



accu-Test™ Micro Chemical Oxygen Demand System

Spectrophotometric Method

1. Scope and Application

1.1 The Chemical Oxygen Demand (COD) test determines the quantity of oxygen required for oxidation of reduced species including organic matter in a water sample using a specific oxidizing agent, temperature and time of reaction.

1.2 Bioscience, Inc. offers prepackaged reagents for use in determining COD in the following ranges:

Low Range COD Vials	5-150 mg/L COD
Standard Range COD Vials	20-900 mg/L COD
High Range COD Vials	100-4500 mg/L COD

These products are for use with the COD method described here. Samples with higher COD can be analyzed after dilution. When the chloride concentration of a sample exceeds 2000 mg/L, the sample may be analyzed after pretreatment with mercuric sulfate or dilution.

1.3 The twist-cap vial COD method has been designated as an accepted procedure for nationwide use^{1,2}. As an accepted procedure, the method is acceptable for use by any person required to use approved procedures under Section 304(g) of the Federal Water Pollution Control Act Amendments of 1972. Only Low Range and Standard Range reagents are accepted for NPDES reporting purposes. The twist-cap vial method is identical to EPA Method 410.4.

2. Summary of Method

2.1 Organic and inorganic compounds are oxidized in a sealed 10 ml borosilicate glass vessel (expendable tube), which contains premixed COD reagents. Chlorides (<2000 mg/L) are complexed by pretreatment with mercuric sulfate. The reagents and catalysts used are in the same proportions as those used in the Standard Titrimetric Methods^{3,4,5} with the exception of the normality of the dichromate oxidizing agent. The normality of the dichromate is that specified by Jirka and Carter⁶ and by EPA Method 410.4². For samples in the range of 20-900 mg/L (Standard Range), and in the range of 100-4500 mg/L (High Range), the COD is determined using a spectrophotometer at 600 nm by measuring the concentration of the produced Cr (III) ion. This wavelength is that specified by Method 410.4 and Jirka and Carter. For samples in the range of 5-150 mg/L, the COD can be determined using a spectrophotometer at 440 nm by measuring the decrease in concentration of the Cr (VI) ion as specified by Jirka and Carter. The path length of the tubes is 1.40 cm.

3. Sampling and Preservation

3.1 Care must be taken to store COD vials in light-proof containers (such as the original box) until use. The guaranteed shelf life of these products is one year if stored in the dark and at

room temperature. The reagents are usable beyond this date if analysis of standards demonstrates a linear calibration curve (over the range of concentrations of interest).

WARNING: Do not expose stored vials to temperatures below 4 degrees Celsius or above 45 degrees Celsius.

- 3.2 Samples should be collected in glass bottles. Plastic containers may be used if the containers are known to contain no contaminants which would interfere with the test.
- 3.3 Samples containing active microorganisms should be analyzed as soon after collection as possible. Bottles containing materials which have settled to the bottom should be well-mixed (preferably homogenized) to permit samples of representative aliquots.
- 3.4 Samples which cannot be run immediately (within 5 hours) after collection should be preserved with a sufficient concentration of H_2SO_4 to lower the pH to below 2 and be refrigerated at 4 degrees Celsius until analysis can be performed.

4. Interferences

- 4.1 Any contamination of the sample with foreign organic matter will cause an error in the analysis.
 - 4.1.1 Water used for dilution and blanks should be as low in organic material as possible. Distilled or type II deionized water is recommended. All water used in any set of analyses should preferably be from the same batch or container.
 - 4.1.2 Any glassware used in sample preparation should be proven to be as free of organics as possible. Rinse with 20% H_2SO_4 and dry at 150°C.
- 4.2 Volatile materials may be lost if the sample is mixed with reagents before sealing the vessel. To avoid this possibility, samples should be carefully added to vials down the side of the vessel to effect the layering of samples over the denser reagents until after the vial is sealed.
- 4.3 Chlorides are among the reduced species oxidized by dichromate and will cause erroneously high COD values if over 2000 mg/L Cl^- and not complexed. Therefore, sufficient mercuric sulfate is added to the reagents to complex chloride before the chloride reacts with the dichromate.
- 4.4 Spectrophotometric interferences in the sample can cause erroneously high COD values. Although samples with such interfering species are not typically encountered, those which might contain such species should be checked against the titration method⁹ to establish comparability.
- 4.5 Spectrophotometric interference in the form of light attenuation can result from smudged or scratched vessels. If a suitable "window" area for light transmission does not exist on the vessel, the sample must be transferred to a cell or to another vessel for reading. When transfer is necessary, care must be taken not to re-suspend or transfer precipitated salts which may be present.
- 4.6 Spectrophotometers with narrow transmission bandwidths (typically the more expensive instruments) may accentuate imperfections in the optical quality of the glass vessel resulting in loss of precision of replicates. Spectrophotometers with bandwidths of 20 nm or more are recommended

5. Apparatus

- 5.1 Premixed COD reagents in screw-cap glass tubes.
- COD Twist-Tubes, Standard Range (20-900 mg/L COD) Part #174-318
COD Twist-Tubes, Low Range (5-150 mg/L COD) Part #174-334
COD Twist-Tubes, High Range (100-4500 mg/L COD).Part #174-326
- 5.2 COD reactor capable of maintaining 150 ± 2 degrees Celsius.
- COD Reactor (15 Tubes).Part #163-486 (110V), Part #163-686 (220V)
COD Reactor (30 Tubes).Part #163-466 (110V), Part #163-666 (220V)
- 5.3 Spectrophotometer or colorimeter with 16 mm sample holder suitable for measurements at 600 nm and 440 nm. Colorimeters with filters may use alternate wavelengths between 600 and 620 nm or between 420 and 440 nm. Verify accuracy using standards of known COD concentration.

6. Reagents

- 6.1 Premixed reagents in the vials are specified in Section 5. These vessels contain all reagents and catalyst needed for the COD test, except those required for chloride pretreatment.
- 6.2 Sulfuric acid (95% H₂SO₄), for chloride pretreatment.
- 6.3 Mercuric sulfate (HgSO₄), for chloride pretreatment (if desired).
- 6.4 Distilled water. Precautions should be taken to insure that water used in the test be low in organic matter, preferably containing less than 0.3 ppm organic carbon.
- 6.5 Distilled or deionized type II water should be used in the preparation of all standard solutions.
- 6.6 Potassium acid phthalate (KHP): KOCOC₆H₄COOH ACS primary standard grade.
- 6.6.1 KHP stock solution: To obtain a 10 g/L COD stock solution, add 8.5034 g of potassium acid phthalate (KHP pre-dried to a constant weight at 110 degrees Celsius) to a 1000 ml volumetric flask and dilute to 1000 ml using deionized water.
- 6.6.2 COD working standards: A series of standard solutions that cover the expected range of sample concentrations is prepared by diluting appropriate volumes of the KHP stock solution. A procedural blank of the distilled water should be prepared with the other standards to zero the spectrophotometer.

7. Procedure

- 7.1 Preheat the reactor (heater block) to 150 degrees Celsius.
- 7.2 This section describes procedures for pretreatment of high chlorides (>2000 mg/L Cl⁻). If pretreatment is not necessary, skip to Section 7.3.
- NOTE: Samples should be diluted when possible to minimize chloride concentration. This procedure is preferred over the pretreatment method.

- 7.2.1 Place 30 ml of the sample into an Erlenmeyer flask.
- 7.2.2 Add 10 mg HgSO₄ for every milligram of Cl⁻ present in the sample.
- 7.2.3 Carefully add 5 ml of 95% H₂SO₄ with stirring to the contents of the flask and mix thoroughly.
- 7.3 Remove the cap from a COD vial.
- CAUTION: Use of safety glasses is recommended.
- 7.4 Carefully add 2.5 ml of sample (or standard solution) down the side of the vessel such that it forms a layer on top of the reagents. For High Range tests, use 0.5 ml of sample.
- NOTE: Samples should be thoroughly homogenized and milled in a blender or similar device before adding the sample to the vessel.
- 7.5 Carefully replace the cap and tighten cautiously.
- NOTE: Caps and seals can be broken by over-tightening.
- 7.6 Thoroughly mix the contents of the sealed vessel by shaking.
- CAUTION: The vessel will get very hot during mixing. Samples should be mixed either in racks or with use of insulated gloves. Appropriate clothing and eye protection should be worn.
- 7.7 Process standards and blanks exactly as the samples.
- 7.8 Place the vessel in the reactor maintaining 150 ±2 degrees Celsius for 2 hours. The 2-hour oxidation period is standard practice²⁻⁷ and is sufficient for complete oxidation of most compounds. (See Reference 5 for relative digestion efficiency of pure compounds.)
- 7.9 Mix vessel contents by shaking and allow to cool. If rapid cooling is desired, the vials may be placed in a water bath. If crystals form during rapid cooling, discontinue and allow vials to cool slowly in the room air.
- 7.10 Carefully wipe the vials clean.
- 7.11 Allow any suspended precipitate to settle for 10 minutes.
- 7.12 Setting the spectrophotometer zero
- 7.12.1 When using Standard and High Range vials, place a procedural (distilled water) blank vessel in the spectrophotometer set at 600 nm wavelength and rezero the absorbance reading.
- 7.12.2 When using Low Range COD vials, use the highest standard (150 mg/L COD) to set the spectrophotometer to zero absorbance at 440 nm.
- 7.13 Read the absorbance of each standard and sample on the spectrophotometer.

- 7.14 As an alternative or for comparison to the spectrophotometric determination, the cap may be removed; the contents may then be removed from the vial and the COD determined by the titrimetric method³⁻⁵. A technical bulletin is available from *Bioscience, Inc.* describing the COD titration method.

8. Calculation

- 8.1 Prepare a graphic calibration curve by plotting the absorbance on the abscissa (X) and the COD (mg/L) of standards on the ordinate (Y). For the Standard and High Range Method, the best-fit curve will have a positive slope. For the Low Range Method, the best-fit curve will have a negative slope.
- 8.2 The absorbance of samples can be converted graphically into measured COD. Linear regression is the preferred data treatment. Alternatively, use a scientific calculator or computer program or a preprogrammed spectrophotometer or colorimeter with built-in calibration curves or user entered calibration curves.
- 8.3 The measured COD must be multiplied by a dilution factor to correct for the dilution of the 30 ml sample if 5 ml of H₂SO₄ was added for complexing chlorides (per Section 7.2).
- 8.4 If a mathematical solution for the COD of samples is required, perform the following calculation:

$$\text{COD (mg/L)} = \frac{DA \times (C_2 - C_1)}{(A_2 - A_1)} + B$$

- C₂ = The COD (mg/L) of any standard solution
C₁ = Any COD (mg/L) of a standard lower than C₂
A₂ = The absorbance of the standard C₂
A₁ = The absorbance of the standard C₁
D = Dilution factor
= 1 if H₂SO₄ was not added to a 30 ml sample
= 35/30 = 1.167 if 5 ml H₂SO₄ was added to a 30 ml sample
A = The absorbance of the sample
B = Zero for Standard and High Range Method
= COD (mg/L) of highest standard for Low Range Method

9. Precision and Accuracy

- 9.1 After a thorough review and evaluation by the Environmental Protection Agency of the results of a comparability testing and other information submitted in accordance with Section 136.5, 40 CFR Part 136, the EPA determined the Standard Range micro-COD vial method to be a suitable alternate procedure to EPA Method 410.1¹. Precision of four replicates of each of 30 samples averaged $\pm 2.7\%$. The data submitted for this evaluation have been published by Messenger⁷. Reprints of this article are available from Bioscience Inc. upon request.
- 9.2 ASTM has published a round-robin study using the micro-COD methods. Results of these tests indicate a standard deviation of 4 mg/l for the low range reagent using KHP standards (5 to 45 mg/l) in Type II water. In the presence of 1000 mg/l sodium chloride, the standard deviation was 8 mg/l. A significant positive bias was demonstrated for low standard concentrations (5 and 12 mg/l) in the presence of sodium chloride.

The standard deviation ranged from 4.5 to 44 mg/l for the standard range reagent using KHP

standards (27 to 750 mg/l) in Type II water. The relative standard deviation decreased from a high of 20 - 25% to less than 5% as the standard concentration increased. The standard deviation ranged from 7.8 to 104 mg/l in the presence of 1000 mg/l sodium chloride. A significant positive bias was noted at the lowest concentration tested (27 mg/l) and a significant negative bias was noted at the highest concentration (750 mg/l). Supporting data are available from ASTM Headquarters, 1916 Race Street, Philadelphia, PA 19103-1187. Request Document RR:D19-1044.

Bibliography

- (1) Environmental Protection Agency, Approval of Alternate Water Pollutant Testing Procedure; Chemical Oxygen Demand (COD), Federal Register, Vol. 43, No. 45, Tuesday, March 7, 1978.
- (2) Methods for Chemical Analysis of Water and Wastes, EPA-600/4-79-020, Environmental Monitoring and Support Laboratory, Method 410.4 (1979).
- (3) Methods for Chemical Analysis of Water and Wastes, EPA-600/4-79-020, Environmental Monitoring and Support Laboratory, Method 410.1 (1979).
- (4) Standard Methods for the Examination of Water and Wastewater, 21st Edition, Method 5220D, (2005).
- (5) Annual Book of ASTM Standards, Vol. 11.02 "Water II," Standard D1252-78, pp 59-63 (1983).
- (6) Andrea M. Jirka and Mark J. Carter, Micro Semi-Automated Analysis of Surface and Wastewaters for Chemical Oxygen Demand, Analytical Chemistry, Vol. 47, No. 8, pp 1397-1402 (1975).
- (7) Allen L. Messenger, Comparisons of Sealed Digestion Chamber and Standard Method COD Tests, J. Water Poll. Contr. Fed., Vol. 53, No. 2, pp 232-236 (1981).
- (8) Environmental Protection Agency, Guidelines for Test Procedures for the Analysis of Pollutants, Federal Register, Vol. 41, No 233, Wednesday, Dec.1, 1976.
- (9) Bioscience Inc. Chemical Oxygen Demand Method for Ampules; Alternate Titration Method, (1986).