## Alternate Test Procedure \#N05-0014

## In-Situ Inc. Method 1002-8-2009 Dissolved Oxygen Measurement by Optical Probe

## TABLE OF CONTENTS

DISSOLVED OXYGEN ..... 3

1. SCOPE AND APPLICATION ..... 3
2. SUMMARY OF METHODS ..... 3
3. DEFINITIONS ..... 3
4. INTERFERENCES ..... 4
5. SAFETY ..... 4
6. EQUIPMENT AND SUPPLIES ..... 4
7. REAGENTS AND STANDARDS ..... 4
8. SAMPLE COLLEC/TION, PRESERVATION AND STORRAGE ..... 7
9. QUALITY CONTROL ..... 8
10. DISSOLVED OXYGEN METER CALIBRATION ..... 9
11. PROCEEDURE ..... 12
12. METHOD PERFORMANCE ..... 12
13. POLLUTION PREVENTION ..... 12
14. WASTE MANAGEMENT ..... 12
15. REFERENCES ..... 12
16. TABLES ..... 14
APPENDIXES ..... i
Dissolved Oxygen in Saturated Water. ..... ii
Density of Water (g/mL) vs. Temperature $\left({ }^{\circ} \mathrm{C}\right)$ ..... vii

## DISSOLVED OXYGEN

## 1. SCOPE AND APPLICATION

1.1. The Dissolved Oxygen determination is an analytical test in which standardized lab procedures are used to determine the oxygen concentration of water, wastewater, effluent and polluted water. These tests will measure the oxygen by means of an optical electrode compared to a membrane electrode.

## 2. SUMMARY OF METHODS

2.1. During the last two decades, a new form of electrode was developed based on the luminescence emission of a photo active chemical compound and the quenching of that emission by oxygen ${ }^{1}$ This quenching photophysics mechanism is described by the Stern-Volmer equation for dissolved oxygen in a solution ${ }^{2}$ :
$\frac{I_{0}}{I}=1+K s v\left[O_{2}\right]$
I and $\mathrm{I}_{0}$ are luminesence in the presence and absence of Oxygen
Ksv is the Stern-Volmer constant for Oxygen quenching
[ $\mathrm{O}_{2}$ ] is the dissolved Oxygen concentration
This equation has been expanded for the fixed photomer on an oxygen optical probe to take into account the 2-dimensional photophysics ${ }^{3}$ :

$$
\frac{I_{0}}{I}=\frac{1+K s v\left[O_{2}\right]}{f_{1}}+\frac{1+K s v\left[O_{2}\right]}{f_{2}}
$$

I and $\mathrm{I}_{0}$ are luminesence in the presence and absence of Oxygen
Ksv is the Stern-Volmer constant for Oxygen quenching
[ $\mathrm{O}_{2}$ ] is the dissolved Oxygen concentration
$\mathrm{f}_{\mathrm{x}}=$ Fraction of each solid state photomer
The determination of oxygen concentration by luminescence quenching has a linear response over a broad range of oxygen concentrations and has excellent accuracy and reproducibility ${ }^{4}$.

## 3. DEFINITIONS

3.1. BOD: Biochemical Oxygen Demand
3.2. CBOD: Carbonaceous Biochemical Oxygen Demand
3.3. DI Water: Deionized water
3.4. Duplicate: Repetitive individual analyses of a sample to measure precision.
3.5. RPD, Relative Percent Difference: Measure of precision and/or matrix effects of Duplicates. The RPD must be less than or equal to ( $\leq$ ) $20 \%$
3.6. Standard Methods: Standard Methods for the Examination of Water and Wastewater, $21^{\text {st }}$ Edition or Online

## 4. INTERFERENCES

4.1. Chemical compounds that compete or interfere can bias the optical electrode or the membrane electrode.
4.2. Optical electrodes respond to $\mathrm{O}_{2}$ partial pressure, which is a function of dissolved inorganic salts. Conversion factors may be calculated from DO concentration verses salinity.
4.3. Chemicals which can pass through or interact with the electrode cap or membrane may interfere with either the membrane electrode or optical electrode.
4.4. High concentrations of oil and grease may plug the membrane or occlude the cap.

## 5. SAFETY

5.1. This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment and a current awareness file of OSHA regulations regarding the safe handling of any chemicals specified in this method. A reference file of material safety data sheets (MSDSs) should be available to all personnel involved in these analyses

## 6. EQUIPMENT AND SUPPLIES

6.1.BOD Incubation bottles, $\sim 300 \mathrm{~mL}$ (capacity to be determined experimentally) with ground glass stoppers.
6.2. Analytical balance that has capacity to measure full BOD bottle to the nearest 0.1 gram.
6.3.Barometer (or internal barometer in the DO meter)
6.4.Stirring plate with stir bars.
6.5.Burette (Class A) with accuracy to the nearest 0.01 mL .
6.6.Dissolved oxygen meter and with In-Situ $\mathrm{RDO}^{\circledR}$ probe or suitable luminescence optical electrode. Note: other manufacturer's luminescence optical electrode may not perform in the non-stirred mode.

## 7. REAGENTS AND STANDARDS

### 7.1.DEIONIZED WATER (DI)

7.1.1. Water from a suitable system that produces water that is low in ions and dissolved solids. Suitable for analytical analyses.

### 7.2.BOD GRADE WATER

7.2.1. Water from a suitable source (Distilled, Deionized or RO) that is free of all chemical interference that would deplete dissolved oxygen or interfere with any dissolved oxygen chemical reaction.

### 7.3. DISSOLVED OXYGEN DILUTION WATER

7.3.1. After filling dilution water storage jug with BOD grade water, sparge with air. The dilution water must sit for approximately 24 hours to allow the dissolved oxygen to come to equilibrium with laboratory temperature and pressure.

### 7.4. BOD OPTICAL AND MEMBRANE PROBE ZERO SOLUTION

7.4.1. Sodium Sulfite Zero Solution Dissolve 10 g of sodium sulfite $\left(\mathrm{Na}_{2} \mathrm{SO}_{3}\right)$ in 200 mL of DI water. Add two drops of saturated cobalt chloride solution to the sodium sulfite solution. (ACS Grade or better)
7.4.2. Saturated Cobalt Chloride solution; Dissolve 4.5 grams of cobalt chloride hexahydrate $\left(\mathrm{CoCl}_{2} \cdot 6 \mathrm{H}_{2} \mathrm{O}\right)$ in 10 mL of DI water. (ACS Grade or better)
7.4.3. A commercial solution of this mixture can be purchased.

### 7.5. SULFURIC ACID

7.5.1. Concentrated $\mathrm{H}_{2} \mathrm{SO}_{4}$, (ACS Grade or better).
7.5.2. Commercial solution can be purchased.

### 7.6.MANGANOUS SULFATE SOLUTION

7.6.1. Dissolve $480 \mathrm{~g} \mathrm{MnSO}_{4} \cdot 4 \mathrm{H} 2 \mathrm{O}, 400 \mathrm{~g} \mathrm{MnSO}_{4} \cdot 2 \mathrm{H}_{2} \mathrm{O}$, or $364 \mathrm{~g} \mathrm{MnSO}_{4} \cdot \mathrm{H}_{2} \mathrm{O}$ (ACS Grade or better) in DI water, filter, and dilute to 1 L . The MnSO4 solution should not give a color with starch when added to an acidified potassium iodide (KI) solution. (ACS Grade or better)
7.6.2. Commercial solution can be purchased.

### 7.7.ALKALINE IODIDE-AZIDE SOLUTION

7.7.1. For saturated DO or less-than-saturated DO samples, Dissolve 500 g NaOH (or 700 g KOH ) and 135 g NaI (or 150 g KI ) in DI water and dilute to 1 L . Add 10 g $\mathrm{NaN}_{3}$ dissolved in 40 mL DI water. This reagent should not give a color with starch solution when diluted and acidified. (ACS Grade or better)
7.7.2. For supersaturated samples-Dissolve $10 \mathrm{~g} \mathrm{NaN}_{3}$ in 500 mL DI water. Add 480 g sodium hydroxide $(\mathrm{NaOH})$ and 750 g sodium iodide $(\mathrm{NaI})$, and stir until dissolved. There will be a white turbidity due to sodium carbonate $\left(\mathrm{Na}_{2} \mathrm{CO}_{3}\right)$. CAUTION, Do not acidify this solution because toxic hydrazoic acid fumes may be produced. (ACS Grade or better on chemicals) Commercial solution can be purchased.
7.7.3. Commercial solution can be purchased.

### 7.8.SODIUM THIOSULFATE

7.8.1. (Standard Titrant, 0.025 M) Purchase to meet Standard Methods $4500-\mathrm{O}$ C criteria or dissolve 6.205 g sodium thiosulfate-pentahydrate in $\sim 500 \mathrm{~mL}$ DI water in a 1000 mL volumetric flask, add 1.5 mL of 6 N NaOH or 0.4 grams solid NaOH , dilute to 1000 mL , mix well, and standardize. (ACS Grade or better)
7.8.2. (Standard Titrant, 0.0375 M), Purchase to meet Standard Methods 4500-O C criteria or dissolve 9.304 grams of sodium thiosulfate-pentahydrate in $\sim 500 \mathrm{~mL}$ DI water in a 1000 mL volumetric flask, add 1.5 mL of 6 N NaOH or 0.4 grams solid NaOH , dilute to 1000 mL , mix well, and standardize. (ACS Grade or better)
7.8.3. Commercial solution can be purchased.

### 7.9.STARCH SOLUTION

7.9.1. Use either an aqueous solution or soluble starch powder mixtures. To prepare an aqueous solution, dissolve 2 g laboratory-grade soluble starch and 0.2 g salicylic acid, as a preservative, in 100 mL hot DI water. (ACS Grade or better)
7.9.2. Commercial solution can be purchased.

### 7.10. POTASSIUM BI-IODATE

7.10.1. 0.0021 Molar potassium bi-iodate solution, ( 0.0021 M ): Dissolve 818.8 mg $\mathrm{KH}\left(\mathrm{IO}_{3}\right)_{2}$ in DI water and dilute to 1000 mL in a volumetric flask and mix well. (ACS Grade or better)
7.10.2. 0.00021 Molar potassium bi-iodate solution, ( 0.00021 M ): Transfer 100 mL of 0.0021 M potassium bi-iodate solution to a 1000 mL volumetric flask, dilute to 1000 mL with DI water and mix well (ACS Grade or better)
7.10.3. Commercial solution can be purchased.

### 7.11. THIOSULFATE STANDARDIZATION

7.11.1. Dissolve approximately 2 g KI, free from iodate, in a 500 mL Erlenmeyer flask with 100 to 150 mL DI water.
7.11.2. Add 1 mL 6 N H 2 SO 4 or a few drops of conc $\mathrm{H}_{2} \mathrm{SO}_{4}$.
7.11.3. Add 20.00 mL of 0.0021 M bi-iodate solution with a Class A pipette and mix well.
7.11.4. Titrate with an appropriate Class A burette the liberated iodine with thiosulfate titrant, adding starch toward end of titration (when a pale straw color is reached). Continue to titrate the solution until the blue color just disappears.
7.11.5. Record the mL of thiosulfate titrant used. Read the burette to an accuracy of only one half ( $1 / 2$ ) of a volume unit between each demarcation.
7.11.6. Calculate the Molarity of the thiosulfate.

Molarity $($ thiosulfate $)=\frac{20 \mathrm{ml} \times 12 \times 0.0021(\text { Molarity potassium bi }- \text { iodate })}{m l(\text { thiosulfate })}$
7.11.7. Balanced Equation

$$
\begin{aligned}
& \mathrm{IO}_{3}^{-}+5 \mathrm{I}^{-}+6 \mathrm{H}^{+} \rightarrow 3 \mathrm{I}_{2}+3 \mathrm{H}_{2} \mathrm{O} \\
& 6 \mathrm{~S}_{2} \mathrm{O}_{3}{ }^{2-}+3 \mathrm{I}_{2} \rightarrow 3 \mathrm{~S}_{4} \mathrm{O}_{6}{ }^{2-}+6 \mathrm{I}^{-} \\
& \text {Molar Ratio } \mathrm{IO}_{3}^{-}: 6 \mathrm{~S}_{2} \mathrm{O}_{3}{ }^{2-}
\end{aligned}
$$

### 7.12. BOD BOTTLE VOLUME DETERMINATION (Titrametric)

## (Note: Volume determination is required for Winkler Titration accuracy. See 9.7 for QA/QC requirements)

7.12.1. Fill a BOD bottle to the top with 0.00021 M potassium bi-iodate solution. Make sure no bubbles are present. Cap with stopper and pour off excess from the water seal.
7.12.2. Dissolve approximately 4 g KI, free from iodate, in a 500 mL Erlenmeyer flask with 50 to 100 mL DI water.
7.12.3. Add 2 mL 6 N H 2 SO 4 or a few drops of concentrated $\mathrm{H}_{2} \mathrm{SO}_{4}$.
7.12.4. Add the potassium bi-iodate solution from the BOD bottle and mix well.
7.12.5. Rinse the BOD bottle with DI water and add to the Erlenmeyer flask.
7.12.6. Titrate with a Class A burette the liberated iodine with standardized thiosulfate titrant, adding starch toward end of titration (when a pale straw color is reached). Continue to titrate the solution until the blue color just disappears.
7.12.7. Record the mL of standardized thiosulfate titrant used. Read the burette to an accuracy of only one half $(1 / 2)$ of a volume unit between each demarcation.
7.12.8. Make sure cap used for volume determination is mated to the BOD bottle used.
7.12.9. Calculate the BOD bottle volume to the nearest 0.1 mL

$$
\text { BOD Bottle }(\text { volume }, m l)=\frac{M(\text { Molarity thiosulfate }) \times m l(\text { thiosulfate })}{0.00021(\text { Molarity potassium bi }- \text { iodate }) \times 12}
$$

### 7.13. BOD BOTTLE VOLUME DETERMINATION (Gravimetric)

7.13.1. Weigh a BOD bottle and cap to the nearest 0.1 grams. Record the weight.
7.13.2. Fill a BOD bottle to the top with DI water. Make sure no bubbles are present. Cap with stopper and pour off excess from the water seal.
7.13.3. Weigh the full BOD bottle and cap to the nearest 0.1 grams. Record the weight.
7.13.4. Make sure cap used for volume determination is mated to the BOD bottle used.
7.13.5. Calculate the volume of the BOD bottle using the Density of Water (Appendix 2) at the temperature of the DI water. Record the volume to the nearest 0.1 mL .

$$
\text { BOD Bottle }(\text { volume }, m l)=\frac{[B O D \text { Bottle }(\text { Final weight, } g)-\text { BOD Bottle }(\text { Initial Weight, } g)]}{\text { Density of Water }}
$$

## 8. SAMPLE COLLEC/TION, PRESERVATION AND STORRAGE

8.1.See Title 40 of the Code of Federal Regulations Part 136.3, Table II for information regarding required sample collection containers.
8.2.Determine DO as soon as possible on all samples. Acid and azide preservations/stabilization are not suitable for samples utilizing the RDO probe.

## 9. QUALITY CONTROL

### 9.1. INITIAL AND ONGOING DEMONSTRATION OF CAPABILITY FOR DISSOLVED OXYGEN

9.1.1. Before new analysts perform the dissolved oxygen analyses or to confirm their continuing capability, verify their performance. Have them determine the dissolved oxygen with a calibrated meter of four BOD bottles of air saturated water. Utilizing the table in the appendixes, calculate the concentration of dissolved oxygen from the barometric pressure, temperature and salinity of the air saturated water. Calculate the standard deviation for the combined values for each analysis. This value is $\mathrm{S}_{\text {Pooled. }}$. Utilize this value to calculate the t -Test for the two experimental means. The value determined should be $\leq 2.31$ for $95 \%$ confidence. The ongoing demonstration of capability will be performed at a minimum on a quarterly basis.

$$
S_{\text {Pooled }}=\left[\frac{\sum(X-\bar{X})}{(8-1)}\right]^{\frac{1}{2}}
$$

Note: Calculate mean from all 8 Data points
Use all 8 data points to calculate pooled standard deviation

$$
\begin{aligned}
& t=\frac{\left|\bar{X}_{1}-\bar{X}_{2}\right|}{S_{\text {Pooled }} \sqrt{\frac{1}{2}}} \\
& \bar{X}_{1}=\text { Mean of the DO Meter Measurements } \\
& \bar{X}_{2}=\text { Mean of the DOCalculated Measurements } \\
& t \leq 2.31 \text { at } 95 \% \text { confidence }
\end{aligned}
$$

### 9.2. DAILY DATA ANALYSES

9.2.1. Data analysis to be performed on a daily basis or with each set of samples will consist of the dissolved oxygen measurement of a duplicate samples and the RPD calculated. Typical RPD values based on multiple laboratory analysis of varied sample types are illustrated below.

$$
\frac{\left(C_{I}-C_{D}\right)}{\left(\left(C_{I}+C_{D}\right) / 2\right)} \times 100=\mathrm{RPD}
$$

$$
\begin{aligned}
& C_{I}=\text { Concentration determined for a sample } \\
& C_{D}=\text { Concentration determined for the duplicate }
\end{aligned}
$$

### 9.3. LOWER LIMIT OF OPERATIONAL RANGE OF THE DO METER

9.3.1. Determine the lower limit of the meters operational range weekly or every time the meter or probe are serviced. Measure the dissolved oxygen with a calibrated
meter of four BOD bottles of zero solution. Calculate the standard deviation for the values for the analysis. This value is S . Utilize this value to calculate the confidence interval for the mean of the zero solution. The mean and confidence level must be below the minimum dissolved oxygen measurement required for the analysis.

$$
\begin{aligned}
& \qquad S=\left[\frac{\sum(X-\bar{X})}{(4-1)}\right]^{\frac{1}{2}} \\
& \text { Confidence Interval }=\bar{X} \pm \frac{t \times S}{2} \\
& t=\text { Student } 2 \text { Sided } t \text { Distribution }
\end{aligned}
$$

### 9.4.DO Spiking of Sample

9.4.1. No DO spiking of samples will be required as accurate spiking of a gaseous analyte into a laboratory sample is not easily achieved in most commercial and municipal laboratories.

### 9.5.BOD Bottle Volume

9.5.1. The use of a BOD bottle for Winkler titration volume requires that the bottle volume be known. Sections 7.12 through 7.13 describe how to calculate the volume.
9.5.2. The laboratory can determine the precision and bias of a large number of BOD bottles by selecting 10 bottles at random, determine their volume accurately, use a Known Value of 300 mL , and calculate the precision and bias utilizing the equations:
9.5.2.1. $\quad$ Bias $=\frac{\sum_{i=1}^{n}\left(X_{\text {Known Value }}-X_{\text {Experimental Value })}\right.}{n}$
9.5.2.2. $\quad$ Precision $=\sqrt{\frac{\sum_{i=1}^{n}\left(X_{\text {Known Value }}-X_{\text {Experimental Value }}\right)^{2}}{n-1}}$
9.5.3. All DO values calculated with the default Known Value of 300 mL will have the Bias and Precision reported in the final value.
9.5.4. The laboratory will repeat the precision and bias analysis at a minimum on a quarterly basis and keep control records of the precision and bias.

## 10. DISSOLVED OXYGEN METER CALIBRATION

### 10.1. WINKLER TITRATION

10.1.1. Prepare dissolved oxygen dilution water by sparging with air a volume of BOD grade water in a carboy with a venting cap. Allow saturated dilution water to vent to the atmosphere for approximately 24 hours at laboratory temperature. Transfer the air saturated water to the BOD bottle with the minimum of agitation.
10.1.2. Fill a BOD bottle of known volume to the top with air saturated dilution water. Make sure no bubbles are present. Cap with stopper used to calibrate the BOD bottle.
10.1.3. Remove the BOD bottle stopper, add 1 mL of the manganous sulfate solution to the BOD bottle by inserting the pipette tip below the surface of the water or by transferring the solution by touching the tip of the pipette to the side of the BOD bottle above the surface of the water. Care must be taken to transfer the solution quickly with minimum water agitation. Cap with stopper and pour off excess from the water seal. Mix well by inversion.
10.1.4. Remove the BOD bottle stopper, add 1 mL of the alkaline iodide-azide solution to the BOD bottle by inserting the pipette tip below the surface of the water or by transferring the solution by touching the tip of the pipette to the side of the BOD bottle above the surface of the water. Care must be taken to transfer the solution quickly with minimum water agitation. Cap with stopper and pour off excess from the water seal. Mix well by inversion.
10.1.5. Allow a golden brown flock to form and precipitate to the bottom third of the bottle.
10.1.6. Carefully remove the stopper and immediately add 1 mL of concentrated $\mathrm{H}_{2} \mathrm{SO}_{4}$ solution to the BOD bottle by inserting the pipette tip below the surface of the water. Care must be taken to transfer the solution quickly with minimum water agitation. Cap with stopper and pour off excess from the water seal. Mix well by inversion. The solution will be a golden brown color
10.1.7. Transfer by Class A volumetric pipette a measured amount of the contents of the BOD bottle into a 500 mL wide mouth flask quickly, but without introducing air bubbles.
10.1.8. Titrate the solution using standardized thiosulfate titrant in a Class A burette until it is a pale yellow.
10.1.9. Add approximately 3 drops of the starch solution to the pale yellow solution. The solution should turn dark blue.
10.1.10. Continue to titrate the solution until the blue color just disappears.
10.1.11. Record the mL of standardized thiosulfate titrant used. Read the burette to an accuracy of only one half $(1 / 2)$ of a volume unit between each demarcation.
10.1.12. Calculate the concentration of dissolved oxygen:
$\frac{m l(\text { thiosulfate }) \times \text { Molarity }(\text { thiosulfate }) \times 32}{m l(\text { sample }) \times 4 \times\left(\frac{\text { volume }(\text { initial sample }, \mathrm{ml})-1 \mathrm{ml}}{\text { volume }(\text { initial sample }, \mathrm{ml})}\right)^{2}}=$ Dissolved oxygen $(\mathrm{mg} / \mathrm{l})$

1. $\mathrm{ml}($ thiosulfate $)=\mathrm{ml}$ of titrant used
2. Molarity (thiosulfate) $=$ Molarity of standardized thiosulfate
3. ml (sample) $=\mathrm{ml}$ of Winkler sample titrated
4. volume (initial sample) = volume of BOD bottle
10.1.13. Record this dissolved oxygen value to the nearest $0.01 \mathrm{mg} / \mathrm{L}$.

### 10.2. BAROMETRIC DETERMINATION: AIR SATURATED WATER

10.2.1. Determine the barometric pressure for your laboratory in atmospheres or mm Hg (Torr).
10.2.2. Convert laboratory barometric pressure to atmospheres.
10.2.3. Determine the temperature for your laboratory in centigrade and Kelvin.
10.2.4. Determine the DO in air saturated water from the tables and formulas in Appendix 1.

### 10.3. BAROMETRIC DETERMINATION: WATER SATURATED AIR

10.3.1. Determine the barometric pressure for your laboratory in atmospheres or mm Hg (Torr).
10.3.2 Convert laboratory barometric pressure to atmospheres.
10.3.3. Determine the temperature for your laboratory in centigrade and Kelvin.
10.3.4. Determine the DO in water saturated water from the tables and formulas in Appendix 1.

### 10.4. CALIBRATION OF DISSOLVED OXYGEN METER

10.4.1. Operate the meter and electrode as per instrument manufacturer's instructions. Sample may be stirred on not. Allow the meter to come to equilibrium prior to accepting dissolved oxygen value.
10.4.2. Calibrate the meter as per instrument manufacturer's instructions with both the following standards:
10.4.2.1. High Dissolved Oxygen Standard consisting of one of the following 10.4.2.1.1. Winkler titration.
10.4.2.1.2. Water Saturated Air.
10.4.2.1.3. Air Saturated Water.
10.4.2.2. Low Dissolved Oxygen Standard as required by instrument response or Lower Reporting Limit.

### 10.4.2.2.1. Sodium Sulfite with $\mathrm{CoCl}_{2}-6 \mathrm{H}_{2} \mathrm{O}$

## 11. PROCEEDURE

### 11.1. DISSSOLVED OXYGEN DETERMINATION

11.2. Calibrate the DO meter as per manufacturer's directions and Section 10 above.
11.3. Fill a BOD bottle to the top with sample or place dissolved oxygen probe in the bottle it was collected in or place dissolved oxygen probe in sample stream.
11.4. Allow the dissolved oxygen meter to come to equilibrium with the sample. Stirring, agitation, or flow may accelerate the dissolved oxygen meter equilibrium.
11.5. Record the dissolved oxygen concentration.

## 12. METHOD PERFORMANCE

12.1. Blanks from Biochemical Oxygen Demand (BOD) and Carbonaceous Biochemical Oxygen Demand (CBOD) analyses.
12.1.1. Dissolved oxygen data analysis performed for both BOD and CBOD Blanks with the RDO compared to the membrane electrode from a multiple laboratory study are in Section 17.1.
12.1.2. Bias and precision for RDO blank measurement compared to the membrane electrode for multiple laboratory study are in Section 17.1.
12.2. Lower of operational range of the RDO DO Meter
12.2.1. The lower limit of operational range of the DO Meter must be below the lowest dissolved oxygen value required. Typical values for RDO confidence intervals based on multiple laboratory analysis are illustrated Section 17.2.

## 13. POLLUTION PREVENTION

13.1. There are no standards or reagents used in this method at the concentrations required that pose a threat to the environment. Refer to Waste Management for correct disposal of all chemicals.

## 14. WASTE MANAGEMENT

14.1. It is the laboratory's responsibility to comply with all federal, state, and local regulations governing waste management, particularly the hazardous waste identification rules and land disposal restrictions, and to protect air, water, and land by minimizing and control all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

## 15. REFERENCES

1. Mingoarranz, F. J.; Moreno-Bondi, M. C.; Garcia-Fresnadillo, D.; de Dios, C.; Orellana, G., Oxygen-sensitive layers for optical fiber devices. Mikrochim. Acta 1995, 121, (1-4), 107-18.
2. Garcia-Fresnadillo, D.; Marazuela, M. D.; Moreno-Bondi, M. C.; Orellana, G., Luminescent Nafion Membranes Dyed with Ruthenium(II) Complexes as Sensing Materials for Dissolved Oxygen. Langmuir 1999, 15, (19), 6451-6459.
3. Borisov, S. M.; Klimant, I., Ultrabright Oxygen Optodes Based on Cyclometalated Iridium(III) Coumarin Complexes. Anal. Chem. (Washington, DC, U. S.) 2007, 79, (19), 7501-7509.
4. Titze, J.; Walter, H.; Jacob, F.; Friess, A.; Parlar, H., Evaluation of a new optical sensor for measuring dissolved oxygen by comparison with standard analytical methods. Monatsschr. Brauwiss. 2008, (Mar./Apr.), 66-80.

## 16. TABLES

16.1. DO Blank Performance.

| RPD RDO to Membrane: BOD Blanks |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  | RPD NSRDO <br> Initial | RPD SRDO <br> Initial | RPD NSRDO <br> Final | RPD SRDO <br> Final |  |
| Average | 1.25 | 1.00 | 1.43 | 0.99 |  |
| Standard <br> Deviation | 1.51 | 1.58 | 1.71 | 1.74 |  |


| RPD RDO to Membrane: CBOD Blanks |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: |
|  | RPD NSRDO <br> Initial | RPD SRDO <br> Initial | RPD NSRDO <br> Final | RPD SRDO <br> Final |
| Average | 1.34 | 0.73 | 1.54 | 0.73 |
| Standard <br> Deviation | 1.18 | 0.62 | 1.57 | 0.93 |


| Bias and Precision |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| BOD Blank |  |  |  |  |  |  |
|  | NSRDO |  | SRDO |  | Membrane |  |
| Overall Bias (mg/L) | -0.10 |  | -0.10 |  | -0.10 |  |
| Overall Precision (mg/L) |  | 0.12 |  | 0.12 |  | 0.12 |
| CBOD Blank |  |  |  |  |  |  |
|  | NSRDO |  | SRDO |  | Membrane |  |
| Overall Bias (mg/L) | -0.10 |  | -0.10 |  | -0.09 |  |
| Overall Precision (mg/L) |  | 0.12 |  | 0.12 |  | 0.11 |

16.2. Lower of operational range of the RDO DO Meter

| RDO Stirred and Non-Stirred Lower Limit of Determination |  |  |  |
| :---: | :---: | :---: | :---: |
| Zero Solution Reading |  |  |  |
| Laboratory \# | Mean <br> (mg/L) | Pooled Standard <br> Deviation <br> $(\mathbf{m g} / \mathrm{L})$ | Calculated <br> Confidence Interval <br> (mg/L) |
| $\mathbf{1}$ | 0.053 | 0.025 | 0.03 |
| $\mathbf{2}$ | 0.072 | 0.053 | 0.07 |
| $\mathbf{3}$ | 0.043 | 0.006 | 0.01 |
| $\mathbf{4}$ | 0.022 | 0.007 | 0.01 |
| $\mathbf{5}$ | 0.047 | 0.021 | 0.04 |
| $\mathbf{6}$ | 0.162 | 0.008 | 0.01 |
| $\mathbf{7}$ | 0.067 | 0.047 | 0.10 |
| $\mathbf{O v e r a l l}$ | $\mathbf{0 . 0 7 1}$ | $\mathbf{0 . 0 3 7}$ | 0.04 |

## APPENDIXES

Dissolved Oxygen in Saturated Water

| SOLUBILITY OF OXYGEN IN WATER EXPOSED TO WATER-SATURATED AIR AT ATMOSPHERIC PRESSURE (101.3 KPA) ${ }^{1}$ |  |  |  |  |  |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Oxygen Solubility mg/L |  |  |  |  |  |  | Oxygen Solubility mg/L |  |  |  |  |  |  |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Temperature ${ }^{\circ} \mathrm{C}$ | Chlorinity: 0 | 5.0 | 10.0 | 15.0 | 20.0 | 25.0 | Temperature ${ }^{\circ} \mathrm{C}$ | Chlorinity: 0 | 5.0 | 10.0 | 15.0 | 20.0 | 25.0 |
| 0.0 | 14.621 | 13.728 | 12.888 | 12.097 | 11.355 | 10.657 | 26.0 | 8.113 | 7.711 | 7.327 | 6.962 | 6.615 | 6.285 |
| 1.0 | 14.216 | 13.356 | 12.545 | 11.783 | 11.066 | 10.392 | 27.0 | 7.968 | 7.575 | 7.201 | 6.845 | 6.506 | 6.184 |
| 2.0 | 13.829 | 13.000 | 12.218 | 11.483 | 10.790 | 10.139 | 28.0 | 7.827 | 7.444 | 7.079 | 6.731 | 6.400 | 6.085 |
| 3.0 | 13.460 | 12.660 | 11.906 | 11.195 | 10.526 | 9.897 | 29.0 | 7.691 | 7.317 | 6.961 | 6.621 | 6.297 | 5.990 |
| 4.0 | 13.107 | 12.335 | 11.607 | 10.920 | 10.273 | 9.664 | 30.0 | 7.559 | 7.194 | 6.845 | 6.513 | 6.197 | 5.896 |
| 5.0 | 12.770 | 12.024 | 11.320 | 10.656 | 10.031 | 9.441 | 31.0 | 7.430 | 7.073 | 6.733 | 6.409 | 6.100 | 5.806 |
| 6.0 | 12.447 | 11.727 | 11.046 | 10.404 | 9.799 | 9.228 | 32.0 | 7.305 | 6.957 | 6.624 | 6.307 | 6.005 | 5.717 |
| 7.0 | 12.139 | 11.442 | 10.783 | 10.162 | 9.576 | 9.023 | 33.0 | 7.183 | 6.843 | 6.518 | 6.208 | 5.912 | 5.631 |
| 8.0 | 11.843 | 11.169 | 10.531 | 9.930 | 9.362 | 8.826 | 34.0 | 7.065 | 6.732 | 6.415 | 6.111 | 5.822 | 5.546 |
| 9.0 | 11.559 | 10.907 | 10.290 | 9.707 | 9.156 | 8.636 | 35.0 | 6.950 | 6.624 | 6.314 | 6.017 | 5.734 | 5.464 |
| 10.0 | 11.288 | 10.656 | 10.058 | 9.493 | 8.959 | 8.454 | 36.0 | 6.837 | 6.519 | 6.215 | 5.925 | 5.648 | 5.384 |
| 11.0 | 11.027 | 10.415 | 9.835 | 9.287 | 8.769 | 8.279 | 37.0 | 6.727 | 6.416 | 6.119 | 5.835 | 5.564 | 5.305 |
| 12.0 | 10.777 | 10.183 | 9.621 | 9.089 | 8.586 | 8.111 | 38.0 | 6.620 | 6.316 | 6.025 | 5.747 | 5.481 | 5.228 |
| 13.0 | 10.537 | 9.961 | 9.416 | 8.899 | 8.411 | 7.949 | 39.0 | 6.515 | 6.217 | 5.932 | 5.660 | 5.400 | 5.152 |
| 14.0 | 10.306 | 9.747 | 9.218 | 8.716 | 8.242 | 7.792 | 40.0 | 6.412 | 6.121 | 5.842 | 5.576 | 5.321 | 5.078 |
| 15.0 | 10.084 | 9.541 | 9.027 | 8.540 | 8.079 | 7.642 | 41.0 | 6.312 | 6.026 | 5.753 | 5.493 | 5.243 | 5.005 |
| 16.0 | 9.870 | 9.344 | 8.844 | 8.370 | 7.922 | 7.496 | 42.0 | 6.213 | 5.934 | 5.667 | 5.411 | 5.167 | 4.933 |
| 17.0 | 9.665 | 9.153 | 8.667 | 8.207 | 7.770 | 7.356 | 43.0 | 6.116 | 5.843 | 5.581 | 5.331 | 5.091 | 4.862 |
| 18.0 | 9.467 | 8.969 | 8.497 | 8.049 | 7.624 | 7.221 | 44.0 | 6.021 | 5.753 | 5.497 | 5.252 | 5.017 | 4.793 |
| 19.0 | 9.276 | 8.792 | 8.333 | 7.896 | 7.483 | 7.090 | 45.0 | 5.927 | 5.665 | 5.414 | 5.174 | 4.944 | 4.724 |
| 20.0 | 9.092 | 8.621 | 8.174 | 7.749 | 7.346 | 6.964 | 46.0 | 5.835 | 5.578 | 5.333 | 5.097 | 4.872 | 4.656 |
| 21.0 | 8.915 | 8.456 | 8.021 | 7.607 | 7.214 | 6.842 | 47.0 | 5.744 | 5.493 | 5.252 | 5.021 | 4.801 | 4.589 |
| 22.0 | 8.743 | 8.297 | 7.873 | 7.470 | 7.087 | 6.723 | 48.0 | 5.654 | 5.408 | 5.172 | 4.947 | 4.730 | 4.523 |
| 23.0 | 8.578 | 8.143 | 7.730 | 7.337 | 6.963 | 6.609 | 49.0 | 5.565 | 5.324 | 5.094 | 4.872 | 4.660 | 4.457 |
| 24.0 | 8.418 | 7.994 | 7.591 | 7.208 | 6.844 | 6.498 | 50.0 | 5.477 | 5.242 | 5.016 | 4.799 | 4.591 | 4.392 |
| 25.0 | 8.263 | 7.850 | 7.457 | 7.083 | 6.728 | 6.390 |  |  |  |  |  |  |  |

## NOTE:

1. The table provides three decimal places to aid interpolation. When computing saturation values to be used with measured values, such as in computing DO deficit in a receiving water, precision of measured values will control choice of decimal places to be used.
2. Equations are available to compute DO concentration in fresh water ${ }^{1-3}$ and in seawaterl at equilibrium with water-saturated air. Figures and tables also are
available. ${ }^{3}$
Calculate the equilibrium oxygen concentration, $C^{*}$, from equation:
$\ln C^{*}=-139.34411+\left(1.575701 \times 10^{5} / T\right)-\left(6.642308 \times 10^{7} / T^{2}\right)+\left(1.243800 \times 10^{10} / T^{3}\right)-$ $\left.\left(8.621949 \times 10^{11} / T^{4}\right)-\operatorname{Chl}\left[(3.1929) \times 10^{2}\right)-\left(1.9428 \_10^{1} / T\right)+\left(3.8673 \times 10^{3} / T^{2}\right)\right]$
where:
$C^{*}=$ equilibrium oxygen concentration at $101.325 \mathrm{kPa}, \mathrm{mg} / \mathrm{L}$,
$T$ _temperature $\left({ }^{\circ} \mathrm{K}\right)={ }^{\circ} \mathrm{C}+273.150$, $\left({ }^{\circ} \mathrm{C}\right.$ is between 0.0 and 40.0 in the equation; the table is accurate up to 50.0), and
$\mathrm{Chl}=$ Chlorinity $($ see definition in Note 4, below $)$.
Example 1: At $20^{\circ} \mathrm{C}$ and $0.000 \mathrm{Chl}, \ln C^{*}=2.207442$ and $C^{*}=9.092 \mathrm{mg} / \mathrm{L}$;
Example 2: At $20^{\circ} \mathrm{C}$ and 15.000 ChL ,
$\ln C^{*}=(2.207442)-[15.000 \times(0.010657)]$
$=2.0476$ and $C^{*}=7.749 \mathrm{mg} / \mathrm{L}$.
When salinity is used, replace the chlorinity term (-Chl[. . .]) by:
$-S\left(1.7674 \times 10^{-2}\right)-\left(1.0754 \times 10^{1} / T\right)+\left(2.1407 \times 10^{3} / T^{2}\right)$
where:
$S=$ salinity (see definition in Note 4, below).
3. For nonstandard conditions of pressure: (Excel worksheet is available)

$$
C_{P}=C^{*} P\left[\frac{\left(1-P_{\mathrm{WV}} / P\right)(1-\theta P)}{\left(1-P_{\mathrm{WV}}\right)(1-\theta)}\right]
$$

where:
$C p=$ equilibrium oxygen concentration at nonstandard pressure, $\mathrm{mg} / \mathrm{L}$,
$C^{*}=$ equilibrium oxygen concentration at standard pressure of $1 \mathrm{~atm}, \mathrm{mg} / \mathrm{L}$.
$P=$ nonstandard pressure, atm,
$P_{W V}=$ partial pressure of water vapor, atm, computed from: $\ln P_{W V}=11.8571 \mathrm{x}$ (3840.70/T) - (216961/T ${ }^{2}$ ),
$T=$ temperature, ${ }^{\circ} \mathrm{K}$,
$\theta=0.000975-\left(1.426 \times 10^{-5} t\right)+\left(6.436 \times 10^{-8} t^{2}\right)$, and
$t=$ temperature,${ }^{\circ} \mathrm{C}$.
N.B.: Although not explicit in the above, the quantity in brackets in the equation for $C p$ has dimensions of $\operatorname{atm}^{-1} 1$ per Reference 4 , so that $P$ multiplied by this quantity is dimensionless.

Also, the equation for $\ln P_{W V}$ is strictly valid for fresh water only, but for practical purposes no error is made by neglecting the effect of salinity.

An equation for $P w v$ that includes the salinity factor may be found in Reference 1.
Example 3: At $20^{\circ} \mathrm{C}, 0.000 \mathrm{Chl}$, and 0.700 atm ,
$C p=C^{*} P(0.990092)=6.30 \mathrm{mg} / \mathrm{L}$.

## 4. Definitions:

Salinity: Although salinity has been defined traditionally as the total solids in water after all carbonates have been converted to oxides, all bromide and iodide have been replaced by chloride, and all organic matter has been oxidized (see Section 2520), the new scale used to define salinity is based on the electrical conductivity of seawater relative to a specified solution of KCl in water. ${ }^{5}$ The scale is dimensionless and the traditional dimension of parts per thousand (i.e., $\mathrm{g} / \mathrm{kg}$ of solution) no longer applies.

Chlorinity: Chlorinity is defined in relation to salinity as follows:

$$
\text { Salinity }=1.80655 x \text { chlorinity }
$$

Although chlorinity is not equivalent to chloride concentration, the factor for converting a chloride concentration in seawater to include bromide, for example, is only 1.0045 (based on the relative molecular weights and amounts of the two ions). Therefore, for practical purposes, chloride concentration (in $\mathrm{g} / \mathrm{kg}$ of solution) is nearly equal to chlorinity in seawater. For wastewater, it is necessary to know the ions responsible for the solution's electrical conductivity to correct for their effect on oxygen solubility and use of the tabular value. If this is not done, the equation is inappropriate unless the relative composition of the wastewater is similar to that of seawater.

Appendix References

1. BENSON, B.B. \& D. KRAUSE, JR. 1984. The concentration and isotopic fractionation of oxygen dissolved in freshwater and seawater in equilibrium with the atmosphere. Limnol. Oceanogr. 29:620.
2. BENSON, B.B. \& D. KRAUSE, JR. 1980. The concentration and isotopic fractionation of gases dissolved in fresh water in equilibrium with the atmosphere: I. Oxygen. Limnol. Oceanogr. 25:662.
3. MORTIMER, C.H. 1981. The oxygen content of air-saturated fresh waters over ranges of temperature and atmospheric pressure of limnological interest. Int. Assoc. Theoret. Appl. Limnol., Communication No. 22, Stuttgart, West Germany.
4. SULZER, F. \& W.M. WESTGARTH. 1962. Continuous D. O. recording in activated sludge. Water Sewage Works 109: 376.
5. UNITED NATIONS EDUCATIONAL, SCIENTIFIC \& CULTURAL ORGANIZATION. 1981. Background Papers and Supporting Data on the Practical Salinity Scale 1978. Tech. Paper Mar. Sci. No. 37. 4500-O D. Permanganate Modification

## Density of Water ( $\mathrm{g} / \mathrm{mL}$ ) vs. Temperature ( ${ }^{\circ} \mathrm{C}$ )

Whole degrees are listed down the left hand side of the table, while tenths of a degree are listed across the top. So to find the density of water at say $5.4^{\circ} \mathrm{C}$, you would first find the whole degree by searching down the left hand column until you reach '5'. Then you would slide across that row until you reach the column labeled '0.4'. The density of water at $5.4^{\circ} \mathrm{C}$ is $0.999957 \mathrm{~g} / \mathrm{mL}$.

|  | 0.0 | 0.1 | 0.2 | 0.3 | 0.4 | 0.5 | 0.6 | 0.7 | 0.8 | 0.9 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 0 | 0.999841 | 0.999847 | 0.999854 | 0.999860 | 0.999866 | 0.999872 | 0.999878 | 0.999884 | 0.999889 | 0.999895 |
| 1 | 0.999900 | 0.999905 | 0.999909 | 0.999914 | 0.999918 | 0.999923 | 0.999927 | 0.999930 | 0.999934 | 0.999938 |
| 2 | 0.999941 | 0.999944 | 0.999947 | 0.999950 | 0.999953 | 0.999955 | 0.999958 | 0.999960 | 0.999962 | 0.999964 |
| 3 | 0.999965 | 0.999967 | 0.999968 | 0.999969 | 0.999970 | 0.999971 | 0.999972 | 0.999972 | 0.999973 | 0.999973 |
| 4 | 0.999973 | 0.999973 | 0.999973 | 0.999972 | 0.999972 | 0.999972 | 0.999970 | 0.999969 | 0.999968 | 0.999966 |
| 5 | 0.999965 | 0.999963 | 0.999961 | 0.999959 | 0.999957 | 0.999955 | 0.999952 | 0.999950 | 0.999947 | 0.999944 |
| 6 | 0.999941 | 0.999938 | 0.999935 | 0.999931 | 0.999927 | 0.999924 | 0.999920 | 0.999916 | 0.999911 | 0.999907 |
| 7 | 0.999902 | 0.999898 | 0.999893 | 0.999888 | 0.999883 | 0.999877 | 0.999872 | 0.999866 | 0.999861 | 0.999855 |
| 8 | 0.999849 | 0.999843 | 0.999837 | 0.999830 | 0.999824 | 0.999817 | 0.999810 | 0.999803 | 0.999796 | 0.999789 |
| 9 | 0.999781 | 0.999774 | 0.999766 | 0.999758 | 0.999751 | 0.999742 | 0.999734 | 0.999726 | 0.999717 | 0.999709 |
| 10 | 0.999700 | 0.999691 | 0.999682 | 0.999673 | 0.999664 | 0.999654 | 0.999645 | 0.999635 | 0.999625 | 0.999615 |
| 11 | 0.999605 | 0.999595 | 0.999585 | 0.999574 | 0.999564 | 0.999553 | 0.999542 | 0.999531 | 0.999520 | 0.999509 |
| 12 | 0.999498 | 0.999486 | 0.999475 | 0.999463 | 0.999451 | 0.999439 | 0.999427 | 0.999415 | 0.999402 | 0.999390 |
| 13 | 0.999377 | 0.999364 | 0.999352 | 0.999339 | 0.999326 | 0.999312 | 0.999299 | 0.999285 | 0.999272 | 0.999258 |
| 14 | 0.999244 | 0.999230 | 0.999216 | 0.999202 | 0.999188 | 0.999173 | 0.999159 | 0.999144 | 0.999129 | 0.999114 |
| 15 | 0.999099 | 0.999084 | 0.999069 | 0.999054 | 0.999038 | 0.999023 | 0.999007 | 0.998991 | 0.998975 | 0.998959 |
| 16 | 0.998943 | 0.998926 | 0.998910 | 0.998893 | 0.998877 | 0.998860 | 0.998843 | 0.998826 | 0.998809 | 0.998792 |
| 17 | 0.998774 | 0.998757 | 0.998739 | 0.998722 | 0.998704 | 0.998686 | 0.998668 | 0.998650 | 0.998632 | 0.998613 |
| 18 | 0.998595 | 0.998576 | 0.998558 | 0.998539 | 0.998520 | 0.998501 | 0.998482 | 0.998463 | 0.998444 | 0.998424 |
| 19 | 0.998405 | 0.998385 | 0.998365 | 0.998345 | 0.998325 | 0.998305 | 0.998285 | 0.998265 | 0.998244 | 0.998224 |
| 20 | 0.998203 | 0.998183 | 0.998162 | 0.998141 | 0.998120 | 0.998099 | 0.998078 | 0.998056 | 0.998035 | 0.998013 |
| 21 | 0.997992 | 0.997970 | 0.997948 | 0.997926 | 0.997904 | 0.997882 | 0.997860 | 0.997837 | 0.997815 | 0.997792 |
| 22 | 0.997770 | 0.997747 | 0.997724 | 0.997701 | 0.997678 | 0.997655 | 0.997632 | 0.997608 | 0.997585 | 0.997561 |
| 23 | 0.997538 | 0.997514 | 0.997490 | 0.997466 | 0.997442 | 0.997418 | 0.997394 | 0.997369 | 0.997345 | 0.997320 |
| 24 | 0.997296 | 0.997271 | 0.997246 | 0.997221 | 0.997196 | 0.997171 | 0.997146 | 0.997120 | 0.997095 | 0.997069 |
| 25 | 0.997044 | 0.997018 | 0.996992 | 0.996967 | 0.996941 | 0.996914 | 0.996888 | 0.996862 | 0.996836 | 0.996809 |
| 26 | 0.996783 | 0.996756 | 0.996729 | 0.996703 | 0.996676 | 0.996649 | 0.996621 | 0.996594 | 0.996567 | 0.996540 |
| 27 | 0.996512 | 0.996485 | 0.996457 | 0.996429 | 0.996401 | 0.996373 | 0.996345 | 0.996317 | 0.996289 | 0.996261 |


|  | $\mathbf{0 . 0}$ | $\mathbf{0 . 1}$ | $\mathbf{0 . 2}$ | $\mathbf{0 . 3}$ | $\mathbf{0 . 4}$ | $\mathbf{0 . 5}$ | $\mathbf{0 . 6}$ | $\mathbf{0 . 7}$ | $\mathbf{0 . 8}$ | $\mathbf{0 . 9}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| $\mathbf{2 8}$ | 0.996232 | 0.996204 | 0.996175 | 0.996147 | 0.996118 | 0.996089 | 0.996060 | 0.996031 | 0.996002 | 0.995973 |
| $\mathbf{2 9}$ | 0.995944 | 0.995914 | 0.995885 | 0.995855 | 0.995826 | 0.995796 | 0.995766 | 0.995736 | 0.995706 | 0.995676 |
| $\mathbf{3 0}$ | 0.995646 | 0.995616 | 0.995586 | 0.995555 | 0.995525 | 0.995494 | 0.995464 | 0.995433 | 0.995402 | 0.995371 |

