



Albuquerque Bernalillo County

Water Utility Department

WATER RECLAMATION DIVISION
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**WATER QUALITY LABORATORY
STANDARD OPERATING PROCEDURE APPROVAL FORM**

WQL SOP 211 Chemical Oxygen Demand

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*New WQL Management Staff
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STANDARD OPERATING PROCEDURE

SOP 211

CHEMICAL OXYGEN DEMAND

SCOPE AND APPLICATION: This method is applicable to COD in ground and surface water, domestic and industrial wastes. The practical range of determination is 10 mg/L to 1,500 mg/L.

APPLICABLE METHOD REFERENCES:

18th ed. of Standard Methods, 5220 A,D
Hach COD Procedure, for the Hach DR4000 spectrophotometer

DISPOSAL OF SAMPLES: Dispose of samples at an the sample compositing sink located in the sample preparation area.

**CHEMICAL OXYGEN DEMAND
CLOSED REFLUX, COLORIMETRIC METHOD**

1.0 GENERAL DISCUSSION

1.1 Principle: Most types of organic matter are oxidized by a boiling mixture of chromic and sulfuric acids. A sample is refluxed in strongly acidic solution with a known excess of potassium dichromate ($K_2Cr_2O_7$), for two hours at 150NC in a tightly capped culture tube. After digestion, oxygen consumed is measured against standards at 620 nm with a spectrophotometer.

1.2 Interferences:

Volatile straight-chain aliphatic compounds, are not oxidized to any appreciable extent. This failure occurs partly because volatile organics are present in the vapor space and do not come in contact with the oxidizing liquid. Straight-chain aliphatic compounds are oxidized more effectively when silver sulfate is added as a catalyst. However silver sulfate reacts with chloride, bromide, and iodide to produce precipitates that are oxidized only partially. The difficulties caused by the presence of the halides can be overcome largely, though not completely, by complexing with mercuric sulfate before the refluxing procedure. Although 1 g mercuric sulfate is specified for 50 mL sample, a lesser amount may be used where sodium chloride concentration is known to be less than 2000 mg/L, as long as a 10:1 ratio of mercuric sulfate to chloride is maintained. Do not use the test for samples containing more than 2000 mg/L chloride per liter.

Nitrite, exerts a COD of 1.1 mg O_2 /mg Nitrite-Nitrogen. Because concentrations of nitrite in waters rarely exceed 1 or 2 mg nitrite-nitrogen/L, the interference is considered insignificant and usually is ignored. To eliminate a significant interference due to nitrite, add 10 mg sulfamic acid for each mg nitrite-nitrogen present in the sample volume used; add the same amount of sulfamic acid to the reflux vessel containing the reagent water blank.

Reduced inorganic species, such as ferrous iron, sulfide, manganous manganese, etc., are oxidized quantitatively under the test conditions. For samples containing significant levels of these species, stoichiometric oxidation can be assumed from known initial concentration of the interfering species and corrections can be made to the COD value obtained.

1.3 Safety Considerations: The toxicity or carcinogenicity of each reagent used in this method have not been fully established. Each chemical should be regarded as a potential health hazard and exposure should be as low as reasonably achievable. Wear protective gloves, laboratory coat and eye protection. Dispose of all samples and reagents in an acid sink. Reagents used are potentially caustic or corrosive, avoid ingestion or inhalation and contact with the skin. For specific hazards consult the MSDS sheet, located in the laboratory office for the following compounds: sulfuric acid, silver sulfate, mercuric sulfate, potassium dichromate, potassium hydrogen phthalate.

1.4 Sample Preservation & Storage: Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water. Volume collected should be sufficient to insure a representative sample; allow for replicate analysis. Test unstable samples without delay. If delay before analysis is unavoidable, preserve sample by acidification to a pH of equal to or less than 2, using concentrated sulfuric acid. Samples preserved in this manner can be stored for up to seven days.

1.5 Sample Preparation: Blend samples containing settleable solids with a homogenizer to permit representative sampling. Make preliminary dilutions for wastes containing a high COD to reduce the error inherent in measuring small sample volumes.

1.6 Method Performance Criteria: The reference method cites the method precision and bias by stating, forty-eight synthetic samples containing potassium hydrogen phthalate and sodium chloride were tested by five laboratories. At an average COD of 193 mg O₂/L in the absence of chloride, the standard deviation was $\sqrt{17}$ mg O₂/L (coefficient of variation 8.7%). At an average COD of 212 mg O₂/L and 100 mg Cl⁻/L, the standard deviation was $\sqrt{20}$ mg O₂/L (coefficient of variation 9.6%).

2.0 APPARATUS & EQUIPMENT: Digestion vessels, 16x100 mm borosilicate culture tubes with TFE-lined screw caps. Heating block with block heater operated at 150 $\sqrt{2}$ NC, two mL class A volumetric pipet and auto pipet, sample homogenizer, vortex stirrer, spectrophotometer @ 620nm.

Auto pipet calibration procedure: To a class A 10mL volumetric flask add repeated 2mL volumes of reagent-grade water using the auto pipet. Check final volume of flask. Final volume must be 10mL, if not adjust pipet volume select knob and recalibrate using this procedure until the exact volume is delivered. Calibration of the auto pipet shall be conducted monthly and the date and analyst conducting the calibration documented on the COD bench sheet.

3.0 REAGENTS & SUPPLIES:

3.1 Deionized water, free of the analyte of interest.

3.2 HACH, Digestion solution for COD, HACH catalog #21259-15, 0-1500 ppm.

3.3 Digestion solution, Add to about 500 mL reagent water 10.216 g K₂Cr₂O₇, primary standard grade, previously dried at 103NC for two hours, 167 mL sulfuric acid reagent, and 33.3 g HgSO₄. Dissolve, cool to room temperature, and dilute to 1000 mL.

3.4 Sulfamic acid, powdered.

3.5 Mercuric sulfate, Crystal or powder.

3.6 Potassium hydrogen phthalate, HOCC₆H₄COOK, lightly crush and then dry potassium hydrogen phthalate to constant weight at 120NC. Dissolve 425 mg in reagent water and dilute to 1000 mL. KHP has a theoretical COD of 500 ug O₂/mL. This solution is stable when refrigerated for up to three months in the absence of viable biological growth.

3.7 Sulfuric acid reagent, add Silver sulfate reagent or technical grade, crystals or powder, to concentrated sulfuric acid at the rate of 5.5 g Ag₂SO₄/Kg H₂SO₄. Let stand 1 to 2 days to dissolve Ag₂SO₄.

4.0 QUALITY CONTROL PROCEDURE:

4.1 Calibration Blank (CB) are to be used at a frequency of one per each calibration curve produced and precede each CCVS used. The CB is a volume of reagent water fortified with the same matrix as the calibration standards but without the analyte. The CB must have a quantification less than the method detection limit. If the acceptability limit is exceeded, then a new calibration blank must be prepared and recalibration must be instituted.

4.2 Initial Calibration Standard (ICAL) is a solution prepared from the primary dilution standard solution or stock standard solution. The ICAL solutions are used to calibrate the instrument response with respect to analyte concentration. A minimum of three ICALs are to be used to establish a valid calibration curve. The ICALs must be of concentrations that represent the high range, mid-range and low range of the calibration curve.

4.3 Continuing Calibration Verification Standards (CCVS) a mid-range ICAL used to verify continued calibration, are to be conducted with a frequency of one CCVS per ten samples, with a minimum of one CCVS for less than ten samples. A CCVS must be run last in each series of ten samples and must always be included at the end of any batch run regardless of the number of samples run or number of CCVS's used, i.e. use a 'End of Run CCVS'. CCVS's must have a percent difference of no greater than 10%. If the 10% acceptability limit is exceeded, then recalibration must be instituted. The samples subsequent to the last successful CCVS must be retested with a reinstated calibration curve.

4.4 Initial Calibration Verification Standard (ICVS) is a solution of method analyte of known concentration that is used to validate calibration curves. The ICVS is obtained from a source external to the laboratory and different from the source of calibration standards. ICVS is to be used at a frequency of one per each calibration curve produced. The ICVS must have a percent difference of not greater than 10%. If the 10% acceptability limit is exceeded, then recalibration must be instituted, using freshly prepared ICAL's and verified with a ICVS.

4.5 Laboratory Reagent Blank (LRB) is an aliquot of reagent water or other blank matrices that are treated exactly as a sample including exposure to all glassware, equipment, solvents, reagents, that are used with other samples. The LRB is used to determine if method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus. The laboratory must analyze at least one LRB with each batch of samples. Data produced are used to assess contamination from the laboratory environment. When the LRB value is 2.2 times the analyte MDL, a fresh aliquot of the sample/s must be prepared and analyzed again for the affected analyte after the source of contamination has been corrected and acceptable LRB values have been obtained. If the affected sample are beyond their holding time or lack adequate volume to retest, these samples will not be re-analyzed. The results of the affected samples will be corrected by subtracting out the measured level of contamination and reporting the difference. The corrected samples will require text to qualify the data. The text should state the sample has been corrected due to a LRB greater than 2.2 times the MDL.

4.6 Laboratory Control Sample (LCS) is an aliquot of reagent water or other blank matrices to which known quantities of method analytes are added in the

laboratory. The LCS is analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements. The laboratory must analyze at least one LCS with each batch of samples. Calculate accuracy as percent recovery. If the recovery of any analyte falls outside the required control limits of 85-115%, that analyte is judged out of control, and the source of the problem should be identified and resolved, any corrective action taken must be documented in the bound work sheet book concomitant with the analysis data. The laboratory must use LCS analyses data to assess laboratory performance against the required control limits of 85-115%. When sufficient internal performance data become available, usually a minimum of 20-30 analyses, optional control limits can be developed from the percent mean recovery (X) and the standard deviation (S) of the mean recovery. These data can be used to establish the upper and lower control limits as follows:

$$\begin{aligned}\text{UPPER CONTROL LIMIT} &= X + 3S \\ \text{LOWER CONTROL LIMIT} &= X - 3S\end{aligned}$$

After each five to ten new recovery measurements, new control limits can be calculated using only the most recent 20-30 data points. Also, the standard deviation (S) data should be used to establish an on-going precision statement for the level of concentrations included in the LCS. These data must be kept on file and be available for review. If the LCS is not within its control limits, then the affected samples must have text to qualify the data. The text should state the percent recovery achieved for the LCS.

Calculation of percent recovery for an LFB:

$$R = \frac{\text{LCS} - \text{LRB}}{s} \times 100$$

where: R = Percent recovery
LCS = Laboratory control sample.
LRB = Laboratory reagent blank.
s = Concentration equivalent of analyte added to fortify the LRB solution

4.7 Laboratory Control Sample Duplicate (LCSD) is prepped exactly like the LCS and measures precision of the methodology.

4.8 Quality Control Calculations:

To calculate a % Difference: $\frac{2(\text{Sample Result} - \text{Sample Result}') \times 100}{\text{Sample Result} + \text{Sample Result}'}$

To calculate a % Recovery: $\frac{(\text{Spiked Sample Result} - \text{Sample Result}) \times 100}{\text{Final Concentration of spike added}^*}$

* Use the dilution formula to compute the final concentration of spike added ie. $V_1 \times C_1 = V_2 \times C_2$. The volume of spike solution added should not exceed 1-2% of the final volume.

where: C_1 = Concentration of spike solution
 V_1 = Volume of spike solution added to sample
 C_2 = **Final Concentration of spike added**
 V_2 = Volume of sample

4.9 Method Detection Limit (MDL): MDLs must be established for all analytes, using reagent water (blank) fortified at a concentration of two to three times the estimated instrument detection limit. To determine MDL values, take seven replicate aliquot of the fortified reagent water and process through the entire analytical method. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$\text{MDL} = (t) \times (S)$$

where, t = Student's t value for a 99% confidence level and a standard deviation estimate with n-1 degrees of freedom, t = 3.14 for seven replicates.

S = Standard deviation of the replicate analyses.

MDLs should be determined annually.

4.10 Control Charts- All quality control data will be entered in the lab share drive by analysts performing this test. Quality assurance reviews are performed weekly, for complete details of control chart performance evaluations see QA SOP-005.

5.0 INSTRUMENT CALIBRATION: Calibration is conducted by generation of a calibration curve via concentration mode on the spectrophotometer. The calibration curve must be produced using three or greater Initial Calibration Standards (ICAL) and a Calibration Blank (CB). The calibration curve generated must have a regression analysis performed on the calibration curve. A correlation coefficient, resultant from the regression analysis, must be greater than or equal to 0.995 for the calibration curve generated. A mid-range ICVS must be run to validate the calibration curve. The percent difference must be no greater than 10%. If the acceptability limits are not met for the correlation coefficient and/or the ICVS, then a new calibration curve must be generated which meets the acceptability criteria.

6.0 PROCEDURE:

6.1 Digestion: Place approximately 250 mL of sample in a clean blender cup and homogenize at a high speed for two minutes. Blending the sample ensures a uniform distribution of suspended solids and thus improves the accuracy and reproducibility of test results.

Turn on the COD block heater to preheat to 150NC. WARNING: Protective clothing, gloves and eye protection must be worn when adding sample to vials, placing vials in a heating block and removing vials from heating block to cool. Vials contain sulfuric acid and toxic mercury salts. Cautiously remove the cap of a COD digestion vial. While holding the vial at a 45-degree angle, carefully pipet 2.0 mL of sample into the vial. Replace the cap to the vial after sample has been added, ensure that the cap is well tightened. Using a vortex stirrer, stir the vial. Place the stirred sample vial in the block heater and allow it to reflux for two hours at 150NC.

Prepare a Calibration Blank by adding 2 mL reagent water to a digestion vial and refluxing for two hours at 150NC. Prepare ICAL's and a ICVS by adding individually, 2 mL prepared calibration standard and control standard to a

digestion vial and refluxing for two hours at 150NC.

6.2 Calibration & Procedure:

- 1) Turn on Hach DR4000 by depressing rocker switch at rear left of instrument.
- 2) Lift up the display panel and allow instrument to complete internal check.
- 3) From the main screen depress the soft key to select **USER PROGRAM**.
- 4) From the user program screen using the up and down page keys place the highlighted bar on user method 2, COD. Then depress the **EDIT** soft key, followed by depression of the **ENTER** key.
- 5) From the user program 2 edit program screen using the up and down keys place the highlighted bar on Calib. Table and depress the **EDIT TABLE** soft key.
- 6) The table appears on the display and the soft key menu changes to **ENTRY DONE**, **EDIT ABS** and **DELETE LINE**. The cursor prompts for a new concentration value or an adjustment to an existing concentration value on the CONC(mg/L) ?_ _ _ _ prompt line. Use the up and down arrow keys to highlight any line of data that requires a change. Enter the concentration using the digit keys. If the standards needs to be re-read and a new absorbance reported, highlight the line using the arrows keys, press the **EDIT ABS** soft key, then press the **CE** key and the **ENTER** key. When the **ENTRY DONE** soft key is pressed, the instrument will prompt to read the edited standards.
- 7) Add the zero concentration standard to the sample compartment and press the **ZERO** soft key. Press the **READ** soft key to accept the concentration reading for the zero absorbance. Place each standard concentration sample cell into the sample cell holder in the order listed and press the **READ** soft key. The instrument will read the standards and report an absorbance value. The information will be added to the calibration table. When the last standard is read, the display changes to show a graphical representation of the data.
- 8) Press **ENTRY DONE** soft key. Press the **EXIT** key twice and return to the main screen.
- 9) From the main screen depress the soft key to select **USER PROGRAM**.
- 10) From the user program screen using the up and down page keys place the highlighted bar on user method 2, COD. Then depress the **ENTER** key.
- 11) Add the zero concentration standard to the sample compartment and press the **ZERO** soft key. Press the **READ** soft key to accept the concentration reading for the zero absorbance.
- 12) Place sample cells into the sample compartment and read concentration values.

6.3 Sample Analysis: Place a cooled digested sample tube into the cuvette holder with the logo aligned with alignment mark and cover with the light exclusion cap. Read the concentration displayed and record the result.

7.0 CALCULATIONS: Using direct reading mode, no calculation is needed to obtain a result. If a sample has been diluted to achieve a reading in the analytical range of the calibration curve, then the displayed result must be adjusted to give a final result by multiplying the displayed result by a dilution factor.

To calculate a dilution factor:
$$\frac{\text{mL Sample} + \text{mL Diluent}}{\text{mL Sample}} = \text{Dilution Factor}$$

7.0 REPORTING: All measurements and results are to be reported to three
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significant figures and will be transcribed from the instrument display and recorded in the bound work sheet book for Chemical Oxygen Demand. The determined results for each sample tested will be entered on the electronic data system, SOLLMS. All samples requiring qualification will be text at the sample level in SOLLMS. All analyses requiring corrective actions will have the documentation of the corrective action in the bound work sheet book, concomitant with the sample results, calibration and QC results.