2.1 INTRODUCTION, SCOPE AND PRINCIPLE OF METHOD

The $B.O.D._5$ test is defined as " the mass of dissolved oxygen required by a specific volume of liquid for the process of biochemical oxidation, under prescribed conditions, over 5 days at 20^o C in the dark" (DoE, 1983). The result is expressed as milligrams of oxygen per litre of sample.

Biochemical Oxygen Demand ($B.O.D._5$) is a standard quality test for the presence of natural or introduced organic matter, including pollutants, in waters and effluents. B.O.D. is a test in which standardised laboratory procedures are used to determine the oxygen requirements of bacteria and other micro-organisms which, in the presence of oxygen, cause the biodegradation of organic matter (carbonaceous oxygen demand). The test will also measure the oxygen used to oxidise inorganic material such as sulphides and ferrous iron where these are present. It may also measure the oxygen used to oxidise reduced forms of nitrogen, unless an inhibitor reduces their oxidation.

A 250 to 300ml sample of water or waste water (diluted, as appropriate) is incubated at 20^o C in the dark for 5 days. Sufficient micro-organisms and nutrients must be present in the sample or diluted sample to allow for the biodegradation of the organic matter present; hence for some samples, the dilution water will need to be seeded with micro-organisms. The dissolved oxygen in the sample is measured at the beginning and end of the test period. The reduction in D.O. during the incubation period yields a measure of the B.O.D. Dissolved oxygen must be present throughout the test with a recommended minimum of 2mg/l Oxygen remaining after 5 days, samples with a BOD greater than 7mg/l must be diluted using dilution water.

2.1.1 Type of Application: This method is applicable for determining B.O.D. in clean and polluted surface waters, and in municipal, agricultural and industrial effluents.

2.2 SAMPLE STORAGE AND PRESERVATION

Analyse within 24 hours of sampling. Samples may be preserved for up to 48 hours by cooling to 2-4 ^{o} C. in the dark, (refrigerated).

2.3 EQUIPMENT

a) Glass BOD bottles 250 – 300 ml and stoppers
b) B.O.D. stopper P.V.C. covers or tin foil or *parafilm*.
c) Calibrated dissolved 0xygen meter and probe with stirrer.
d) Incubator set to 20^{o} C $\pm$ 1 ^{o}C.
e) 1000ml graduated cylinder and other assorted glassware.
f) Air pumps with air stones attached to the polyethylene tubing.
g) 25 litre or 50 litre P.V.C. graduated container with tap for dilution water
h) Thermometer
i) Auto pipette 0 to 5 mls or disposable syringes 0-1 ml, 0-5 ml & 0-10 ml.
j) Disposable gloves.
k) One Large Black Bin Bag.

2.4 REAGENTS

a) Phosphate Buffer solution – purchased from an Approved Vendor, (E.g-Reagecon, product no. WTR5005)
b) Magnesium Sulphate solution 0.09M – purchased from an Approved Vendor (E.g.- Reagecon product no. MS0091).
c) Calcium Chloride solution 0.01M – purchased from an Approved Vendor (E.g.- Reagecon, product no. CA20011)
d) Ferric Chloride Solution 0.001M – purchased from an Approved Vendor, (E.g-Reagecon, product no. F00011)
e) Dilution Water – Mains water is distilled into a PVC container no. 1. The distilled water is drawn from PVC Container no. 1 to PVC graduated container no. 2. Nutrients are added to the distilled water in no. 2. The container (no. 2) is then agitated, using the airpump, by immersing the ends of the PVC tubing, with the airstones attached, into the distilled water and agitate for 15mins. When required, add nitrification inhibitor (see 2.4.f) and/or seed material (see 2.4.h) after adding the nutrients and then proceed to agitate for 15 mins. The container of dilution water is usually made up 24 hours before it is required, the container covered with a black plastic bag and left on the lab. bench overnight. On occasion, the dilution water is used one hour after being made up.
f) Nitrification Inhibitor, purchased from an Approved Vendor (E.g.-Hach product purchased from Celtic Engineering – Formula 2533, Cat. No. 2533-34) – Use 0.16g per 300ml sample, 5.3g per 10 litres dilution water, 8g per 15 litres dilution water, or 10.6 g per 20 litres dilution water.
g) Glucose Glutamic Acid Solution – This may be produced by two methods.

(1) Vials: This is purchased from an Approved Vendor (E.g.- a Hach product, ex Celtic Engineering, Cat. No. 14865-10). The vial is broken and the contents added to dilution water (see procedure section below)

(2) The solution may be prepared directly from Glucose (BDH Analar) and Glutamic acid (Merck for biochemistry) powder. These materials must be dried prior to use by placing a suitable quantity of both materials into beakers and drying at 103^{o} C for one hour. The beakers and their contents are then allowed to cool in the desiccators. Add 150mg of each to a 1 litre volumetric flask and make up to the mark with deionised water. Place magnetic stirrer into volumetric flask. Place flask onto magnetic stirring plate. Stir well for one hour.

h) Seed material – either from the outlet of an activated sludge sewage treatment plant or polyseed capsules. Empty one polyseed capsule into 500mls of dilution water. Aerate solution for 30mins or stir with magnetic stirrer. For best results, the Polyseed should be used within 6 hours of re-hydration. When using the outlet of an activated sludge treatment plant, add either 0.2ml of the outlet sample to 800ml of dilution water in the graduated cylinder or 1.25 ml of the outlet sample to 5 litres of dilution water.

i) Sodium Thiosulphite granules

j) Alkali and Acid solutions 1N for neutralisation of waste samples when required. $\underline{H_2SO_4}$ $\underline{1N}$ - add slowly, while stirring, 28mls conc. Sulphuric acid to distilled water in a volumetric flask and dilute to 1 Litre. $\underline{NaOH\ 1N}$ – Dissolve 40g NaOH in distilled water and dilute to 1 litre in a volumetric flask. Alternatively the 1N acid and alkali solutions may be purchased.

2.5 PROCEDURE

2.5.1 Pre-treatment

a) pH Adjustment – if the pH of the sample is outside the range 6.5-8.5, neutralize to pH 6.5-7.5 with a solution of H_2SO_4 or NaOH of such strength that the quantity of reagent used does not dilute the sample by more than 0.5%.

b) Removal of Residual Chlorine – Residual chlorine may dissipate by standing the sample in the light for one to two hours. If the residual chlorine does not dissipate on standing, add about 5-6 granules of Sodium Thiosulphate to the B.O.D. bottle containing the sample. Dilution water used for testing chlorinated/de-chlorinated samples must be seeded, or the samples must be seeded directly into the BOD bottle.

c) Saline samples – Add about 5-6 granules of Sodium Thiosulfate to each B.O.D. bottle, record salinity and, when possible, adjust D.O. meter for the salinity.

d) Removal of Algae – Remove algae by filtration or centrifugation. Report the results as BOD (filtered) or BOD (centrifuged).
e) Samples saturated with Dissolved Oxygen – Samples containing more than 9 mg/l D.O. at 20° C may be reduced to saturation by vigorous shaking/agitation in a partially filled corked sample bottle.

2.5.2 Calibration

a) Turn on Dissolved Oxygen Meter (YSI).
b) Wait 15 minutes.
c) Press Calibrate
d) Press D.O. Cal.
e) Press up or down as necessary to achieve 100%
f) Press enter
g) Press Mode – calibration is now complete.

2.5.3 Test Procedure

a) Check that samples are at 20° C ± 1° C
b) Check that the pH is within required range.
c) Place BOD bottles in a row on the bench in front of the DO Meter
d) Mark each sample identification log number on each bottle; also mark dilution on each BOD bottle.
e) Fill two BOD bottles with dilution water from each batch and call Blank 1 & Blank 2. When batches of dilution water are seeded, blanks are put on of each batch.
f) Mark one BOD bottle A/C. Pour dilution water into a 1000ml graduated cylinder to 990mls. Break **acid check** vial and pour into cylinder.
g) Place gloved hand, (clean disposable plastic glove), on top of cylinder and gently rock the cylinder from side to side about six times until mixing occurs without allowing air entrapment. The sample is then poured into a B.O.D. bottle.
h) or, use pipettor/ syringe and put 10mls of glucose glutamic **acid** standard (as made up in volumetric flask – see 2.4.g) into cylinder.
i) When samples do not require dilution, pour sample into BOD bottle gently without allowing air bubbles to form. Samples with an expected BOD of <7 do not require dilution.
j) For dilution purposes, the BOD's of effluents are estimated at 0.7 times the C.O.D. demand and dilutions are prepared accordingly. C.O.D. results are not often available when doing large numbers of B.O.D's, so through experience the analyst learns what dilutions to give the various effluents. The relevant

amount of dilution water is poured into a 1000ml graduated cylinder, the required amount of sample is added using a pipettor or syringe, e.g., when doing a 1/100 dilution pour the dilution water into the graduated cylinder to 990 mls, then using a pipettor or syringe add 10mls of the sample. Place gloved (clean disposable plastic glove) hand on top of cylinder and gently rock the cylinder from side to side about six times until mixing occurs without allowing air entrapment. The sample is then poured into a B.O.D. bottle.

k) Ensure the D.O. meter is calibrated as per 2.6.1. The D.O. probe is placed into each B.O.D. bottle and the D.O. is recorded.
l) The B.O.D. bottles are placed in the incubator, which is set to 20^{o} C.
m) After 5 days, the B.O.D. bottles are removed from the incubator.
n) The D.O. meter is calibrated and the D.O. level of each sample, in each bottle, is measured.

2.6 REPORTING OF RESULTS

a) The D.O. reading on day 1 minus the D.O. reading on day 5 gives the B.O.D. in mg/l
b) For diluted samples, the difference in the D.O. readings, minus the blank, multiplied by the dilution factor gives the B.O.D. in mg/l. Where samples had been seeded, subtract the seeded blank value before multiplying by the dilution factor.

2.7 PRECISION

As this test is a biological test, there are many parameters, which are outside the analyst's control. The glucose/glutamic acid standard should give a result of 198mg/l B.O.D. $\pm$ 30.5. The results of this test are plotted on a Control Chart in the Lab.

2.8 TROUBLESHOOTING

In the event that DO readings are lower than expected, or results for blanks differ significantly from the initial readings, it is imperative to check the physical condition of the membrane cap and to change the tip if there is any concern about its reliability. The stirrer paddle must be removed prior to unscrewing the old membrane cap. The probe tip should then be rinsed with deionised water to remove any contaminants. The new membrane cap is half filled with electrolyte solution, (KCl – purchased), prior to screwing on to the probe, (moderately tight), and a small amount of electrolyte should overflow. This excess is rinsed off with DI water and the paddle re-fitted. Ensure that the membrane is not wrinkled or torn and does not contain air bubbles >1/8". The average replacement interval is 1-2 weeks, depending on use.
A complete troubleshooting guide may be found in the Probe Instruction Manual.

2.9 REFERENCES

Standard Methods for the examination of water and wastewater, 19th edition 1995.
YSI 5905/5010 BOD Probe Instruction Manual
YSI

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