondary treated effluents, 45-75 mL samples should be used. Refer to Table I for other ranges.

TABLE I: EXPECTED BOD, RANGE

Sample added to 300 mL bottle (mL)	Minimum (mg/L)	Maximum (mg/L)
3	210	560
6	105	280
9	70	187
12	53	140
15	42	112
18	35	94
21	30	80
24	26	70
27	24	62
30	21	56
45	14	37
60	11	28
75	8	22
150	4	12

- 2. Fill two 300 mL BOD bottles (2-2033) about half full with dilution water. With an appropriately sized pipet or graduated cylinder dispense the precalculated amount of the sample into each of the two 300 mL BOD bottles. Fill each bottle with dilution water and insert stopper. Exclude all air bubbles.
- 3. Fill an additional two 300 mL BOD bottles (2-2033) only with dilution water. Insert stoppers.
- 4. Incubate one bottle containing diluted sample (Step 2) and one bottle containing only dilution water (Step 3) at 20°C for 5 days. Ensure that the area above the stopper contains distilled water. Check daily or use bottle covers to prevent evaporation (not supplied).
- 5. Run a dissolved oxygen determination on the remaining BOD bottle from Step 2. Record the initial dissolved oxygen content as D<sub>1</sub>. Run another dissolved oxygen determination on the remaining BOD bottle from Step 3. Record initial dissolved oxygen content for control as C<sub>1</sub>. See Dissolved Oxygen Determination Procedure.

## DISSOLVED OXYGEN DETERMINATION PROCEDURE

- **A.** Use a 1.0 mL transfer pipet (2-2170) to add 1.0 mL of the \*Manganous Sulfate Solution (4167) to the sample in the BOD bottle. Hold the tip of the pipet under the surface of the sample. Rinse pipet.
- **B.** Use a second pipet to add 1.0 mL of \*Alkaline Potassium Iodide Azide Reagent (7166). Stopper and invert several times to mix.
- C. Allow sample to stand to settle precipitate.
- $D \cdot$  Use a third pipet to add 1.0 mL of \*Sulfuric Acid, Conc. (5172). Stopper and invert several times to mix.
- **E.** Use the 100 mL graduated cylinder (0419) to measure and transfer 202 mL of the treated sample. Transfer to 500 mL Erlenmeyer flask (0435).
- **F.** Fill the Automatic Buret (0997) with \*Sodium Thiosulfate, 0.025N (4169). Titrate the sample in the flask to a light yellow color.
- **G.** Use the fourth pipet to add 5 drops of Starch Solution (4170). The sample will turn blue. Continue titration until the blue color just disappears. Record buret reading in mL.
- H. Dissolved Oxygen, mg/L = mL 0.025N Sodium Thiosulfate
- 6. After 5 days, run a dissolved oxygen determination on the two incubated BOD bottles (Step 4). Record the dissolved oxygen content of the incubated diluted sample (Step 2) as D<sub>2</sub>. Record the dissolved oxygen content of the incubated dilution water for control (Step 3) as C<sub>2</sub>.
  - **NOTE:** There should not be an increase or decrease of more than 0.2 mg/L of dissolved oxygen between  $C_1$  and  $C_2$ . Use Dissolved Oxygen Determination procedure described above.
- 7. Use the following equation to determine BOD<sub>5</sub>:

mg/L BOD<sub>5</sub> = 100 (D<sub>1</sub> – D<sub>2</sub>)/P = 300(D<sub>1</sub> – D<sub>2</sub>)/S P = % of sample added (S/300)100

S = Volume of sample added to 300 mL BOD Bottle  $D_1 = DO$  of diluted sample immediately after preparation

D<sub>2</sub> = DO of diluted sample after 5 day incubation at 20°C

Data attained is considered reliable if there is a minimum residual dissolved oxygen level of 1 mg/L and minimum dissolved oxygen depletion ( $D_1-D_2$ ) of 2 mg/L.

## LaMOTTE COMPANY

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