Dissolved Oxygen (DO)

All gases of the atmosphere are soluble in water to some degree. Oxygen is classified as poorly soluble, and its solubility is affected both by atmospheric pressure, and physical and chemical properties of water such as temperature, salinity, pollutants, etc. The solubility of atmospheric oxygen in fresh waters ranges from 14.6 mg/L at 0o C to about 7 mg/L at 35o C under 1 atm. of pressure. Most of the critical conditions related to dissolved-oxygen deficiency, both in natural waters and biological wastewater treatment, occur during the warmer months when temperatures are high and solubility of oxygen is at a minimum. The low solubility of oxygen is a major factor limiting the purification capacity of natural waters. In aerobic biological treatment processes, the limited solubility of oxygen is also of great importance, because it governs the rate at which oxygen will be absorbed by the medium and therefore the cost of aeration. Hence, DO analysis is a key test both in natural waters and water pollution control practice.

Equipment Dissolved Oxygen Probe

The use of DO probes or electrodes which allow in-situ measurements to be made has become standard practice in recent years. They are especially useful for taking DO profiles of reservoirs and streams, for monitoring DO levels in aerobic biological wastewater treatment processes, and for conducting BOD analyses. An inert metal such as gold or platinum serves as the cathode, and silver is used for the anode. These are electrically connected with a potassium chloride solution, and the cell is separated from the sample by means of a gas-permeable membrane, usually polyethylene. The membrane shields the cathode and anode from contamination by interfering liquids and solids. When a potential of about 0.5 to 0.8 volt is applied across the anode and cathode, any oxygen which passes through the membrane will be reduced at the cathode, causing a current to flow. The magnitude of the current produced is proportional to the amount of oxygen in the sample. Dissolved-oxygen electrodes are very sensitive to temperature, and thus either temperature measurements must be made along with dissolved-oxygen measurements so that a correction can be applied, or else instruments which are equipped with a thermistor or other device to compensate automatically for temperature changes must be used

Dissolved oxygen measurements using DO probe

(i) Remove glass stopper of the BOD bottle, and place DO probe into the sample.
(ii) Switch on the stirrer of the DO probe.
(iii) Read the DO value in mg/L when the meter reading has stabilized.

Biochemical Oxygen Demand (BOD)

Biochemical oxygen demand (BOD) is defined as the amount of oxygen required by bacteria while stabilizing decomposable organic matter under aerobic conditions. The BOD test is widely used to determine the pollutional strength of domestic and industrial wastewaters in terms of the oxygen that they will require if discharged into natural watercourses in which aerobic conditions exist. The test is one of the most important both in regulatory work and in studies designed to evaluate the purification capacity of receiving water bodies. Its disadvantage is the long time required by the test, generally taking 5 days.

The BOD test is essentially a bioassay procedure involving the measurement of oxygen consumed by living organisms (mainly bacteria) while utilizing the organic matter present in a waste, under conditions as similar as possible to those that occur in nature. The requirements of the environmental conditions for the test can be summarized as follows:

- Sufficient nutrients, e. g. N, P, S, K, Na, and certain trace elements.
- Free from toxins.
- Presence of a mixed culture of microorganisms (seed).
- Dissolved oxygen must be available in the sample throughout the period of the test.
- No interference due to re-aeration.

- 20o C incubation.

It is possible to interpret BOD data in terms of organic matter, as well as the amount of oxygen used during its oxidation. This concept is fundamental to an understanding of the rate at which BOD is exerted. Typical BOD5 values in mg/L for various wastewaters and sewage are given below:

Raw Sewage

- Weak 110
- Medium 220
- strong 440
- typical in Singapore 300 – 350

Primary effluent 150 – 200
Secondary effluent 20 – 50
Tertiary effluent 5 –10
Raw reservoir water

- Kranji 5 –15
- Upper Pierce 3 –7

BOD measurements

The following procedure applies to both total BOD5 and soluble BOD5 determinations.

No dilution is required for samples whose 5-day BOD is less than 6 mg/L. Standard dilution technique should be applied to samples with DO depletion greater than 6 mg/L. The analyst has to decide what dilution should be set for determination of BOD. In most instances, three dilutions will be sufficient to cover the possible range of a sample with unknown strength.

The BOD value is not affected by oxygen concentrations greater than 0.5 mg/L. Dilutions that produce a depletion of oxygen less than 2 mg/L should not be used. Hence, it is customary to base calculations of BOD on samples that produce a depletion of at least 2 mg/L and have at least 0.5mg/L of dissolved oxygen remaining at the end of the incubation period. This restriction usually means a DO range of 2 to 6 mg/L.

Table presents suitable dilutions prepared by direct pipetting into bottles of about 300 mL capacity. It is customary to estimate the BOD of a sample and set one dilution based upon the estimate. Two other dilutions, one higher and one lower, are also set up. For example, a sample is estimated to have a BOD of 1000 mg/L. From Table 2, a 0.5% mixture should be used. If a 0.2 and a 1.0% mixture are included, the range of measurable BOD is extended from 200 to 3000 mg/L and should compensate for any errors in the original estimate.

Table BOD measurable with various dilutions of samples.

Using percent mixtures		By direct pipetting into 300 mL bottles	
% mixture	Range of BOD (mg/L)	mL	Range of BOD (mg/L)
0.01	20,000 - 60,000	0.02	30,000 - 90,000
0.02	10,000 - 30,000	0.05	12,000 - 36,000
0.05	4,000 - 12,000	0.10	6,000 - 18,000
0.1	2,000 - 6,000	0.20	3,000 - 9,000
0.2	1,000 - 3,000	0.50	1,200 - 3,600
0.5	400 - 1,200	1.0	600 - 1,800
1.0	200 – 600	2.0	300 - 900
2.0	100 - 300	5.0	120 - 360
5.0	40 – 120	10.0	60 - 180
10.0	20 - 60	20.0	30 - 90
20.0	10 - 30	50.0	12 - 36
50.0	4 - 12	100	6 – 18

100.0	0 - 6	300	0 - 6

In the direct-pipetting technique, preliminary dilutions should be made of all samples that require less than 0.5 mL of the sample, so that amounts added to the bottles can be measured without serious error. The volumes of all bottles must be known in order to allow calculation of the BOD when this method is used.

Procedure for Standard Dilution Technique

(i) Prepare dilution water by adding the following per litre of required dilution water, then aerate to oxygen saturation (approx. 1 hour),
- 1 mL phosphate buffer,
- 1 mL magnesium sulfate solution,
- 1 mL calcium chloride solution,
- 1 mL ferric chloride solution,
- 2 mL of settled raw sewage seed.

(ii) Set up three seeded dilution water blanks. Always siphon dilution water into BOD bottles to avoid entrapping air bubbles.
Note, BOD5 of seeded dilution water should range between 0.6~1.0 mg/L.

(i) Prepare three dilutions for each sample.

(ii) Measure the initial DO of each diluted sample and blank using a calibrated DO probe.

(iii) Incubate blanks, the remaining samples at 20o C for five days.

(iv) After five days incubation, measure DO in each bottle by DO probe, and calculate BOD5 as follows:

$$BOD_5 \text{ as } mgO_2 / L = \frac{(D_1 - D_2) - (B_1 - B_2)f}{P}$$

where: D1 = initial DO of sample, mg/L

D2 = Final DO of incubated sample after 5 days, mg/L

B1 = DO of seed control before incubation, mg/L

B2 = DO of seed control after incubation, mg/L

P = Decimal volumetric fraction of sample used

Note: Only consider dilutions where:

(1) depletion is $\geq$ 2.0 mg/L, and

(2) final DO ≥ 1.0 mg/L.

If more than one dilution satisfies (1) and (2) above, selects dilution with greatest DO depletion.

Chemical Oxygen Demand (COD)

Chemical oxygen demand (COD) is another parameter used widely to measure the pollutional strength of domestic and industrial wastewaters. COD is defined as the amount of oxygen required to oxidize organic matter chemically. Potassium dichromate (K2Cr2O7) is generally chosen for this purpose due to its strong chemical oxidizing capability. Almost all organic compounds (except for ammonia, aromatic hydrocarbons, pyridine and their related compounds) can be oxidized by dichromate under heated acidic and AgSO4-catalysed conditions, equivalent to 95 – 100% of the theoretical values. 2 One of the main limitations of the COD test is its inability to differentiate between biologically oxidizable and biologically inert organic matter. Nor can it provide any evidence of the biological decomposition rate that proceeds either in natural or man-made conditions. The major advantage of COD test is the short time required for evaluation. The determination can be made in about 3 hr rather than the usual 5 days required for the measurement of BOD.

In the test of COD, potassium dichromate is used to oxidize the organic matter. In order for the potassium dichromate to oxidize organic matter completely, the solution must be strongly acidic and is refluxed with a silver catalyst at an elevated temperature.

Chemical Oxygen Demand (using the closed reflux colorimetric method)

Preparation of Samples

(i) Turn on the COD reactor. Preheat to 150o C.
(ii) Remove the cap of a COD digestion reagent vial (Vial type 0-1500 mg/L). Hold the vial at a 45o angle. Pipette 2 mL of sample into the vial. For greater accuracy, a minimum of three replicates per sample should be analyzed and the results averaged.
(iii) Close the vial cap tightly. Hold the vial by the cap and invert gently several times to mix the contents.
(iv) Place the blank and sample vials in the preheated COD reactor. Heat the vials for 1 hour.
(v) After 1 hour, turn the reactor off. Invert each vial several times while still warm. Place the vials into a rack and cool for 1 hour at the room temperature.

Sample measurement

(i) Switch on visible spectrophotometer and set wavelength to 600nm. Enter the value for “K factor” to 2941. The constant K factor is derived from the standard calibration curve.
(ii) Pour blank into the glass cell and place it in the sample compartment. Press “Autozero” key to set the zero absorbance. (iii)

(iii) Discard blank and place the cell containing the digested sample in the measuring position. Close sample compartment lid and press “Start” key to measure the absorbance. The result will show the direct COD concentration reading of sample measured as mg/L or ppm.