Dissolved Oxygen

(DO)

VT WSMD Wastewater Program Lab Manual Section #11

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Dissolved Oxygen

Background

Oxygen is present in solution as both the dissolved form and in compounds, including sulfates (SO_4^{-2}), nitrates (NO_3^-), etc. When we measure dissolved oxygen in a solution, we are measuring the free oxygen gas dissolved in the solution. The dissolved oxygen is the oxygen that is available for use in respiration, or breathing, by fish, aquatic insects, plants, and aerobic bacteria. Without dissolved oxygen, the waters go septic, and the result can be unsightly and foul-smelling. Oxygen enters water from the air through the surface by diffusion and happens much more rapidly in waterfalls, rapids, and riffle areas than in quiet pools. Oxygen also enters the water from the daytime photosynthetic activities of aquatic plants. The levels of dissolved oxygen or DO in streams varies from a low of 0-3 mg/l for polluted waters to a high of 12-15 mg/l in clear, very cold streams. Seven to nine (7-9) mg/l is the average in natural waters. The levels are dependent on: 1) physical conditions such as rapids, dams, pools and temperature of the water; 2) chemical action such as rusting, etc.; and 3) biological activity going on in the water such as that of fish, insects, bacteria, and plants.

We test for DO in treatment plant effluents to determine the amount there, to aid in the stream's natural waste stabilization process; in secondary plant processes to determine if the amount of DO present is sufficient to maintain good aerobic biological treatment; and in influents to avoid septic conditions which may result in the formation of hydrogen sulfide and its corrosive results.

Methods of Measurement

The three methods of dissolved oxygen measurement described in this document are the Iodometric /Winkler titration method with azide modification, the membrane electrode method, and the Luminescent Dissolved Oxygen method. Since the acceptance of the Luminescent Dissolved Oxygen Method by EPA, it has become the most popular and commonly used method for dissolved oxygen and Biochemical Oxygen Demand measurement in Vermont (and with good reason)! The meter and probes are capable of reliable self-calibration, there are no membranes to change, and very little maintenance is required. Unfortunately, the equipment is more expensive than the alternative membrane versions. The method has proven to be very accurate and user friendly and will be described first.

Luminescent Dissolved Oxygen/Optical Probe Method

The Luminescent Dissolved Oxygen (LDO) probe measures the time it takes for a specific luminescent reaction to take place. Blue light is transmitted from light emitting diodes (LED) on the probe to a sensor. Luminescent material on the probe is “excited” by the blue light. As the material becomes less excited at a specific point it emits a red light. The probe measures the amount of time from when the material becomes excited by the blue light until it relaxes enough to emit the red light. The higher the oxygen concentration the shorter the time it takes for the red light to be emitted. (Oxygen “quenches” the luminescence). That amount of time is directly proportional to the concentration of oxygen. Between the blue light flashes, a separate LED emits red light onto the probe sensor. This serves as an internal standard.

These LDO probes are capable of very accurate measurements from 0.05 mg/Liter to 20.00 mg/Liter. After the initial setup the probes are self-calibrating and require very little maintenance besides thorough rinsing of the probe between readings.

There are some interferences with this type of probe that must be considered. High concentrations of Chlorine dioxide can have a detrimental effect. Bacterial or algal growth can cause problems, but this can be avoided by good rinsing practice. Oils are a problem with all DO probes. Again, rinsing minimizes this problem. Finally, alcohols and some organic solvents can damage the probe.

Equipment

- Luminescent Oxygen probe with stirrer and appropriate meter

Reagents

- Sodium Sulfite solution - 2M solution for blank
- Cobalt chloride 0.03 g/L

Add the 0.03 g/L Cobalt chloride to the 2M Sodium sulfite solution. This makes a blank solution of zero mg/L Dissolved Oxygen. * The 23rd edition of Standard Methods for the Examination of Water states "Add excess sodium sulfite and a trace of cobalt chloride to bring DO to zero."

Procedure

Calibrate the probe per manufacturer's instructions. Note: Many of the LDO probes have built in calibration. For daily or more frequent quality control purposes the instrument's automatic calibration is enough. Occasional checks with a blank and a control are recommended but not required.

Note: If analyzing for Biochemical Oxygen Demand, more frequent verification of calibration is required.

To prepare a water- saturated air sample, simply add a small amount (10 to 25 mL) of reagent grade water to a 300 mL BOD bottle. Place the glass stopper on the bottle and shake it vigorously for about 30 seconds. Let the bottle sit for approximately 30 minutes to let the water equilibrate to room temperature (which should be 20 °C +/- 2 °C) then place the DO probe into the BOD bottle and take the reading. You must note the barometric pressure and temperature at your laboratory at the time of analysis and use table 4500-O:II (from 23rd edition Standard Methods for the Examination of Water and Waste) to get the theoretical DO concentration. The meter reading must be within +/- 10% of the theoretical value. If not, the probe must be re-calibrated.

Example I: Assume you have completed the calibration procedure described above at a temperature of 20 °C with a barometric pressure reading (at your lab) of 750 mm of mercury. The meter reading is 9.1 mg/L. Check this reading against the theoretical value in Table 4500-O:II. You will see that the meter is reading exactly as it should. The calibration has verified the meter reading.

Example II: Assume you have completed the calibration at a temperature of 25 °C with a barometric pressure reading (at your lab) of 625 mm of mercury. The meter reading is 9.1 mg/L. Check the reading against the theoretical value in Table 4500-O:II. In this case you see that the theoretical value is 6.8 mg/L. The meter reading is NOT within +/-10% of the theoretical value. The meter must be recalibrated.

Once the calibration is completed, fill each BOD bottle to the top of the neck being very careful not to introduce oxygen. (This is best accomplished by letting the sample slowly flow down the inside of the bottle). Place the probe in the sample being careful not to entrap air, turn on the stirrer and take the reading.

Quality Control

Unless this analysis is required for reporting purposes in your permit, quality control procedures are not required. Recommended quality control procedures include the use of a blank, a control and 10% replication or duplication of sample analysis.

Reporting Dissolved Oxygen Results: Luminescent Electrode Method

Dissolved Oxygen (Luminescent Electrode Method) Bench Sheet
ANALYST:
SAMPLE TIME and DATE:
SAMPLE LOCATION:
ANALYSIS TIME and DATE
SAMPLE VOLUME (Method):
SAMPLE TEMPERATURE: METER STANDARDIZATION METHOD: (atmospheric, Winkler, auto, etc.)
RESULT in mg/L:
Reporting is the same as described for the membrane electrode method

Membrane Electrode Method for Dissolved Oxygen Analysis

The membrane electrode method is based on the fact that the diffusion of dissolved oxygen across the electrode membrane produces a change in the potential of the electrode. This voltage change is measured by the DO meter. These electrodes, like pH electrodes, are sensitive to changes in temperature but most modern DO meters are equipped with automatic temperature compensation circuits. This method is especially useful for sludges, fast measurements in the plant, respiration rates and, when equipped with a stirring bottle probe, for BOD measurements.

Equipment and Reagent

- DO meter, YSI model 51, 54, 57, etc. or equivalent
- DO probe
- spare membranes
- thermometer
- probe filling solution
- razor blade (if using older style probe)

Calibration of DO Meter

DO meters can be calibrated by any of three methods. These are: atmospheric calibration, barometric calibration and calibration against the Winkler method. The atmospheric calibration method is fast and simple and is fine for in-plant process control testing but it is not generally a recommended method when calibrating for BOD readings. For BOD analysis, the DO meter should be calibrated using either the barometric or Winkler method. Each of these methods will be discussed below. *NOTE: Many of the newer Dissolved Oxygen meters have built in atmospheric calibration. The meter atmospheric pressure reading should be checked against a certified barometer at least annually (more often if there is reason to believe the reading is inaccurate).

Before going through the entire process of calibration it is always a good idea to check the membrane on the DO probe. Membranes must be replaced quite often. Some indications of the need for membrane replacement are: any sign of bubbles under the membrane, drifting of the meter and the inability to reach calibration. To replace the membrane, follow the manufacturer's instructions closely. Look for bubbles!

Once the membrane has been replaced:

1) Attach probe to meter.
2) Set master control to "red line" and adjust needle so that it lines up with the red line on the face with the "red line" knob.
3) Set the master control to "zero" and adjust the needle to zero with the zero knob.
4) Switch the master control to "calibrate" and allow the probe 15 minutes to polarize. Then proceed with one of the following methods of calibration.

NOTE: The probe should be stored in a wet environment; the sponge in the plastic cap provided, should be wetted.

Atmospheric Calibration

With the probe polarized, read the room temperature and consult the saturation value either in Standard Methods or printed on the back of the meter. This value should be corrected by the atmospheric correction factor given on the back of the meter and this corrected value set on the meter with the "calibration" knob. For example, if the thermometer reads 19 °C and the plant is located at about 500 feet above sea level:

- 19 °C saturation value is 9.3 mg/l
- 500 feet correction factor is 0.98
- 9.3 mg/l x .98 - 9.1 mg/l

The value of 9.1 mg/l should be set on the meter. The meter is now calibrated and ready to use.

Winkler Calibration

(Use with DO bottle probes with stirrer for BODs). With the probe polarized, fill a graduated cylinder with distilled water. Siphon this out carefully into 3 DO bottles. Put the probe into one bottle and perform a Winkler titration on the other two bottles as described previously. Calibrate the meter to the average of the two Winkler results.

Most DO probes need a minimum flow rate past the probe in order to read accurately. In moving water, this is not so much a problem but when taking DOs on clarifiers, lakes, or BOD bottles, etc., some means of agitation must be provided. In BOD bottles, magnetic stirrers can be used if your bottle probe is not equipped with its own stirrer. In large, quiet bodies of water, the lead to the probe can be moved up and down to provide an artificial flow.

Barometric Calibration

The third method for calibrating a DO meter is called barometric calibration. It is quicker to calibrate the meter by this method than by the Winkler calibration and it is permissible to utilize this method when using the meter for BOD analysis.

The meter should be warmed and polarized. The DO probe is put in a DO bottle partially filled (50 mL or so) with water and is allowed 15 minutes to equilibrate. The temperature of this water vapor is then taken and by using the chart below the solubility of oxygen at this temperature is determined. For example, assume the temperature is 21 °C. The chart below tells you that the solubility of saturated water vapor(s) at 21 °C is 8.9 mg/L. **However, most current DO probes automatically correct for temperature – check with the manufacturer to determine whether you need to manually perform this step.**

Solubility of Oxygen in Water Exposed to Water-Saturated Air

Temperature	Oxygen Concentration in Water
° C	mg/L
10	11.27
11	11.01
12	10.76
13	10.52
14	10.29
15	10.07
16	9.85
17	9.64
18	9.45
19	9.26
20	9.07
21	8.90
22	8.72
23	8.56
24	8.40
25	8.24
26	8.09
27	7.95
28	7.81
29	7.67
30	7.54

If the barometer being used gives the barometric pressure reading in inches, use the following equation to calibrate the solubility at which the DO meter should be set:

$$S^1 = \frac{S * P}{29.92}$$

Where:

- S^1 = The solubility that you will set the meter at
- S = the solubility of saturated water vapor at 101.3 KPa (from the chart in column under “oxygen concentration in water”)
- P = Barometric pressure, in inches
- 29.92 = a constant

In the example, the calculation works out:

$$S^1 = \frac{8.90 * 29.75}{29.92}$$

$$S^1 = 8.849, rounds\ to\ 8.85\ considering\ significant\ digits$$

A different equation is used when the barometric pressure is measured in mm Hg. In this case the following equation should be used.

$$S^1 = \frac{S * P}{760}$$

Where:

- S^1 = The solubility that you will set the meter at
- S = the solubility of saturated water vapor at 101.3 KPa (from the chart in column under "oxygen concentration in water")
- P = Barometric pressure, in mmHg
- 760 = a constant

At the same temperature (21 °C) and same barometric pressure (29.75" = 745 mm Hg), the example calculation becomes:

$$S^1 = \frac{8.90 * 756}{760}$$

$$S^1 = 8.853\ (rounds\ to\ 8.85)$$

These directions hold true for elevations less than 1,000 meters (3,281') and for temperatures below 25 °C.

Quality Control

For accurate data, it is important that all reagents are standardized correctly in the frequency required. The DO meter is standardized daily (each use) by the means outlined above and properly maintained to insure optimum performance.

Dissolved Oxygen Troubleshooting Guide: Membrane Electrode Method

PROBLEM	MOST PROBABLE CAUSE	SOLUTION
Air bubbles appear at the top of the BOD bottle after probe is inserted.	Membrane not cut around 'o' ring - (older type probes). Air is captured under membrane	Trim excess membrane just above 'o' ring.
Drifting, erratic reading **OR** Cannot get meter properly standardized.	Crease in membrane **OR** filling solution low. Flow rate of sample past the probe membrane is insufficient. Probe is not allowed to polarize before analysis.	Replace membrane; add fresh potassium chloride filling solution – Re-standardize meter and re-run analysis. Increase meter speed - If probe stirrer is malfunctioning use a magnetic stirrer. Turn meter on 15 minutes before standardization or analysis.

Quality Control for Dissolved Oxygen Analysis: Membrane Electrode Method

Document the Following (Including by not limited to):

Sampling

- Grab -
    - Exact Time and Date Sampled
    - Exact Time and Date Analyzed
- Location
- Temperature

Glassware

- Properly washed and rinsed

Instrument

- Calibration and Maintenance
- Method of Standardization
    - Date
    - Analyst's initials

Duplication/Replication Schedule

- Minimum of 5%, 10% is recommended

Reporting Dissolved Oxygen Results: Membrane Electrode Method

Dissolved Oxygen (Membrane Electrode Method) Bench Sheet
ANALYST:
SAMPLE TIME and DATE:
SAMPLE LOCATION:
ANALYSIS TIME and DATE:
SAMPLE VOLUME (method):
SAMPLE TEMPERATURE:
METER STANDARDIZATION METHOD: (Atmospheric, Winkler, etc.)
RESULT in mg/L:

Winkler Titration Method

The Winkler procedure is based on the oxidizing property of DO or the tendency of free oxygen to attach to certain ions. The azide modification is used to eliminate the interference of nitrite, which is found in many biologically treated effluents, some streams at certain times of the year, and in the BOD test.

In the presence of dissolved oxygen, Mn2+ (the manganous ion) from DO solution #1 (manganous sulfate) reacts with the dissolved oxygen under alkaline conditions supplied by the addition of DO solution #2 (the alkali-azide-iodide solution) to form a brown (manganic hydroxide) floc.

$$2Mn^{2+} + O_2 + 4OH \rightarrow 2MnO(OH)_2$$

With the addition of DO solution #3 (sulfuric acid), the oxidized manganese is reduced back to the divalent form, and iodine is released (from the iodide ions supplied in DO solution #2.)

$$MnO(OH)_2 + 6I^- + 6H^+ \rightarrow Mn^{2+} + 2I_3^- + 3H_2O$$

The amount of iodine formed is proportional to the amount of dissolved oxygen originally present in the solution.

By titrating with a standard solution of Sodium Thiosulfate we can measure the amount of iodine present which as stated is directly proportional to the DO concentration.

$$H^+ + I_3^- + 2Na_2S_2O_3 \rightarrow 2NaI + Na_2S_4O_6 + HI$$

The addition of starch indicator to the solution before titration makes endpoint determination easier by producing a dark blue color which contrasts the colorless endpoint.

Sampling Handling and Preservation

Samples for DO should be taken with a minimum of turbulence, to avoid air entrapment, into a 300 mL DO bottle. The bottle should be filled into the neck and stoppered tightly. * It is very important NOT to agitate the sample or allow it to remain in contact with the ambient air. Samples should be doped (the DO solutions added) and titrated immediately, although they can be held for up to eight (8) hours if fixed (Manganous sulfate and alkali-iodide solutions added) and stored in the dark (true for Winkler Method only). Then add the sulfuric acid and shake the sample. *Note: If there is an appreciable iodine demand in the sample (an iodine demand might be expected if there is a high concentration of alkali metals such as aluminum or mercury in the sample) preserve the sample with 0.7 mL of sulfuric acid and 1 milliliter of sodium azide solution. Place the glass stopper in the bottle, create a water seal and cover with the plastic cap. Perform the analysis as soon as possible!

When a Kemmerer sampler is used, the BOD sample bottle should be filled to overflowing. (Overflow for approximately 10 seconds.) The outlet tube of Kemmerer should be inserted to bottom of BOD bottle. Care must be taken to prevent turbulence and the formation of bubbles when filling the bottle.

At the time of sampling, the sample temperature should be recorded as precisely as required.

Do not delay the determination of dissolved oxygen in samples having an appreciable iodine demand or containing ferrous iron.

Winkler-Iodometric Method (w/ azide modification)

300 mL (Full Bottle)

Equipment

- 3 2 mL automatic or graduated pipets
- 2 500 mL Erlenmeyer flasks
- burette, 50 mL or 10 or 25 mL automatic filling burette
- 2 dropping bottles, for starch and back titrant solution
- 10 mL volumetric pipet
- 100 mL graduated cylinder
- magnetic stirrer (optional)
- sample bottles - 300 mL (+ 3 mL) capacity BOD incubation bottles with tapered round glass pointed stoppers and flared mouths

Reagents

- Manganous Sulfate ($MnSO_4$) Solution DO Solution #1

  Dissolve 480 g MnSO4 - 4H2O in distilled water and dilute to 1 liter.

  Alternatively, use 400 g of MnSO4 2H2O or 364 g of MnSO4H2O per liter.

- Alkaline Iodide-Azide (OH-,I-,NaN_3) Solution DO Solution #2

  Gradually add and dissolve 500 grams of reagent grade Sodium Hydroxide (NaOH) OR 700 grams Potassium Hydroxide (KOH) in 600 mL distilled water in a 2 liter beaker. In another beaker add 135 grams Sodium Iodide (NaI) OR (NaN3) in 40 mL of distilled water. Carefully add this solution to the Sodium Hydroxide solution, cool to room temperature, pour into one-liter volumetric flask, and dilute to one-liter with distilled water.

- Sulfuric Acid (H_2SO_4) Concentrated, 36N DO Solution #3
- Sodium Thiosulfate ($Na_2S_2O_3$), Stock Solution, 0.75N:

  Dissolve 186.15 g $Na_2S_2O_3 \bullet 5H_2O$ in boiled and cooled distilled water and dilute to 1 liter. Preserve by adding 5 mL chloroform.

- Sodium Thiosulfate ($Na_2S_2O_3$), Standard Titrant, 0.0375N:

  Prepare by diluting 50.0 mL of stock solution to 1 liter. Preserve by adding 5 mL of chloroform. Standard sodium thiosulfate, exactly 0.0375N is equivalent to 1.0 mg of DO per 1.00 mL. Standardize with 0.0375N potassium bi-iodate. SEE STANDARDIZATION PROCESS IN APPENDIX I AT THE END OF THE DISSOLVED OXYGEN SECTION.

  ***NOTE**: 0.0375 N Phenylarsine Oxide can be used in place of the 0.0375 N Sodium Thiosulfate solution as a titrant. To prepare this solution, dissolve 6.30 grams of Phenylarsine Oxide crystals in 700 mL of freshly boiled and cooled distilled water. Dilute to 1 Liter with distilled water. Preserve with 2.5 mL Chloroform.

- Potassium Bi-Iodate ($KH(IO_3)_2$) Stock Solution, 0.15N

This stock solution of Potassium Bi-iodate if stored in an amber glass bottle with screw cap, and refrigerated, has a shelf life of approximately 6 months and can be used to make up the working solution of Potassium Bi-iodate every 2 weeks to 1 month.

After drying 5 grams of reagent grade Potassium Bi-iodate for 2 hours at 103 °C, weigh out 4.873 grams and dissolve this in 600 mL distilled water. Dilute to 1 liter with distilled water.

- Potassium Bi-Iodate ($KH(IO_3)_2$) Primary Standard, 0.0375N

  Dilute exactly 250 mL of the 0.15N Potassium Bi-iodate solution to 1 liter with distilled water. Store in an amber glass bottle with screw top. Discard after 1 month.

  *NOTE: The primary standard potassium bi-iodate KH(IO3)2 should be used in place of potassium dichromate, for standardization of the thiosulfate solution. Potassium bi-iodate seems to eliminate the return of the blue color that is titrated out.

- Starch Indicator Solution

  Prepare an emulsion of 10 grams soluble (potato) starch in a beaker with a small amount of distilled water. Pour this emulsion into 1 liter of boiling distilled water and allow to boil for a few minutes. Let the solution settle overnight and pour off the semi-clear portion. Discard the thick substance left in the bottom of the container. Preserve the starch solution by adding 1 g salicylic acid, or 5 mL Chloroform or a couple of drops of Toluene. This solution should be kept refrigerated.

Procedure: 300 mL Method

1) Remove the stopper and add 2 mL solution #1 (MnSO4). Hold the pipet just above the liquid surface and add 2 mL solution #2 (OH-, I-, N3-) the same way, slowly and just above the sample surface, to avoid adding any air bubbles.
2) Re-stopper and mix by inverting the bottle 10 to 15 times.
3) Allow the bottle to settle so that the brown floc occupies ½ the bottle volume or less.
4) Invert the bottle again 10 to 15 times.
5) Allow the floc layer to settle again to ½ the bottle volume or less.
6) Carefully, add 2 mL concentrated H2SO4, solution #3.
7) Stopper and shake until the solution is uniformly mixed.
8) Pour the entire contents into a 500 mL Erlenmeyer flask.
9) While swirling the flask, titrate with 0.0375N sodium thiosulfate solution from the starting orange-yellow until it becomes a pale-yellow color. (0.0375N phenylarsine oxide (PAO) may be substituted as a titrant.)
10) Add 1 mL starch solution, the sample will turn blue.
11) Continue titration until the blue goes to clear.
12) Check the endpoint by back-titration (as described on page 6 of this section).

NOTE: Occasionally, a dark brown or black precipitate persists in the bottle after acidification. This precipitate will dissolve if the solution is kept for a few minutes longer than usual or, if particularly persistent, a few drops of H2SO4 will effect dissolution.

Calculation

When using a 300 mL sample (full DO bottle) and 0.0375N sodium thiosulfate, then 1 mL thiosulfate used = 1 mg/l DO present in sample.

The Winkler method with azide modification is not applicable under the following conditions: a) samples containing sulfite, thiosulfate, polythionate, appreciable quantities of free chlorine or hypochlorite; b) samples high in suspended solids; c) samples containing organic substances which are readily oxidized in a highly alkaline solution or which are oxidized by free iodine in an acid solution; d) untreated domestic sewage; e) biological flocs; and f) where sample color interferes with endpoint detection. In instances where the azide modification is not applicable, the DO probe should be used.

Dissolved Oxygen Troubleshooting Guide for Winkler Titration Method with Azide Modification

PROBLEM	MOST PROBABLE CAUSE	SOLUTION
Cannot get floc to dissolve completely after addition of sulfuric acid (DO #3)	High DO in sample. Insufficient quantity of acid added to dissolve the iodine formed.	Add more sulfuric acid until the entire floc is dissolved. Mix by inversion.
	Insufficient mixing.	Continue to mix by inversion.
A white floc forms after the addition of manganous sulfate (DO #1) and the alkaline iodide - azide (DO #2) solutions. * Referred to as a "snowball" or "white-out".	Very little or no dissolve oxygen present in sample.	Add the sulfuric acid solution after allowing the floc to settle. If the sample turns clear (or milky) there is no dissolved oxygen in the sample.
Difficult to determine trace endpoint - "Fading" endpoint.	Background color interfering with reading.	Use a white burette stand or place a white piece of paper under the flask to help differentiate clear from light blue sample appearance.
	Starch solution weak.	Prepare fresh solution - Re-run analysis.
	The starch indicator solution was added without first titrating the sample to a pale straw yellow color.	Re-run the analysis. Before adding the starch indicator solution titrate the sample to a light - straw yellow color. Then add starch indicator and continue the titration to the clear endpoint.

PROBLEM	MOST PROBABLE CAUSE	SOLUTION
Result is unreasonably high.	Improper concentration of Sodium Thiosulfate titrant (too weak).	Re-standardize the titrant to 0.025N for the 203 mL version of the test or 0.0375N for the 300 mL version. Re-run analysis.
	Sample has a chlorine residual.	Neutralize any chlorine residual before performing the analysis.
Result is unreasonably low.	Improper concentration of sodium thiosulfate titrant (too strong).	Re-standardize the titrant. Re-run analysis.
	The sample contains >5 mg/l ferric iron salts.	Add potassium fluoride (approximately 1 milliliter) to the sample before adding the manganous sulfate (DO #1) solution **OR** Add an 80% to 85% solution of phosphoric acid in place of the sulfuric acid for acidification of the sample (approximately 1 milliliter).

Quality Control for Dissolved Oxygen: Winkler Titration w/ azide modification

Document the following:

Sampling

- Grab
  - Exact time and Date sampled
  - Exact time and Date analyzed
- Volume
- Location
- Temperature

Glassware

- 300 mL version
- Glassware properly washed and rinsed

Reagents

- NIST traceable
- Preparation and Expiration dates

Reporting Dissolved Oxygen: Winkler Titrant Method

Dissolved Oxygen (Winkler Method) Bench Sheet
ANALYST:
SAMPLE TIME and DATE:
SAMPLE LOCATION:
ANALYSIS TIME and DATE:
SAMPLE VOLUME (method):
SAMPLE TEMPERATURE:
ANY MODIFICATION TO NEGATE INTERFERENCES:
BURET READING: Start _____ Endpoint _____
AMOUNT OF BACK TITRANT USED TO PRODUCE BLUE COLOR:
CALCULATIONS (Back Titration):
RESULTS IN mg/L: Reagent lot #s and expiration dates:

Dissolved Oxygen on Activated Sludge Using Winkler Method

Since activated sludge is a biological floc that will continue to use oxygen after taking a sample, it is necessary to stop the oxygen uptake as soon as possible to get a valid result. This is done by adding copper sulfate-sulfamic acid to the flocculate and settling out the activated sludge providing a clear supernatant on which to perform the Winkler method.

Equipment

In addition to the equipment and reagents required for the Winkler Method, you will need:

- 1 liter glass stoppered bottle

Reagents

- Copper Sulfate-Sulfamic Acid ($CuSO_4$-NH_2SO_2OH) Inhibitor Solution

  Dissolve 32 g of technical-grade NH2SO2OH in 475 mL distilled water. (DO NOT use heat to help dissolve). Dissolve 50 g CuSO4 - 5H2O in 500 mL distilled water. Mix these two solutions and carefully add 25 mL concentrated acetic acid.

Procedure

1) Add 10 mL CuSO4-NH2SO2OH inhibitor to the 1 liter glass stoppered bottle.
2) Take a sample of the activated sludge by placing the 1 liter bottle in a special sampler that fills the bottle via a tube near the bottom.
3) Fill the bottle to the very top and stopper the bottle immediately and mix by inverting several times.
4) After suspended solids have settled, siphon off the clear layer (supernatant) into a 300 mL DO bottle being careful not to introduce any air by turbulence. Stopper the bottle tightly.
5) Continue Winkler method as described in Section 11 on Page 3 or Page 7 of this manual.

It is important to be aware of the difficulty of avoiding air entrapment into the bottle. It is recommended that a DO probe be used in the actual tank when DOs less than 1 mg/l are present.

Troubleshooting, quality control and reporting are the same as described for in the Winkler Titration method with azide modification here.

References

Winkler Method (with Azide Modification): Standard Methods 23rd Edition 4500-OC (General Reference)

The Membrane Electrode Method for the analysis of dissolved oxygen can be found in pages 4-149 through 4-152 in the 23rd Edition of Standard Methods for the Examination of Water and Wastewater.

The Copper Sulfate-Sulfamic Acid method used for analysis of DO in activated sludge is described on page 4-149 of Standard Methods for the Examination of Water and Wastewater – 23rd Edition.

APPENDIX I

Standardization of 0.0375N Sodium Thiosulfate

Run in duplicate:

1. Dissolve 2 g potassium iodide, KI, in 100 mL distilled water in a 500 mL Erlenmeyer flask.
2. Slowly, while stirring, add 10 mL 10% sulfuric acid, H2SO4.
3. Add 20.0 mL (use a volumetric pipet) of 0.0375N potassium bi-iodate (KH(IO3)2 and mix.
4. Place flasks in dark for 5 minutes
5. Dilute to approximately 400 mL with distilled water.
6. Titrate with approximately 0.0375N sodium thiosulfate, to a pale straw color, add starch and titrate until blue color disappears.
7. Should use exactly 20 mL of thiosulfate if the normality is exactly 0.0375.

If you use more than 20 mL, the thiosulfate is weaker than 0.0375N, if you use less than 20 mL, it is stronger. If the thiosulfate is too weak, adjust the normality to 0.0375N by adding thiosulfate crystals; if it is too strong, adjust by diluting with distilled water. Two examples follow:

Example 1

For 0.0375N Sodium Thiosulfate:

$$N_1V_1 = N_2V_2$$

$$0.0375N * 20\ mL = N_2 * 21.40\ mL$$

$$0.75 = N_2 * 21.40\ mL$$

$$N_2 = \frac{0.75}{21.40}$$

$$N_2 = 0.350N$$

The thiosulfate solution is too weak and must be adjusted upward by adding thiosulfate crystals. For every increase of 0.0001N you wish to make in a liter of thiosulfate, add 0.0248g of crystals. In this example, 0.0375N - 0.035N is an increase of .0025N desired. Therefore, you want to add 25 x .0288g of crystals or 0.62g to a liter.

Example 2

For 0.0375N Sodium Thiosulfate: 19.35 mL thiosulfate used in the standard titration. Again, using the equation $N_1V_1 = N_2V_2$, the normality of the thiosulfate is calculated:

$$N_1V_1 = N_2V_2$$

$$0.0375N * 20\ mL = N_2 * 19.35\ mL$$

$$0.75 = N_2 * 19.35\ mL$$

$$N_2 = \frac{0.75}{19.35}$$

$$N_2 = 0.388N$$

The thiosulfate solution is too strong, and the solution must be diluted to the proper strength. To find out show much distilled water to add, again use the formula $N_1V_1 – N_2V_2$

$$N_1V_1 = N_2V_2$$

$$0.0388N * 961\ mL = 0.0375N * V_2$$

$$V_2 = \frac{37.29}{0.0375}$$

$$V_2 = 994.4\ mL$$

We need to add 994.4 - 961 mL of distilled water = 33.4 mL.

That is, to the 961 mL of sodium thiosulfate remaining we add 33.4 mL of distilled water.