

METHOD #: 360.2 Approved for NPDES (Issued 1971)

TITLE: Oxygen, Dissolved (Modified Winkler, Full-Bottle Technique)

ANALYTE: CAS # O Oxygen 7782-44-7

INSTRUMENTATION: Titration, Probe

STORET No. 00300

1.0 Scope and Application

- 1.1 This method is applicable for use with most wastewaters and streams that contain nitrate nitrogen and not more than 1 mg/L of ferrous iron. Other reducing or oxidizing materials should be absent. If 1 mL of fluoride solution is added before acidifying the sample and there is no delay in titration, the method is also applicable in the presence of 100 200 mg/L ferric iron.
- 1.2 The Dissolved Oxygen (DO) Probe technique gives comparable results on all samples types.
- 1.3 The azide modification is not applicable under the following conditions: (a) samples containing sulfite, thiosulfate, polythionate, appreciable quantities of free chlorine or hypochlorite; (b) samples high in suspended solids; (c) samples containing organic substances which are readily oxidized in a highly alkaline solution, or which are oxidized by free iodine in an acid solution; (d) untreated domestic sewage; (e) biological flocs; and (f) where sample color interferes with endpoint detection. In instances where the azide modification is not applicable, the DO probe should be used.

2.0 Summary of Method

- 2.1 The sample is treated with manganous sulfate, potassium hydroxide, and potassium iodide (the latter two reagents combined in one solution) and finally sulfuric acid. The initial precipitate of manganous hydroxide, $Mn(OH)_2$, combines with the dissolved oxygen in the sample to form a brown precipitate, manganic hydroxide, $MnO(OH)_2$. Upon acidification, the manganic hydroxide forms manganic sulfate which acts as an oxidizing agent to release free iodine from the potassium iodide. The iodine, which is stoichiometrically equivalent to the dissolved oxygen in the sample is then titrated with sodium thiosulfate or phenylarsine oxide (PAO).

3.0 Interferences

- 3.1 There are a number of interferences to the dissolved oxygen test, including oxidizing and reducing agents, nitrate ion, ferrous iron, and organic matter.
- 3.2 Various modifications of the original Winkler procedure for dissolved oxygen have been developed to compensate for or eliminate interferences. The Alsterberg modification is commonly used to successfully eliminate the nitrite

interference, the Rideal-Stewart modification is designed to eliminate ferrous iron interference, and the Theriault procedure is used to compensate for high concentration of organic materials.

- 3.3 Most of the common interferences in the Winkler procedure may be overcome by use of the dissolved oxygen probe.

4.0 Sample Handling and Preservation

- 4.1 Where possible, collect the sample in a 300 mL BOD incubation bottle. Special precautions are required to avoid entrainment or solution of atmospheric oxygen or loss of dissolved oxygen.
- 4.2 Where samples are collected from shallow depths (less than 5 feet), use of an APHA-type sampler is recommended. Use of a Kemmerer type sampler is recommended for samples collected from depths of greater than 5 feet.
- 4.3 When a Kemmerer sampler is used, the BOD sample bottle should be filled to overflowing. (overflow for approximately 10 seconds). Outlet tube of Kemmerer should be inserted to bottom of BOD bottle. Care must be taken to prevent turbulence and the formation of bubbles when filling bottle.
- 4.4 At time of sampling, the sample temperature should be recorded as precisely as required.
- 4.5 Do not delay the determination of dissolved oxygen in samples having an appreciable iodine demand or containing ferrous iron. If samples must be preserved either method (4.5.1) or (4.5.2) below, may be employed.
- 4.5.1 Add 2 mL of manganous sulfate solution (6.1) and then 2 mL of alkaline iodide-azide solution (6.2) to the sample contained in the BOD bottle. Both reagents must be added well below the surface of the liquid. Stopper the bottle immediately and mix the contents thoroughly. The sample should be stored at the temperature of the collection water, or water sealed and kept at a temperature of 10 to 20°C, in the dark. Complete the procedure by adding 2 mL H_2SO_4 (see 7.1) at time of analysis.
- 4.5.2 Add 0.7 mL of conc. H_2SO_4 (6.3) and 1 mL sodium azide solution (2 g NaN_3 in 100 mL distilled water) to sample in the BOD bottle. Store sample as in (4.5.1). Complete the procedure using 2 mL of manganous sulfate solution (6.1), 3 mL alkaline iodide-azide solution (6.2), and 2 mL of conc. H_2SO_4 (6.3) at time of analysis.
- 4.6 If either preservation technique is employed, complete the analysis within 4-8 hours after sampling.

5.0 Apparatus

- 5.1 Sample bottles-300 mL \pm 3 mL capacity BOD incubation bottles with tapered ground glass pointed stoppers and flared mouths.
- 5.2 Pipets-with elongated tips capable of delivering 2.0 mL \pm 0.10 mL of reagent.

6.0 Reagents

- 6.1 Manganous sulfate solution: Dissolve 480 g manganous sulfate ($MnSO_4 \cdot 4H_2O$) in distilled water and dilute to 1 liter.
- 6.1.1 Alternatively, use 400 g of $MnSO_4 \cdot 4H_2O$ or 364 g of $MnSO_4 \cdot 4H_2O$ per

liter. When uncertainty exists regarding the water of crystallization, a solution of equivalent strength may be obtained by adjusting the specific gravity of the solution to 1.270 at 20°C.

- 6.2 Alkaline iodide-azide solution: Dissolve 500 g of sodium hydroxide (NaOH) or 700 g of potassium hydroxide (KOH) and 135 g of sodium iodide (NaI) or 150 g of potassium iodide (KI) in distilled water and dilute to 1 liter. To this solution add 10 g of sodium azide (NaN₃) dissolved in 40 mL of distilled water.
- 6.3 Sulfuric acid: concentrated.
- 6.4 Starch solution: Prepare an emulsion of 10 g soluble starch in a mortar or beaker with a small quantity of distilled water. Pour this emulsion into 1 liter of boiling water, allow to boil a few minutes, and let settle overnight. Use the clear supernate. This solution may be preserved by the addition of 5 mL per liter of chloroform and storage in a 10°C refrigerator.
 - 6.4.1 Dry, powdered starch indicators such as "thyodene" may be used in place of starch solution.
- 6.5 Potassium fluoride solution: Dissolve 40 g KF · 2H₂O in distilled water and dilute to 100 mL.
- 6.6 Sodium thiosulfate, stock solution, 0.75 N: Dissolve 186.15 g Na₂S₂O₃ · 5H₂O in boiled and cooled distilled water and dilute to 1 liter. Preserve by adding 5 mL chloroform.
- 6.7 Sodium thiosulfate standard titrant, 0.0375 N: Prepare by diluting 50.0 mL of stock solution to 1 liter. Preserve by adding 5 mL of chloroform. Standard sodium thiosulfate, exactly 0.0375 N is equivalent to 0.300 mg of DO per 1.00 mL. Standardize with 0.0375 N potassium biiodate.
- 6.8 Potassium biiodate standard, 0.0375 N: For stock solution, dissolve 4.873 g of potassium, biiodate, previously dried 2 hours at 103°C, in 1000 mL of distilled water. To prepare working standard, dilute 250 mL to 1000 mL for 0.0375 N biiodate solution.
- 6.9 Standardization of 0.0375 N sodium thiosulfate: Dissolve approximately 2 g (±1.0 g) KI in 100 to 150 mL distilled water; add 10 mL of 10% H₂SO₄ followed by 20.0 mL standard potassium biiodate (6.8). Place in dark for 5 minutes, dilute to 300 mL, and titrate with the standard sodium thiosulfate (6.7) to a pale straw color. Add 1-2 mL starch solution and continue the titration drop by drop until the blue color disappears. Run in duplicate. Duplicate determinations should agree within ± 0.05 mL.
- 6.10 As an alternative to the sodium thiosulfate, phenylarsine oxide (PAO) may be used. This is available, already standardized, from commercial sources.

7.0 Procedure

- 7.1 To the sample collected in the BOD incubation bottle, add 2 mL of the manganous sulfate solution (6.1) followed by 2 mL of the alkaline iodide-azide solution (6.2), well below the surface of the liquid; stopper with care to exclude air bubbles, and mix well by inverting the bottle several times. When the precipitate settles, leaving a clear supernatant above the manganese hydroxide floc, shake again. When settling has produced at least 200 mL of clear supernatant, carefully remove the stopper and immediately add 2 mL of conc. H₂SO₄ (6.3) (sulfamic acid packets, 3 g may be substituted for H₂SO₄) ⁽¹⁾ by allowing the acid to run down the neck of the bottle, re-stopper, and mix by

gentle inversion until the iodine is uniformly distributed throughout the bottle. Complete the analysis within 45 minutes.

- 7.2 Transfer the entire bottle contents by inversion into a 500 mL wide mouth flask and titrate with 0.0375 N thiosulfate solution (6.7) (0.0375 N phenylarsine oxide (PAO) may be substituted as titrant) to pale straw color. Add 1-2 mL of starch solution (6.4) or 0.1 g of powdered indicator and continue to titrate to the first disappearance of the blue color.
- 7.3 If ferric iron is present (100 to 200 mg/L), add 1.0 mL of KF (6.5) solution before acidification.
- 7.4 Occasionally, a dark brown or black precipitate persists in the bottle after acidification. This precipitate will dissolve if the solution is kept for a few minutes longer than usual or, if particularly persistent, a few more drops of H_2SO_4 will effect dissolution.

8.0 Calculation

- 8.1 Each mL of 0.0375N sodium thiosulfate (or PAO) titrant is equivalent to 1 mg DO when the entire bottle contents are titrated.
- 8.2 If the results are desired in milliliters of oxygen gas per liter at 0°C and 760 mm pressure multiply mg/L DO by 0.698.
- 8.3 To express the results as percent saturation at 760 mm atmospheric pressure, the solubility data in Table 422:1 (Whipple & Whipple, p 446-447, Standard Methods, 14th Edition) may be used. Equations for correcting the solubilities to barometric pressures other than mean sea level are given below the table.
- 8.4 The solubility of DO in distilled water at any barometric pressure, p (mm Hg), temperature, T °C, and saturated vapor pressure, μ (mm Hg), for the given T, may be calculated between the temperature of 0° and 30°C by:

$$\text{ml/L DO} = \frac{(P - \mu) \times 0.678}{35 + T}$$

and between 30° and 50°C by:

$$\text{ml/L DO} = \frac{(P - \mu) \times 0.827}{49 + T}$$

9.0 Precision and Accuracy

- 9.1 Exact data are unavailable on the precision and accuracy of this technique; however, reproducibility is approximately 0.2 mg/L of DO at the 7.5 mg/L level due to equipment tolerances and uncompensated displacement errors.

Bibliography

1. Kroner, R. C., Longbottom, J. E., Gorman, R.A., "A Comparison of Various Reagents Proposed for Use in the Winkler Procedure for Dissolved Oxygen", PHS Water

Pollution Surveillance System Applications and Development, Report #12, Water Quality Section, Basic Data Branch, July 1964.

2. Annual Book of ASTM Standards, Part 31, "Water", Standard D1589-60, Method A, p 373 (1976).
3. Standard Methods for the Examination of Water and Wastewater, 14th Edition, p 443, method 422 B (1975).