Oxygen Demand, Biochemical

DOC316.53.01201

Respirometric Method **Method 10099**

0–700 mg/L **BODTrak™II Apparatus**

Scope and application: For water and wastewater.

Test preparation

Before starting

This test is a 5-day test. Complete all the steps carefully to make sure that the test does not have to be done again.
The American Public Health Association (APHA) recommends an incubation temperature of 20 ±1 °C (68 ±1 °F) for the BOD test. Adjust the incubator to the applicable temperature setting. This setting can change with incubator circulation.
When the BOD range of a sample is unknown, use the results from the Chemical Oxygen Demand (COD) test, or the results from a series of BOD tests with the same sample but different volumes, or dilution ratios to select an applicable BOD range. Effluent is usually in the 0–70 mg/L range while influent is in the 0–700 mg/L range. When the BOD of the sample is more than 700 mg/L, prepare a sample dilution.
Refer to the BODTrak Instrument documentation for information on how to download the test results to a computer or printer.
Carbonaceous BOD (CBOD) can be determined by the addition of nitrification inhibitor. A test for CBOD is recommended for biologically-treated effluents, samples with bacterial seed, samples with biologically treated effluents and river water.
Review the Safety Data Sheets (MSDS/SDS) for the chemicals that are used. Use the recommended personal protective equipment.
Dispose of reacted solutions according to local, state and federal regulations. Refer to the Safety Data Sheets for disposal information for unused reagents. Refer to the environmental, health and safety staff for your facility and/or local regulatory agencies for further disposal information.

Items to collect

Description	Quantity
BODTrak apparatus	1
BODTrak bottles, 300 mL, glass	6
BOD Nutrient Buffer Pillow	6
Graduated cylinder	1
Grease, stopcock, tube	1
Incubator	1
Lithium Hydroxide Powder Pillow	6
Nitrification inhibitor (for CBOD only)	1 bottle
Stir bars	6

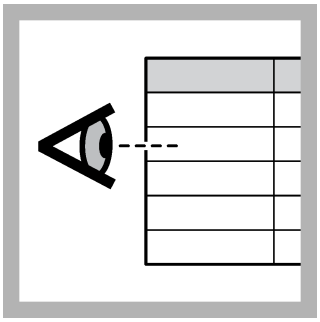
Refer to Consumables and replacement items on page 9 for order information.

Sample collection and storage

- Collect samples in clean glass or plastic bottles.
- To preserve samples for later analysis, keep the samples at or below 6 °C (43 °F) for up to 24 hours.
- Let the sample temperature increase to room temperature before analysis.

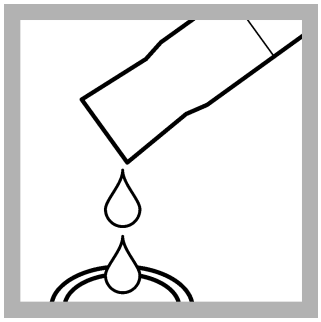
Test procedure

1. Increase or decrease the sample temperature to within 2 °C of its incubation temperature typically 20 °C (68 °F).



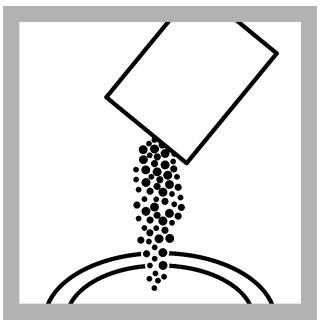
2. Select a BOD range and sample volume.

Note: *Effluent is usually in the 0–70 mg/L range. Influent is usually in the 0–700 mg/L range.*



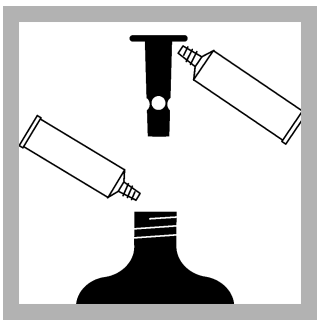
3. Use a clean graduated cylinder to measure and add the sample volume in six BODTrak bottles.

4. Put a 3.8-cm (1.5-in.) stir bar in each bottle.

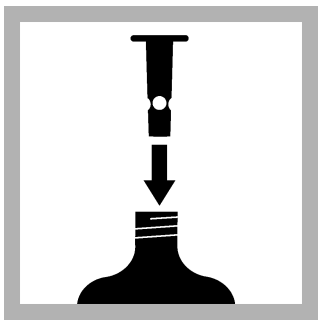


5. Add the contents of one BOD Nutrient Buffer Pillow to each bottle for optimal bacteria growth.

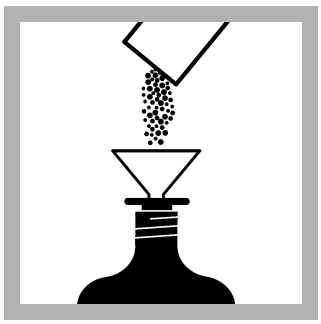
Note: *Ignore this step if accurate simulation of the original sample qualities is necessary.*



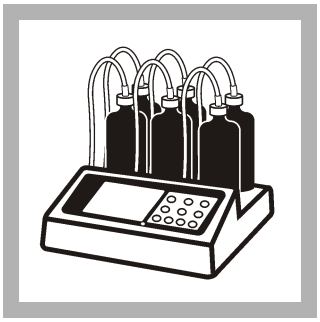
6. Apply stopcock grease to the seal lip of each bottle and to the top of each seal cup.



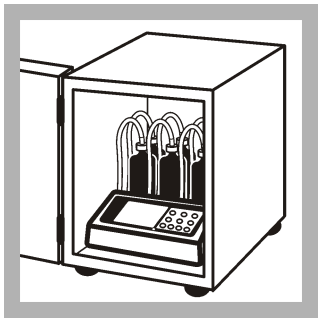
7. Put a seal cup in the neck of each bottle.



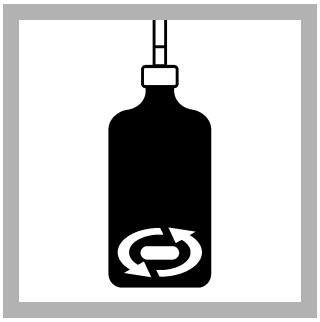
8. Use a funnel to add the contents of one Lithium Hydroxide Powder Pillow to each seal cup.



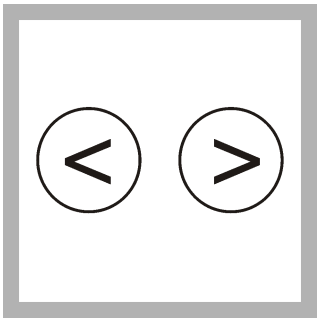
9. Put the bottles on the base of the BODTrak apparatus. Connect the tubes to the bottles and tighten the caps. Each tube is identified with the channel number. The channel numbers will show on the BODTrak display.



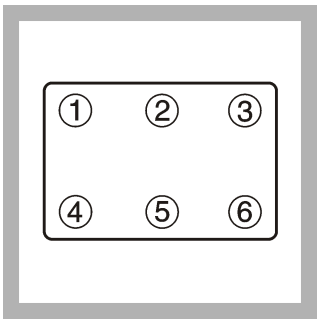
10. Put the instrument into the incubator. Set the instrument to on.



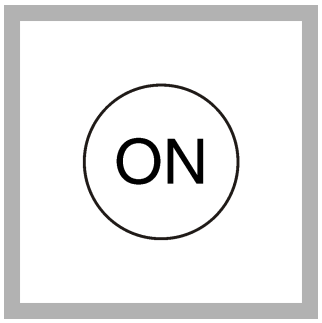
11. Make sure that all of the stir bars are in the center of the bottle and are rotating correctly. If a stir bar is on the side of the bottle, lift the bottle from the unit and carefully move it back. Do not start the channel test until the stir bar is rotating correctly.



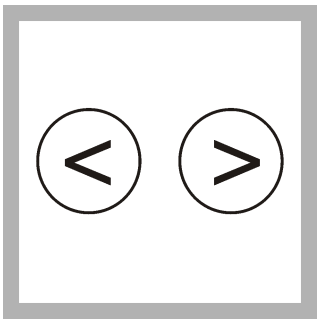
12. Push and hold the left and right arrow keys at the same time until the time menu shows. Push the CHANNEL 6 key to show the test length. Use the arrow keys to choose a 5-, 7-, or 10-day test (test duration is shown on the last line of the display). Push **OFF** to save selections and exit the menu.



13. To start a test, push the channel number of the related bottle.

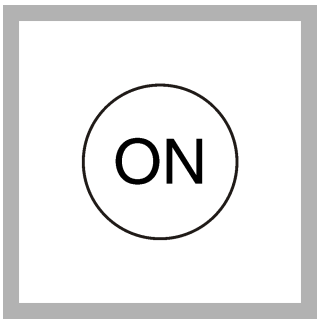


14. Push ON. A menu for the BOD range shows.

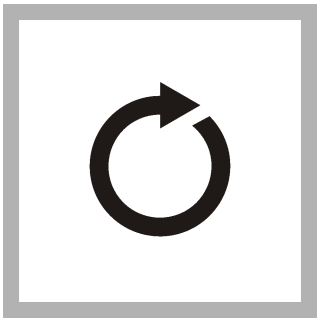


15. For the 0–350 mg/L range, push the right arrow key. For the 0–700 mg/L range, push the right arrow key a second time.

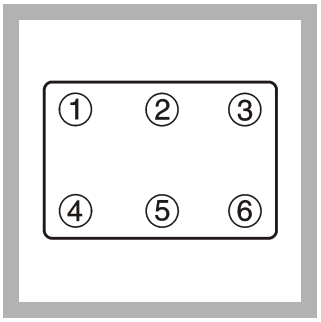
For the 0–35 mg/L range, push the left arrow key. For the 0–70 mg/L range, push the left arrow key a second time.



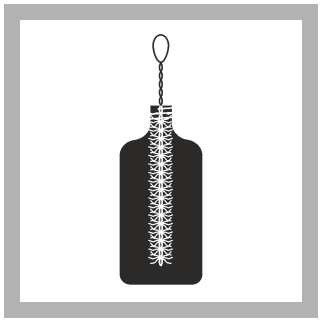
16. Push and hold ON to start a test. A graph shows on the display.



17. Do steps 13–16 again for each channel used, one at a time. To cancel a test, push OFF for several seconds.



18. After the selected test duration expires, the BODTrak apparatus will stop each channel. The display status will change from RUN to END. Push each channel key to read the BOD results.



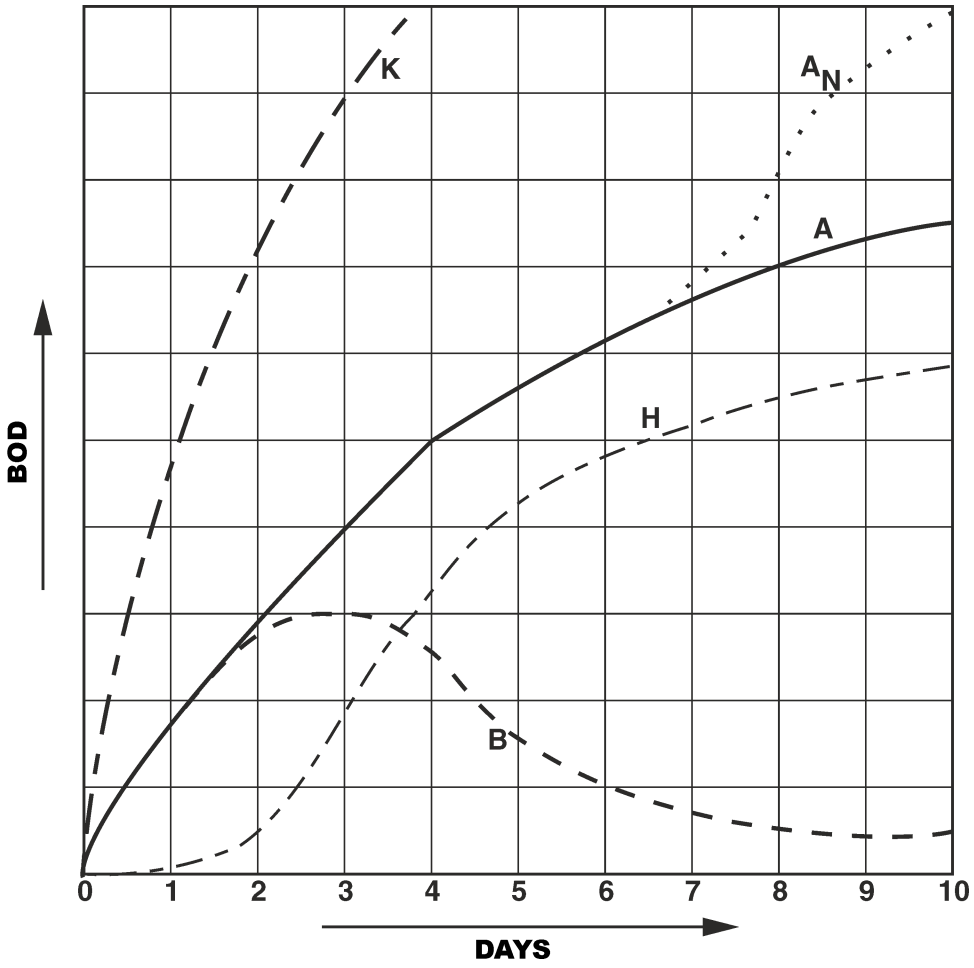
19. When the test is complete, use a brush and hot, soapy water to clean all bottles, stir bars, and seal cups. Fully rinse with distilled water.

Test results

If the test correctly completes, the display will show a curve like Curve A in Figure 1. If such a curve does not occur, look for one or more of the following problems.

- **Bottle leak**—A leak between the bottle cap and seal cup shows Curve B or a flat line. Look for dirt in the inner faces of the bottle cap and below the seal cup.
- **Time lag**—When the sample contains not sufficient bacteria, Curve H shows. Collect a fresh sample, add bacterial seed and repeat the test. Refer to Add bacterial seed to the sample on page 6. If bacteria was not acclimated to the sample this type of curve can also show. Bacteria that is not acclimated to the sample can occur when a standard solution is used even when seed is added.
- **Higher than estimated oxygen demand**—Samples that are above the selected range (e.g., the BOD value is more than 350 mg/L in a 160-mL sample) will cause a curve like Curve K. Use a higher BOD range and a different sample volume or dilute the sample. When the BOD is more than 700 mg/L refer to Dilute the sample on page 5.
- **Nitrification**—A very high BOD value (especially when testing final effluent) is an indication of nitrifying bacteria. Biological oxidation of organic nitrogen usually occurs after five days with normal domestic waste but can contribute to the oxygen demand. To remove the BOD from nitrifying bacteria, add 0.16 g of Nitrification Inhibitor into an empty sample bottle and then add the sample.

Figure 1 Examples of BOD curves



1 Curve A—Good BOD curve	**4** Curve K—BOD value is more than the selected test range
2 Curve B—Possible bottle leak	**5** Curve AN—BOD from nitrifying bacteria
3 Curve H—Not sufficient bacteria or acclimation	

Dilute the sample

If the BOD concentration is more than 700 mg/L, dilute the sample to a lower concentration. Dilute the sample with high-quality dilution water. Refer to Prepare the dilution water on page 5. Do one dilution for all samples when some equivalent samples are necessary. As an example, complete the steps that follow to make a 1:5 (5-fold) dilution.

1. Multiply the sample volume by 5.
   If the sample volume is 200 mL: 5 × 200 = 1000 mL
2. Add dilution water until the calculated sample volume. Mix well.
3. Use the test procedure to measure the BOD concentration of the sample.
   After sample dilution, select a BOD range and sample volume.
4. Multiply the test result by 5 to get the concentration of the sample before dilution.

Prepare the dilution water

Make sure that no source of oxygen demand or toxins are added when the dilution water is prepared.

Items to collect:

- Dilution water (refer to the dilution water guidelines)

- BOD Nutrient Buffer Pillow[1]
- Raw sewage[2] for the bacterial seed, 3 mL (if the sample is low in bacteria)

Dilution water guidelines

- For the best results, use distilled water from an alkaline permanganate distillation.
- Use high-quality water that does not contain organic compounds or toxic compounds (e.g., chlorine, copper and mercury).
- Do not use deionized water from ion exchange columns. The resins in the cartridges (mostly new cartridges) occasionally release organic materials that have an oxygen demand. In addition, bacteria can grow on the columns, which adds contamination to the dilution water.
- The dissolved oxygen concentration of the dilution water must not change by more than 0.2 mg/L when incubated for 5 days at 20 °C (68 °F).

Prepare the dilution water as follows:

1. Keep the dilution water in clean jugs in an incubator at 20 °C (68°F). Shake the jugs to saturate the water with air. As an alternative, loosely put the cap on the jugs and wait a minimum of 24 hours before use.
2. (Optional) Use a small aquarium pump or an air compressor to saturate the water with air. Make sure to use filtered air. Make sure that the filter does not grow bacteria.
3. Shake the BOD Nutrient Buffer Pillow to mix the contents.
4. Add the contents of the BOD Nutrient Buffer Pillow to the distilled water.
5. Put the cap on the jug. Shake the jug vigorously for 1 minute to dissolve the nutrients and to saturate the water with air.
6. If the sample is known to be low in bacteria (e.g., industrial waste or sewage that has been disinfected), immediately before the test, add 3 mL of raw sewage to each liter of the dilution water.

   Measure the BOD of the raw sewage collected. The BOD of the raw sewage will be subtracted from the BOD of the sample.

Conventional method (optional)

As an alternative, prepare the dilution water with the conventional method as follows:

1. Pipet 1 mL of each of the solutions that follow per liter of distilled water at 20 °C:
   Note: *Be careful to prevent contamination of the solutions.*

   - Calcium Chloride Solution
   - Ferric Chloride Solution
   - Magnesium Sulfate Solution
   - Phosphate Buffer Solution[3]
2. Put the cap on the bottle. Shake the bottle vigorously for 1 minute.

Add bacterial seed to the sample

Some types of BOD samples (e.g., industrial discharges) do not contain sufficient bacteria to oxidize the organic matter that is in the sample. Sewage treatment plant effluents that are disinfected do not contain sufficient bacteria to do a direct BOD test. To do a test on this type of sample, add bacterial seed or water that contains a high bacteria population (e.g., domestic sewage) to each bottle.

BOD of the bacterial seed

[1] Different sizes are available for different quantities of water (e.g., 3 L and 6 L).
[2] Keep the raw sewage at 20 °C (68°F) and do not move for 24–36 hours before use. Pipet from the upper portion of the sewage.
[3] Keep the phosphate buffer solution in a refrigerator to decrease the rate of biological growth.

Determine the BOD of the seed to calculate the BOD of the sample. To measure the BOD of the seed, follow the same procedure used to determine the BOD of the sample. Complete a BOD test on the seed and sample at the same time. Refer to seed acclimation information.

When the BOD of the seed is known, use the following formula to determine the sample BOD.

BOD sample = BOD observed – (Decimal fraction of seed used × BOD seed) ÷ Decimal fraction of sample used.

Example:

A seeded sample is 10% seed and 90% sample (by volume). The observed BOD is 60 mg/L, and the pure seed BOD is 150 mg/L.

BOD sample = 60 mg/L – (0.10 × 150 mg/L) ÷ 0.90 = 50 mg/L

Variations in initial bacterial populations

Low seed concentrations are more critical than those that are too high because they delay the start of oxidation and cause low BOD results. Use the trial and error method to determine the optimum concentration of seed for a specific waste material.

Choose the seed concentration that gives the highest sample BOD. This seed percentage can range from 2–30%, based on the waste material tested.

Seed acclimatization

Domestic sewage or freeze-dried BOD bacteria can supply seed for most samples. Freeze-dried BOD bacteria provides a constant seed source and is free of nitrifying microorganisms.

Pour the contents of one inoculum capsule into dilution water to rehydrate (Refer to the intructions on the capsule). Aerate and stir for 1 hour. Prepare sufficient quantity of this solution so that the quantity is between 10–30% of the overall sample volume. Determine the correct percentage of seed for each sample type.

For more information refer to *Standard Methods for the Examination of Water and Wastewater*, it emphasizes the importance of selecting the proper seed for specific wastes.

Use acclimated seed if the sample contains toxic materials like phenol, formaldehyde, or other microbic inhibitory agents. Acclimatize the bacterial seed in one non-metal or stainless steel gallon container fitted with an aeration system. Refer to Acclimatize the bacterial seed on page 8.

Interferences

To get good BOD results, special handling is necessary to analyze chlorinated and industrial effluents. Usually, careful experimentation with the sample shows the changes that should be made to the test procedure. Toxins in the sample have an adverse effect on any microorganisms in the sample, which results in lower BODs.

The substances in Table 1 interfere in the determination of oxygen demand at the given concentrations.

Table 1 Interfering substances

Interfering substance	Interference level
Chlorine	Small quantities of residual chlorine—Let the sample sit for 1 to 2 hours at room temperature. Larger quantities of chlorine—Refer to Remove chlorine from the sample on page 8.
Phenols	Dilute the sample with high quality distilled water. As an alternative, acclimatize the bacterial seed used in the dilution water to tolerate such materials. Refer to Acclimatize the bacterial seed on page 8.
Heavy metals	
Cyanide	

Table 1 Interfering substances (continued)

Interfering substance	Interference level
Highly buffered samples or extreme sample pH	Less than pH 6.5 or more than pH 7.5 interfere. Adjust to pH 7.2 with acid (Sulfuric Acid, 1 N or Phosphate Buffer Solution) or base (Sodium Hydroxide, 1 N).
Cold temperature	Cold samples can be supersaturated with oxygen and will have low BOD results. Fill a 1-liter (1-quart) bottle ½ full with cold sample. Shake the bottle vigorously for 2 minutes. Let the sample temperature increase to 20 °C (68 °F).

Remove chlorine from the sample

Items to collect:

- 250-mL Erlenmeyer flask
- 10-mL serological pipet and a pipet filler
- 25-mL buret
- 0.020 N Sulfuric Acid Standard Solution, 10 mL
- 100-g/L Potassium Iodide Solution, 10 mL
- 0.025 N Sodium Thiosulfate Standard Solution, 25 mL
- Starch Indicator Solution, 3 full droppers

1. Add 100 mL of sample to a 250-mL Erlenmeyer flask.
2. Use a 10-mL serological pipet and a pipet filler to add 10 mL of 0.020 N Sulfuric Acid Standard Solution to the flask.
3. Use a 10-mL serological pipet and a pipet filler to add 10 mL of 100-g/L Potassium Iodide Solution to the flask.
4. Add 3 full droppers of Starch Indicator Solution. Swirl to mix.
5. Fill a 25-mL buret with 0.025 N Sodium Thiosulfate Standard Solution.
6. Titrate the sample from dark blue to colorless.
7. Calculate the amount of 0.025 N Sodium Thiosulfate Standard Solution to add to the sample.

   mL 0.025 N Sodium Thiosulfate Standard Solution = (mL titrant used × volume of remaining sample) ÷ 100
8. Add the calculated quantity of 0.025 N Sodium Thiosulfate Standard Solution to the sample.
9. Mix fully. Wait 10–20 minutes before the test is done.

Acclimatize the bacterial seed

1. Fill a 4-liter (1-gallon) stainless steel or plastic container with domestic sewage.
2. Aerate the sewage for 24 hours.
3. Let the heavier material collect on the bottom for 1 hour.
4. Use a siphon to remove 3 liters (3 quarts) of the material from the top and discard.
5. Fill the container with a mixture of 90% sewage and 10% wastes that contain the toxic material.
6. Aerate for 24 hours.
7. Do steps 3–5 again with more and more quantities of waste until the container holds 100% toxic waste material.

Accuracy check

Standard solution method

Use the standard solution method to validate the test procedure and the instrument.

Items to collect:

- 300-mg/L BOD Standard Solution[4], Voluette Ampule, 10 mL
- BOD Nutrient Buffer Pillow for 3 L
- Bacterial seed
- Two 300-mL BOD bottles
- Pipet, volumetric, Class A, 7.0 mL

1. Shake 3 L of distilled water for 1 minute to saturate the water with oxygen. Make sure that the container is not fully filled with sample.
2. Add the contents of one BOD Nutrient Buffer Pillow for 3 L. Invert to mix.
3. Open the standard solution ampule.
4. Use a pipet to add 7.0 mL of the standard into a BOD bottle.
5. Add 133 mL of the nutrient buffer solution from step 2.
6. Add 15 mL of bacterial seed. Refer to Add bacterial seed to the sample on page 6.
7. Follow the test procedure and the 0–350 mg/L range to measure the BOD of the solution. Incubate the bottles for 5 days.
8. Prepare a second BOD bottle with bacterial seed. Follow the same procedure used to measure the BOD of the sample.
9. Use the formula in Add bacterial seed to the sample on page 6 to calculate the BOD value. Use the BOD value of the seed in the calculation.

Summary of method

Biochemical oxygen demand (BOD) is a 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.

BOD measures the quantity of oxygen used by bacteria as they oxidize organic matter in the sample. The waste sample is put in an amber BOD bottle with a high quantity of air above the sample. The bottle is connected to a pressure sensor. The bacteria uses dissolved oxygen, which is replaced by the air above the sample. This causes a fall in air pressure in the bottle, which is measured by the pressure sensor. The fall in air pressure is directly read as mg/L BOD from the graphical display. Oxidation of the sample produces carbon dioxide. The lithium hydroxide crystals in the seal cup removes the carbon dioxide.

Consumables and replacement items

Required reagents

Description	Quantity/test	Unit	Item no.
BOD Nutrient Buffer Pillow, 5 mL (for 3 L of dilution water)	1 pillow	50/pkg	1416066
Grease, stopcock, tube	1	75 g	56275
Lithium Hydroxide, Powder Pillows	6	100/pkg	1416369

Required apparatus

Description	Quantity/test	Unit	Item no.
BODTrak™II Apparatus, includes:	1	each	2952400
Bottle, BOD, amber	6	6/pkg	714421
Funnel, powder	6	each	2264467
Power Supply, 110/230 VAC	1	each	2624900

[4] 300-mg/L of glucose and 300-mg/L of glutamic acid

Required apparatus (continued)

Description	Quantity/test	Unit	Item no.
Power cord 115 VAC	1	each	2959200
Power cord 230 VAC	1	each	2959100
Seal cup	6	each	2959500
Stir bar, magnetic	6	each	2959400
Clippers for plastic pillows	1	each	96800

Recommended standards

Description	Unit	Item no.
BOD Standard Solution, Voluette® Ampule, 3000 mg/L, 10 mL	16/pkg	1486610

Optional reagents and apparatus 01201

Description	Unit	Item no.
B.O.D. Incubator, Low Temperature, 120V	each	2636300
B.O.D. Incubator, Low Temperature, 220V	each	2636302
BOD Nutrient Buffer Pillow, 3 mL (for 3 L of dilution water)	50/pkg	1486166
BOD Nutrient Buffer Pillow, 4 mL (for 4 L of dilution water)	50/pkg	2436466
BOD Nutrient Buffer Pillow, 6 mL (for 6 L of dilution water)	50/pkg	1486266
BOD Nutrient Buffer Pillow, 19 mL (for 19 L of dilution water)	25/pkg	1486398
Flask, Erlenmeyer, 250 mL	each	50546
Magnesium Sulfate Solution, APHA, for BOD	500 mL	43049
Nitrification inhibitor	35 g	253335
Nitrification inhibitor	500 g	253334
Nitrification inhibitor, dispenser cap	each	45901
Potassium Iodide Solution, 100 g/L	500 mL	1228949
Sodium Hydroxide, pellets, ACS	500 g	18734
Sodium Hydroxide Standard Solution, 1.00 N	100 mL MDB	104532
Sodium Thiosulfate Standard Solution, 0.025 N	1 L	35253
Sodium Thiosulfate Standard Solution, 0.1 N	100 mL	32332
Starch Indicator Solution	100 mL MDB	34932
Sulfuric Acid Standard Solution, 0.020 N	1 L	20353
Sulfuric Acid Solution, 1.00 N	1000 mL	127053

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