Alternate Test Procedure #N09-0020

In-Situ Inc. Method 1003-8-2009 Biochemical Oxygen Demand (BOD) Measurement by Optical Probe

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DISSOLVED OXYGEN and BOD

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.

1.2. Biochemical Oxygen Demand (BOD) The BOD determination is an empirical test in which standardized laboratory procedures are used to determine the relative oxygen consumption of wastewaters, effluents, and polluted waters.

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]:

$$\frac{I_0}{I} = 1 + K_{SV}[O_2]$$

I and I_0 are luminesence in the presence and absence of Oxygen

K_{SV} is the Stern-Volmer constant for Oxygen quenching

$[O_2]$ 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]:

$$\frac{I_0}{I} = \frac{1 + K_{SV}[O_2]}{f_1} + \frac{1 + K_{SV}[O_2]}{f_2}$$

I and I_0 are luminesence in the presence and absence of Oxygen

K_{SV} is the Stern-Volmer constant for Oxygen quenching

$[O_2]$ is the dissolved Oxygen concentration

f_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].

2.2. The BOD test is applied in measuring waste loadings to treatment plants and in evaluating the BOD-removal efficiency of such treatment systems. The test measures the molecular oxygen utilized during a specified incubation period for the biochemical degradation of organic material (carbonaceous demand) and the oxygen used to oxidize inorganic material such as sulfides and ferrous iron. It also may measure the amount of oxygen used to oxidize reduced forms of nitrogen (nitrogenous demand) unless their oxidation is prevented by an inhibitor. The seeding and dilution procedures provide an estimate of the BOD at pH 6.5 to 7.5.[5]

3. DEFINITIONS

3.1. BOD: Biochemical Oxygen Demand

3.2. DI Water: Deionized water

3.3. Duplicate = Repetitive individual analyses of a sample to measure precision.

3.4. G/GA: A solution of Glucose and Glutamic Acid.

3.5. RPD, Relative Percent Difference: Measure of precision and/or matrix effects of Duplicates. The RPD must be less than or equal to (≤) 20%

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 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, ~300mL (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.BOD air incubator. Thermostatically controlled at 20 ±1°C. Exclude all light to prevent possibility of photosynthetic production of DO (Meets requirements of Standard Methods 5210 B[5])

6.7. Dissolved oxygen meter and an In-Situ RDO® 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 DILUTION WATER

7.4.1. After filling dilution water storage jug with Dissolved Oxygen Dilution Water, add 1 mL of each of the nutrient solutions listed in 7.6 through 7.9 (add them in alphabetical order) per liter of dilution water. Mix well and use that day.

Note: BOD Dilution Water cannot be stored over a standard working day (12 hours)

7.5. BOD SEED PREPARATION

7.5.1. The preferred seed is obtained from a biological treatment system processing the waste. In this case, use supernatant from settled domestic wastewater, effluent from primary clarifiers, diluted mixed liquor from an aeration basin, undisinfected effluent, or receiving water from below the point of discharge. Do not use seed from effluents that have been disinfected by chlorine or other means. Commercial seed sources may be used. Prepare commercial seed as per manufacturer's directions

7.6. PHOSPHATE BUFFER SOLUTION

7.6.1. Dissolve 8.5 g KH_2PO_4, 21.75 g K_2HPO_4, 33.4 g $Na_2HPO_4 \cdot 7H_2O$, and 1.7 g NH_4Cl in about 500 mL DI water and dilute to 1 L. The pH should be 7.2 without further adjustment. (ACS Grade or better)

7.6.2. Alternatively, dissolve 42.5 g KH_2PO_4 and 1.7 g NH_4Cl in about 700 mL distilled water. Adjust pH to 7.2 with 30% NaOH and dilute to 1 L. (ACS Grade or better)

7.6.3. Commercial reagent can be purchased.

7.7. MAGNESIUM SULFATE SOLUTION

7.7.1. Dissolve 22.5 g $MgSO_4 \cdot 7H_2O$ in DI water and dilute to 1 L. (ACS Grade or better)

7.7.2. Commercial reagent can be purchased.

7.8. CALCIUM CHLORIDE SOLUTION

7.8.1. Dissolve 27.5 g $CaCl_2$ in DI water and dilute to 1 L. (ACS Grade or better)

7.8.2. Commercial reagent can be purchased.

7.9. FERRIC CHLORIDE SOLUTION

7.9.1. Dissolve 0.25 g $FeCl_3 \cdot 6H_2O$ in distilled water and dilute to 1 L(ACS Grade or better)

7.9.2. Commercial reagent can be purchased.

7.10. BOD OPTICAL AND MEMBRANE PROBE ZERO SOLUTION

7.10.1. Sodium Sulfite Zero Solution Dissolve 10 g of sodium sulfite (Na_2SO_3) in 200 mL of DI water. Add two drops of saturated cobalt chloride solution to the sodium sulfite solution. (ACS Grade or better)

7.10.2. Saturated Cobalt Chloride solution; Dissolve 4.5 grams of cobalt chloride hexahydrate ($CoCl_2 \cdot 6H_2O$) in 10 mL of DI water. (ACS Grade or better)

7.10.3. A commercial solution of this mixture can be purchased.

7.11. GLUCOSE-GLUTAMIC ACID SOLUTION (G/GA)

7.11.1. Dry reagent-grade glucose and reagent-grade glutamic acid at 103^oC for 1 hour. Store these in a desiccator until needed. Add 0.1500 g (± 0.0001 g) of glucose and 0.1500 g (±0.0001 g) of glutamic acid to a 1 liter volumetric flask and dilute to volume with BOD Grade water. This solution is good for seven days if kept sterile and refrigerated at 4^oC ± 2^oC. Replace if solution becomes cloudy or shows signs of bacterial or chemical contamination. (ACS Grade or better)

7.11.2. A commercial solution can be purchased, but concentrations may vary. Final concentration must be the same as solution above. Keep solution sterile and refrigerated at 4^oC ± 2^oC. Replace if solution becomes cloudy or shows signs of bacterial or chemical contamination

7.12. SULFURIC ACID

7.12.1. Concentrated H_2SO_4, (ACS Grade or better).

7.13. SULFURIC ACID (6N)

7.13.1. Slowly and cautiously add 85mL of concentrated sulfuric acid to approximately 400mL of DI water, allow it to cool to room temperature and then dilute to 500mL. (ACS Grade or better)

7.13.2. Commercial solution can be purchased.

7.14. SODIUM HYDROXIDE (6N)

7.14.1. Dissolve 120gm in 500mL of DI water. Allow to equilibrate to room temperature, transfer to a 1L volumetric flask and dilute to the mark. Mix well by inverting flask. (ACS Grade or better)

7.14.2. Commercial solution can be purchased.

7.15. MANGANOUS SULFATE SOLUTION

7.15.1. Dissolve 480 g $MnSO_4$ • 4H2O, 400 g $MnSO_4$ • $2H_2O$, or 364 g $MnSO_4$ • H_2O (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)

7.15.2. Commercial solution can be purchased.

7.16. ALKALINE IODIDE-AZIDE SOLUTION

7.16.1. 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 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)

7.16.2. For supersaturated samples—Dissolve 10 g NaN_3 in 500 mL DI water. Add 480 g sodium hydroxide (NaOH) and 750 g sodium iodide (NaI), and stir until dissolved. There will be a white turbidity due to sodium carbonate (Na_2CO_3). 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.

7.16.3. Commercial solution can be purchased.

7.17. SODIUM THIOSULFATE

7.17.1. (Standard Titrant, 0.025 M) Purchase to meet Standard Methods 4500-O C criteria or dissolve 6.205 g sodium thiosulfate-pentahydrate in ~ 500 mL DI water in a 1000 mL volumetric flask, add 1.5 mL of 6N NaOH or 0.4 grams solid NaOH, dilute to 1000 mL, mix well, and standardize. (ACS Grade or better)

7.17.2. (Standard Titrant, 0.0375 M), Purchase to meet Standard Methods 4500-O C criteria or dissolve 9.304 grams of sodium thiosulfate-pentahydrate in ~ 500 mL DI water in a 1000 mL volumetric flask, add 1.5 mL of 6N NaOH or 0.4 grams solid NaOH, dilute to 1000 mL, mix well, and standardize. (ACS Grade or better)

7.17.3. Commercial solution can be purchased.

7.18. STARCH SOLUTION

7.18.1. 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)

7.18.2. Commercial solution can be purchased.

7.19. POTASSIUM BI-IODATE

7.19.1. 0.0021 Molar potassium bi-iodate solution, *(*0.0021*M):* Dissolve 818.8 mg $KH(IO_3)_2$ in DI water and dilute to 1000 mL in a volumetric flask and mix well. (ACS Grade or better)

7.19.2. 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)

7.19.3. Commercial solution can be purchased.

7.20. THIOSULFATE STANDARDIZATION

7.20.1. Dissolve approximately 2 g KI, free from iodate, in a 500 mL Erlenmeyer flask with 100 to 150 mL DI water.

7.20.2. Add 1 mL 6*N* H2SO4 or a few drops of conc H_2SO_4.

7.20.3. Add 20.00 mL of 0.0021 M bi-iodate solution with a Class A pipette and mix well.

7.20.4. 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.

7.20.5. 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.

7.20.6. Calculate the Molarity of the thiosulfate.

$$Molarity(thiosulfate) = \frac{20ml \times 12 \times 0.0021(Molarity\ potassium\ bi-iodate)}{ml(thiosulfate\ titrant)}$$

7.20.7. Balanced Equation

$$IO_3^- + 5I^- + 6H^+ \rightarrow 3I_2 + 3H_2O$$

$$6S_2O_3^{2-} + 3I_2 \rightarrow 3S_4O_6^{2-} + 6I^-$$

Molar Ratio IO_3^- : $6S_2O_3^{2-}$

7.21. BOD BOTTLE VOLUME DETERMINATION (Titrametric)

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

7.21.1. 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.

7.21.2. Dissolve approximately 4 g KI, free from iodate, in a 500 mL Erlenmeyer flask with 50 to 100 mL DI water.

7.21.3. Add 2 mL 6*N* H2SO4 or a few drops of concentrated H_2SO_4.

7.21.4. Add the potassium bi-iodate solution from the BOD bottle and mix well.

7.21.5. Rinse the BOD bottle with DI water and add to the Erlenmeyer flask.

7.21.6. 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.

7.21.7. 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.

7.21.8. Make sure cap used for volume determination is mated to the BOD bottle used.

7.21.9. Calculate the BOD bottle volume to the nearest 0.1 mL

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

7.22. BOD BOTTLE VOLUME DETERMINATION (Gravimetric)

7.22.1. Weigh a BOD bottle and cap to the nearest 0.1 grams. Record the weight.

7.22.2. 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.

7.22.3. Weigh the full BOD bottle and cap to the nearest 0.1 grams. Record the weight.

7.22.4. Make sure cap used for volume determination is mated to the BOD bottle used.

7.22.5. 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.

$$BOD\ Bottle\,(volume, ml) = \frac{\left[BOD\ Bottle\,(Final\ weight, g) - BOD\ Bottle\,(Initial\ Weight, g)\right]}{Density\ of\ Water}$$

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, preservation techniques and holding times.

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 S_{Pooled}. Utilize this value to calculate the t-Test for the two experimental means. The value determined should be ≤ 2.31 for 95% confidence. The ongoing demonstration of capability will be performed at a minimum on a quarterly basis.

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

Note: Calculate mean from all 8 Data points

Use all 8 data points to calculate pooled standard deviation

$$t = \frac{\left| \bar{X}_1 - \bar{X}_2 \right|}{S_{Pooled} \sqrt{\frac{1}{2}}}$$

$\bar{X}_1 = Mean\ of\ the\ DO\ Meter\ Measurements$

$\bar{X}_2 = Mean\ of\ the\ DO\ Calculated\ Measurements$

$t \leq 2.31\ at\ 95\%\ confidence$

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.

$$\frac{(C_I - C_D)}{\left((C_I + C_D) / 2 \right)} x100 \quad = \quad \text{RPD}$$

C_I = Concentration determined for a sample

C_D = Concentration determined for the duplicate

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 RDO 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.

$$S = \left[\frac{\sum \left(X - \bar{X} \right)}{\left(4 - 1 \right)} \right]^{\frac{1}{2}}$$

$$Confidence\ Interval = \bar{X} \pm \frac{t \times S}{2}$$

$$t = Student\ 2\ Sided\ t\ Distribution$$

9.4. BOD DILUTION WATER BLANKS

9.4.1. Prepare BOD dilution water blanks to represent each type of test being performed within the batch. Run these QC samples with each batch of BOD.

9.4.2. Unseeded BOD blank: A 300-mL bottle of BOD dilution water. (Minimum 3 per set)

9.4.3. Dilution water blanks must meet the dissolved oxygen levels in Table 1

Table 1: Dilution Water Blanks

Blank Type	Maximum 5 Day Depletion for Passing Blanks
Unseeded BOD	≤ 0.2 mg/L

9.5. GLUCOSE AND GLUTAMIC ACID CHECK (G/GA)

9.5.1. Transfer 6 mL of the glucose - glutamic acid solution to a BOD bottle of known concentration.

9.5.2. Add sufficient seed to achieve acceptable dissolved oxygen depletion.

9.5.3. Fill BOD bottle with BOD dilution water. (Minimum three G-GA checks)

9.5.4. Determine the 5 Day BOD

9.5.5. Passing results will have a BOD of 198 (± 30.5) mg/L.

9.5.6. Run these QC samples with each batch of BOD samples. It is important to realize that GGA is not intended to be an accuracy check in the test. Its sole purpose is to demonstrate that the seed is viable and metabolizing in the proper range of activity under the conditions of the test.

9.6. DUPLICATE SAMPLES

9.6.1. A duplicate sample will be set for each BOD set of seeded and unseeded samples. QA/QC criteria for the Relative Percent Difference and Precision and Bias will be developed.

9.6.2. Duplicate checks will be run on all High and Low DO Calibration Standards each day the dissolved oxygen meter is used. QA/QC criteria for the Relative Percent Difference and Precision and Bias will be developed.

9.7. DO SPIKING OF SAMPLE

9.7.1. 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.

9.8. BOD BOTTLE VOLUME

9.8.1. The use of a BOD bottle for Winkler titration volume or BOD dilution volume requires that the bottle volume be known. Sections 7.21 through 7.22 describe how to calculate the volume.

9.8.2. 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:

$$Bias = \frac{\sum_{i=1}^{n} (X_{\ Known\ Value} - X_{\ Experimental\ Value})}{n}$$

$$\text{Precision} = \sqrt{\frac{\sum_{i=1}^{n} (X_{\ Known\ Value} - X_{\ Experimental\ Value})^2}{n-1}}$$

9.8.3. All DO and BOD values calculated with the default Known Value of 300 mL will have the Bias and Precision reported in the final value.

9.8.4. 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.

9.9. BOD WORKING RANGE AND DETECTION LIMIT

9.9.1. The working range is equal to the difference between the maximum initial DO (7 to 9 mg/L) and minimum DO residual of 1 mg/L corrected for seed, and multiplied by the dilution factor. Detection limits are established by the minimum DO depletion and minimum DO residuals as follows (See formula at 12.2 for formula variable definitions):

9.9.2. The lower limit for seeded samples that require dilution ($S > 0$; $P < 1.0$) is approximately 1 mg/L as established by the minimum depletion of 2.0 mg/L minus the maximum seed correction, which should be less than about 1 mg/L.

9.9.3. The lower limit for seeded samples that require dilution ($S > 0$; $P < 1.0$) is approximately 1 mg/L as established by the minimum depletion of 2.0 mg/L minus the maximum seed correction, which should be less than about 1 mg/L.

9.9.4. The lower limit for unseeded samples that require no dilution ($S = 0$; $P = 1.0$) is equal to the detection limit of the DO measurement method (~0.1 mg/L).

9.9.5. The lower detection limit for seeded samples that require no dilution ($S > 0$; $P = 1.0$) is 0 mg/L, as established by the difference between the sample DO depletion and the seed correction.

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 1mL 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 1mL 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 1mL of concentrated H_2SO_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 500mL 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:

$$\frac{ml\,(thiosulfate)\times Molarity(thiosulfate)\times 32}{ml\,(sample)\times 4\times\left(\frac{volume(initial\ sample,ml)-1\ ml}{volume\,(initial\ sample,ml)}\right)^{2}} = Dissolved\ oxygen\,(mg/l)$$

1. ml (thiosulfate) = ml of titrant used
2. Molarity (thiosulfate) = Molarity of standardized thiosulfate
3. ml (sample) = 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 mg/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 $CoCl_2$-6 H_2O

11. DISSOLVED OXYGEN and BOD PROCEEDURES

11.1. DISSOLVED OXYGEN DETERMINATION

11.1.1. Calibrate the DO meter as per manufacturer's directions and Section 10 above.

11.1.2. Fill a BOD bottle to the top with sample prepared as per 11.2-11.3 leaving no air gaps or bubbles or place dissolved oxygen RDO probe in the bottle it was collected in or place dissolved oxygen probe in sample stream.

11.1.3. Allow the dissolved oxygen meter to come to equilibrium with the sample. Stirring, agitation, or flow may accelerate the dissolved oxygen meter equilibrium.

11.1.4. Record the dissolved oxygen concentration.

11.2. SAMPLE PREPARATION AND DETERMINATION FOR BOD

11.2.1. Bring the sample to ambient room temperature.

Note: Transfer the sample to a cylindrical container if it is not already in one. Non-cylindrical containers do not allow for even mixing by magnetic stirrers.

11.2.2. Add a magnetic stir bar to the sample and mix the sample on a magnetic stirrer.

Note: It is important the sample is allowed to stir until all of the solids are suspended. Failure to do this will cause a bias in the sampling.

11.2.3. If pH of sample is <6.5 or >7.5 neutralize the sample to approximately a pH of 7.0 using either sulfuric acid or sodium hydroxide.

11.2.4. Aliquots of the neutralized sample are transferred to 300 mL BOD bottles using the procedures listed below. These BOD samples must be at concentrations that will deplete by at least 2 mg/L DO and have at least 1 mg/L DO left after five days of incubation. Therefore make enough dilutions (minimum of 3) of the prepared sample to bracket the predicted BOD. See Appendix for dilution charts**.**

11.2.5. Use a magnetic stirrer to stir all samples, including dilutions, while an aliquot is being taken. These samples and their dilutions must be in cylindrical containers to insure an even distribution of solids while being stirred.

11.2.6. Sampling aliquots of the stirred sample or dilution will be taken with a wide tipped pipette.

11.2.7. The minimum aliquot volume transferred to a BOD bottle will be 3 mL. If a smaller volume is needed to meet the DO depletion requirements, then you must make dilutions to the sample.

11.2.8. Only pipette the aliquot volume required for a single BOD bottle. Do not transfer multiple aliquot volumes in your pipette draw. This will lead to solids settling out and sample bias.

11.2.9. Dilutions of a sample will be made by transferring a measured volume of the stirred sample by wide tipped pipette (minimum of a 10 mL pipette) to a volumetric flask and diluting to volume with BOD Dilution water. This sample is then transferred to a cylindrical container and sampled as outlined above.

11.2.10. If the sample is being prepared as a seeded sample, add enough prepared seed to the sample to achieve acceptable dissolved oxygen depletion.

11.2.11. Add BOD Dilution water to each BOD sample bottle so as to completely fill the bottle with no air spaces or bubbles when the stopper is placed in the bottle.

11.2.12. If the DO measurement is to be taken with a non-stirred RDO probe, cap the BOD bottle and invert sample multiple times to mix well.

11.2.13. Place the dissolved oxygen RDO probe in the bottle, make sure no bubbles are introduced to the sample or air gaps in the bottle and allow the dissolved oxygen meter to come to equilibrium. Sample may be stirred or not. Allow the meter to come to equilibrium prior to accepting dissolved oxygen value.

11.2.14. Record the DO of the sample, stopper the bottle making sure no bubbles are introduced to the sample or air gaps in the bottle, add DI water to the water seal if needed, cap with a water snap lid to reduce evaporation, and incubate for 5 days at 20°C ± 1°C. Exclude light to avoid growth of algae in the bottles during incubation.

11.2.15. Upon completion of the 5-day incubation± 6 hours, record the DO's of the depleted samples with a calibrated DO meter. Sample may be stirred or not. Allow the meter to come to equilibrium prior to accepting dissolved oxygen value.

11.2.16. Calculate the BODs from the formula below.

11.2.17. Only bottles, including seed controls, giving a minimum DO depletion of 2.0 mg/L and a residual DO of at least 1.0 mg/L after 5 d of incubation are considered to produce valid data, because at least 2.0 mg oxygen uptake per L is required to give a meaningful measure of oxygen uptake and at least 1.0 mg/L must remain throughout the test to ensure that insufficient DO does not affect the rate of oxidation of waste constituents.

11.3. SEED BOD UPTAKE

11.3.1. Seed BOD Uptake: Typically a 10, 20, and 30 mL sample of seed added to 3 separate BOD bottles and diluted with BOD dilution water. Run these QC samples with each batch of seeded BOD.

11.3.2. Calculate the DO uptake per mL of seed added to each bottle using either the slope method or the ratio method.

11.3.3. For the slope method, plot DO depletion in milligrams per liter versus mLs of seed for all seed control bottles having a 2.0 mg/L depletion and 1.0 minimum residual DO. The plot should present a straight line for which the slope indicates DO depletion per mL of seed. The DO-axis intercept is oxygen depletion caused by the dilution water and should be less than 0.20 mg/L

11.3.4. For the ratio method, divide the DO depletion by the volume of seed in mLs for each seed control bottle having a 2.0 mg/L depletion and greater than 1.0 mg/L minimum residual DO and average the results.

12. BOD CALCULATIONS

12.1. SEED UPTAKE: RATIO METHOD CALCULATION

For each Seed Uptake bottle having 2.0 mg / L minimum Dissolved Oxygen depletion and at least 1.0 mg / L residual Dissolved Oxygen , calculate Seed Uptake as follows :

$$Seed\,Uptake = \frac{\sum_{0}^{n} \Delta\, Dissolved\,Oxygen\,Seed\,Uptake\,Sample}{\sum_{0}^{n} ml\,Seed}$$

12.2. BOD_5 CALCULATION

For each test bottle having 2.0 mg / L minimum Dissolved Oxygen depletion and at least 1.0 mg / L residual Dissolved Oxygen , calculate BOD as follows :

$$BOD_5, mg/l = \frac{(D_1 - D_2) - (S)V_S}{P}$$

D1 = Dissolved Oxygen of diluted sample immediately after preparation, mg / L,

D2 = Dissolved Oxygen ofdiluted sample after 5 d incubation at 20°C, mg / L,

S = seed uptake, Δ DO / mL seed suspension added per bottle

(S = 0 if samples are not seeded),

Vs = volume of seed in the respective test bottle, mL, and

P = decimal volumetric fraction of sample used; 1 / P = dilution factor

13. METHOD PERFORMANCE

13.1. BLANKS

13.1.1. Dissolved oxygen data analysis performed on a daily basis or with each set of samples will consist of the measurement of duplicate samples and the RPD calculated. Blank RPDs for BOD of the RDO to membrane electrode from a multiple laboratory study are in Section 17.1.

13.1.2. Bias and precision for RDO blank measurement compared to the membrane electrode for multiple laboratory study are in Section 17.1.

13.1.3. Lower of operational range of the RDO DO Meter

13.1.4. 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.

13.2. GLUCOSE/GLUTAMIC ACID

13.2.1. The mixture of glucose and glutamic acid was developed in order to provide a standard with a stable metabolic rate that could be used in the 5 day BOD test under a wide variety of applications. F Test results showing method comparability of BOD for the RDO to the membrane electrode are in Section 17.3.

13.2.2. GGA recoveries of BOD for the RDO and membrane from a multiple laboratory study are in Section 17.3.

13.2.3. GGA DO RPDs for the BOD of the RDO to membrane electrode from a multiple laboratory study are in Section 17.3.

13.2.4. Bias and precision for the RDO GGA BOD values are compared to the membrane electrode from a multiple laboratory study in Section 17.3

13.3. OTHER WATER AND WASTEWATER BOD MATRIXES

13.3.1. Typical RPD values of the RDO to membrane electrode based on multiple laboratory analysis of varied sample types are illustrated in Section 17.4 The matrixes include wastewater influent, wastewater effluent, surface water, and industrial waste from Significant Industries and Categorical Industries.

14. POLLUTION PREVENTION

14.1. 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.

15. WASTE MANAGEMENT

15.1. 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.

16. 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.
5. 5210 Biochemical Oxygen Demand. In *Standard Methods for the Examination of Water and Wastewater*, 21 ed.; Eaton, A., Ed. APHA: 2005; pp 5-2 : 5-7.

17. TABLES

17.1. DO BLANK PERFORMANCE

RPD RDO to Membrane: BOD Blanks				
	RPD NSRDO Initial	**RPD SRDO Initial**	**RPD NSRDO Final**	**RPD SRDO Final**
Average	1.25	1.00	1.43	0.99
Standard Deviation	1.51	1.58	1.71	1.74

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

17.2. LOWER OF OPERATIONAL RANGE OF THE RDO DO METER

RDO Stirred and Non-Stirred Lower Limit of Determination			
Zero Solution Reading			
Laboratory #	**Mean (mg/L)**	**Pooled Standard Deviation (mg/L)**	**Calculated Confidence Interval (mg/L)**
1	0.053	0.025	0.03
2	0.072	0.053	0.07
3	0.043	0.006	0.01
4	0.022	0.007	0.01
5	0.047	0.021	0.04
6	0.162	0.008	0.01
7	0.067	0.047	0.10
Overall	**0.071**	**0.037**	0.04

17.3. GLUCOSE/GLUTAMIC ACID PERFORMANCE

Glucose-Glutamic Acid BOD			
Laboratory #	Non Stirred RDO		
	Mean (mg/L)	Standard Deviation (Pooled)	%RSD
1	198	10.03	5.06%
2	197	7.63	3.86%
3	211	19.70	9.32%
4	203	9.25	4.57%
5	173	10.92	6.31%
6	188	21.39	11.35%
7	203	20.89	10.28%
8	194	14.76	7.61%
9	207	23.64	11.40%
Overall	197	17.07	8.66%

Glucose-Glutamic Acid BOD			
Laboratory #	Stirred RDO		
	Mean (mg/L)	Standard Deviation (Pooled)	%RSD
1	203	7.09	3.49%
2	199	7.18	3.61%
3	198	28.36	14.30%
4	179	6.96	3.88%
5	204	9.32	4.56%
6	255	24.10	9.46%
7	231	28.57	12.39%
8	205	23.53	11.47%
Overall	212	24.39	11.50%

Glucose-Glutamic Acid BOD			
Laboratory #	Membrane		
	Mean (mg/L)	Standard Deviation (Pooled)	%RSD
1	201	6.21	3.09%
2	230	9.18	3.99%
3	214	24.66	11.55%
4	205	14.82	7.23%
5	179	6.70	3.74%
6	208	12.92	6.23%
7	257	23.24	9.03%
9	233	27.29	11.71%
10	206	24.09	11.70%
Overall	214	19.97	9.35%

RDO Compared to Membrane Glucose-Glutamic Acid BOD F Tests						
Laboratory #	**# of Data Sets**	**Degrees of Freedom**	**F Test NSRDO**	**Upper F Level 95%**	**F Test SRDO**	**Upper F Level**
1	15	14	2.61	2.48	1.30	2.48
2	3	2	1.45	19.00	0.61	19.00
3	5	4	1.57	6.39	3.02	6.39
4	8	7	2.57	3.79	3.66	3.79
5	4	3	0.38	9.28	1.08	9.28
6	12	11	2.74	2.82	1.92	2.82
7	6	5	1.24	5.05	1.08	5.05
8	10	9	3.42	3.18	1.10	3.18
9	8	7	1.04	3.79	0.95	3.79
Overall	71	62	1.37	1.52	1.49	1.52

Precision and Bias						
Glucose-Glutamic Acid BOD						
	NSRDO		**SRDO**		**Membrane**	
Overall Bias (mg/L)	-0.93		11.32		15.76	
Overall Precision (mg/L)		17.91		39.90		29.97

RPD RDO to Membrane BOD GGA						
	RPD NSRDO Initial	**RPD SRDO Initial**	**RPD NSRDO Final**	**RPD SRDO Final**	**RPD NSRDO Difference**	**RPD SRDO Difference**
Average	2.55	2.31	15.36	6.08	8.72	3.16
Standard Deviation	3.03	2.46	15.34	6.35	8.56	4.05

17.4. OTHER WATER AND WASTEWATER BOD MATRIXES

RPD RDO to Membrane BOD Seed						
	RPD NSRDO Initial	**RPD SRDO Initial**	**RPD NSRDO Final**	**RPD SRDO Final**	**RPD NSRDO Difference**	**RPD SRDO Difference**
Average	2.87	2.34	10.55	6.04	11.86	10.08
Standard Deviation	2.39	2.56	9.50	11.96	16.64	28.59

RPD RDO to Membrane BOD Influent						
	RPD NSRDO Initial	**RPD SRDO Initial**	**RPD NSRDO Final**	**RPD SRDO Final**	**RPD NSRDO Difference**	**RPD SRDO Difference**
Average	2.94	2.45	13.56	5.18	27.49	30.52
Standard Deviation	3.04	2.90	14.53	13.02	65.79	140.62

RPD RDO to Membrane BOD Effluent						
	RPD NSRDO Initial	**RPD SRDO Initial**	**RPD NSRDO Final**	**RPD SRDO Final**	**RPD NSRDO Difference**	**RPD SRDO Difference**
Average	1.25	1.00	1.43	0.99	18.01	6.91
Standard Deviation	2.85	2.60	10.27	6.02	27.45	13.78

RPD RDO to Membrane BOD Industrial						
	RPD NSRDO Initial	**RPD SRDO Initial**	**RPD NSRDO Final**	**RPD SRDO Final**	**RPD NSRDO Difference**	**RPD SRDO Difference**
Average	2.61	2.18	8.85	4.12	18.13	10.28
Standard Deviation	3.67	3.70	11.01	8.61	28.20	24.42

RPD RDO to Membrane BOD Surface Water						
	RPD NSRDO Initial	**RPD SRDO Initial**	**RPD NSRDO Final**	**RPD SRDO Final**	**RPD NSRDO Difference**	**RPD SRDO Difference**
Average	1.25	1.00	1.43	0.99	18.01	6.91
Standard Deviation	1.72	1.35	23.91	2.30	28.82	8.21

Table X: BOD Tap Water				
	RPD NSRDO Initial	**RPD SRDO Initial**	**RPD NSRDO Final**	**RPD SRDO Final**
Average	2.58	2.02	3.60	2.17
Standard Deviation	2.35	1.55	2.49	2.07

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 °C	Chlorinity: 0	5.0	10.0	15.0	20.0	25.0	Temperature °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 seawater1 at equilibrium with water-saturated air. Figures and tables also are

available.[3]

Calculate the equilibrium oxygen concentration, C^*, from equation:

$$\ln C^* = -139.34411 + (1.575701 \times 10^5/T) - (6.642308 \times 10^7/T^2) + (1.243800 \times 10^{10}/T^3) - (8.621949 \times 10^{11}/T^4) - \text{Chl}\,[(3.1929) \times 10^2) - (1.9428\ \_\ 10^1/T) + (3.8673 \times 10^3/T^2)]$$

where:

C^* = equilibrium oxygen concentration at 101.325 kPa, mg/L,

T _ temperature (°K) = °C + 273.150, (°C is between 0.0 and 40.0 in the equation; the table is accurate up to 50.0), and

Chl = Chlorinity (see definition in Note 4, below).

Example 1: At 20°C and 0.000 Chl, ln C^* = 2.207442 and C^* = 9.092 mg/L;

Example 2: At 20°C and 15.000 ChL,

ln C^* = (2.207442) – [15.000 x (0.010 657)]

= 2.0476 and C^* = 7.749 mg/L.

When salinity is used, replace the chlorinity term (-Chl[. . .]) by:

$- S(1.7674 \times 10^{-2}) - (1.0754 \times 10^{1}/T) + (2.1407 \times 10^{3}/T^{2})$

where:

S = salinity (see definition in Note 4, below).

3. For nonstandard conditions of pressure: (*Excel worksheet is available*)

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

where:

Cp = equilibrium oxygen concentration at nonstandard pressure, mg/L,

C^* = equilibrium oxygen concentration at standard pressure of 1 atm, mg/L.

P = nonstandard pressure, atm,

P_{WV} = partial pressure of water vapor, atm, computed from: ln P_{WV} = 11.8571 x (3840.70/T) - (216961/T^2),

T = temperature, °K,

$\theta = 0.000975 - (1.426 \times 10^{-5} t) + (6.436 \times 10^{-8} t^2)$, and

t = temperature, °C.

N.B.: Although not explicit in the above, the quantity in brackets in the equation for *Cp* has dimensions of atm^{-1} per Reference 4, so that *P* multiplied by this quantity is dimensionless.

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

An equation for *Pwv* that includes the salinity factor may be found in Reference 1.

Example 3: At 20°C, 0.000 Chl, and 0.700 atm,

Cp = *C* P* (0.990092) = 6.30 mg/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., g/kg of solution) no longer applies.

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

Salinity = 1.806 55 x 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 g/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 (g/mL) vs. Temperature (°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** °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** °C is 0.999957 g/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

	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
28	0.996232	0.996204	0.996175	0.996147	0.996118	0.996089	0.996060	0.996031	0.996002	0.995973
29	0.995944	0.995914	0.995885	0.995855	0.995826	0.995796	0.995766	0.995736	0.995706	0.995676
30	0.995646	0.995616	0.995586	0.995555	0.995525	0.995494	0.995464	0.995433	0.995402	0.995371

BOD Dilution Tables

Table 1: Dilution Table for Unseeded Samples				
Anticipated BOD	Dilution Factor	% Dilution	ml of Sample per 300 ml Bottle	Dilution BOD Range
5	1	100	300	2-7
9	2	50	150	4-14
13	3	33	100	6-60
16	4	25	75	8-25
22	5	20	60	10-35
32	7.5	13	40	15-50
45	10	10	30	20-70
65	15	607	20	30-100
90	20	5.0	15	40-140
120	25	4.0	12	50-175
130	30	3.3	10	60-200
160	37	2.7	8	75-250
200	42	2.4	7	85-300
225	50	2.0	6	100-350
260	60	1.7	5	120-400
325	75	1.3	4	150-500
475	100	1.0	3	200-700
Table 2: Dilution Table for Seeded Samples Assume Initial DO is > 8.0 mg/L and the Seed Correction is between 0.5 mg/L and 1.0 mg/L				
Anticipated BOD	Dilution Factor	% Dilution	ml of Sample per 300 ml Bottle	Dilution BOD Range
4	1	100	300	2-6
8	2	50	150	4-12
12	3	33	100	6-18
16	4	25	75	8-24
22	5.5	18.3	55	11-33
30	7.5	13.3	40	15-45
40	10	10	30	20-60
48	12	8.3	25	24-72
60	15	6.7	20	30-90
80	20	5	15	40-120
100	25	4	12	50-150
120	30	3.3	10	60-180
150	37.5	2.7	8	75-180
200	50	2	6	100-225
240	60	1.7	5	120-360
300	75	1.3	4	150-450
400	100	1	3	200-600