Technical Note

BOD Testing (Biological Oxygen Demand)

Introduction

BOD is an **empirical** test (ex APHA) which measures

- The molecular oxygen used by bacteria for the biochemical degradation of carbonaceous material (hence carbonaceous BOD or CBOD)
- The oxygen used to oxidise inorganic material such as sulphides and ferrous ion.
- The oxygen used to oxidise reduced forms of nitrogen (nitrogenous BOD, NBOD) such as ammonia and organic nitrogen. This may be prevented by the use of an inhibitor (ATU) during incubation.

A BOD test is often used in conjunction with a Chemical Oxygen Demand (COD) test to provide an indication of the oxygen demand, usually in a waste stream, effluent or surface water such as a river. If the oxygen demand is too high, all the oxygen in the water will be used up and living organisms will die. Note that the COD should always be greater than or equal to the BOD. The ratio of BOD/COD is reasonably constant for a given source.

The BOD and COD tests are often supplemented, and BOD is sometimes being replaced by, a Total Organic Carbon (TOC) test. Because TOC is an instrumental method, as compared with BOD which relies on bacterial activity, it is more reproducible and can also be done more quickly if necessary ie. BOD takes at least 5 days.

An example of data from an Interlaboratory Comparison Programme (ILCP) is shown in the table. Note that the COD test is much more precise than BOD testing, as well as providing results in a shorter timeframe. There is not yet sufficient data on TOC ILCP to draw any firm conclusions.

BOD5		**Sample A [Meat effluent]**	**Sample B [Municipal effluent]**
	# Labs	28	28
	Mean	472	4.62
	Std Dev	91	3.75
	CV	19	81
COD			
	# Labs	23	23
	Mean	2043	22.0
	Std Dev	157	8.7
	CV	8	40
TOC		(example only as few labs do this yet)	
	# Labs	2	2
	Mean	624	6.0
	Std Dev	23	1.7
	CV	4	28

Biochemical Oxygen Demand (BOD) is a test which requires a sample to have its dissolved oxygen (DO) content measured, be incubated under controlled conditions (20°C) for 5 days (hence BOD_5) and then the DO is measured again. The drop in DO is the measure of BOD_5.

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The maximum concentration of DO in water at 20°C is about 7 - 9 mg/L (depending on barometric pressure and sample matrix). APHA specifies a minimum oxygen **depletion** of 2 mg/L, which effectively sets the lower limit of detection (ie. at 2 mg/L), although many laboratories report lower. We accept results with O_2 use of 1 mg/L or greater. Our Detection limit is 1 mg/L for BOD_5 and 0.4 mg/L for $TBOD_5$.

Bacteria

The biochemical processes are carried out by bacteria. These may be present in the sample naturally or are often '**seeded'** into the test bottle by the addition of diluted secondary treated effluent or similar. Seeding is done to ensure there are sufficient numbers of bacteria to digest the organic matter during the test period.

Note that BOD tests carried out using 'seeds' may give different results to tests using a bacterial source from the same site as the sample. This is because the sample site bacteria will be habituated to the chemicals in the sample and may have developed site-specific distributions of different bacteria which selectively use the chemicals present.

Bacterial action may also be affected by the sample matrix (**Toxicity**) and this must be overcome by pre-treatment or dilution of the sample to reduce the toxic effect. It is essential that the laboratory is informed if it is known that any of these may be present in the samples.

- extremes of pH
- sulphides eg from tanneries, paper plants, mining areas
- heavy metals eg from plating waste input
- chlorine from water treatment, etc
- peroxide (which may cause DO to increase not decrease)

Sample bottles and transport

Water samples should be collected into clean plastic containers. A minimum of 500 mL is required.

Samples must be kept cold, not frozen, and delivered to the laboratory as soon as possible after collection. Testing should be started within 48 (preferably 24, refer APHA) hours from sample collection.

The Laboratory Test Process

Our standard practice for samples of unknown origin, when we have no idea of probable levels of BOD, is as follows. Note that samples are diluted with water and a nitrification inhibitor (ATU) added to each bottle, so this test gives results for $CBOD_5$.

The pH of the sample is measured and adjusted to 6.5 - 7.5 if necessary. A series of test bottles is set up with at least three different dilutions of the sample (eg relatively clean samples are diluted 1:2, 1:10, 1:50).

The sample is seeded using a municipal wastewater treatment plant (Hamilton) bacterial source, then saturated with air by shaking. The dissolved oxygen level in each bottle is measured.

The samples are incubated (20±1°C, 5 days) and the DO measured again.

The CBOD5 is calculated and reported.

Immediately after the samples are first prepared, any remaining sample is frozen. If a repeat analysis needs to be done from a frozen sample, it is seeded and ATU added to inhibit any NBOD from the seeding solution. Repeat analyses are mainly carried out because of over dilution or insufficient dilution of the sample originally, but are sometimes required because of equipment failure (DO probe or incubator), because replicates or QCs are unacceptable, or because the sample contains a toxic factor.

If we carry out an analysis using a frozen sample this is always mentioned in the laboratory report.

Total BOD (TBOD)

Samples with very low BOD (less than 7 mg/L), such as clean unpolluted receiving waters, can be analysed 'undiluted'. This means that no nitrification inhibitor is added and so the Total (ie carbonaceous + nitrogenous) BOD is reported. These samples are not seeded.

If a repeat is done from a frozen sample, it is seeded to ensure there is sufficient microbiological population to break down the 'organic matter'.

In these types of samples there is usually minimal contribution of NBOD and so the TBOD = CBOD (but see the next section about recommended determination of Total BOD).

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Nitrogenous BOD

National Water and Soil Conservation Organisation (NAWSCO), "Water and Soil Miscellaneous Publication No. 38", 1982 states;

Ammonia exerts a nitrogenous oxygen demand according to the reaction;

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O$$

This reaction, carried out by certain bacteria, is regarded as being complete under real (field) conditions, but will proceed to varying stages under conditions of the BOD test. It is recommended then that such nitrogeneous oxygen demand be completely inhibited under test conditions and NOD calculated from the measured NH4-N concentration in the sample.

The factor for the conversion of NH4-N to oxygen demand from the stoichiometry of the above equation is 64/14 = 4.57 but confirmatory experiments yield a figure 4.33, so it is recommended that Nitrogenous Oxygen Demand (NOD) be calculated as

g/m³ NOD = 4.33 x [NH4-N]

then

Total BOD_5 = $CBOD_5$ + NOD
This is the preferred method for determining $TBOD_5$

Reporting

Note: Some laboratories simply report “BOD” results. This often means CBOD5, and the laboratory should be requested to clarify whether an inhibitor was used and if the results are for CBOD5 or TBOD5 if this is not clear from the report.

Approximately half the laboratories in New Zealand default to CBOD5, and half to TBOD5, if only “BOD” is requested. Please ensure requests for this test are sufficiently specific.

References

1. Method 5210 B, 5-Day BOD Test, “Standard Methods for the Analysis of Water and Wastewater”, APHA (Online Edition)
2. US EPA Method 405.1, “Biochemical Oxygen Demand (5 days, 20°C)” NB: for the method this refers to APHA.
3. National Water and Soil Conservation Organisation (NAWSCO), "Water and Soil Miscellaneous Publication No. 38", 1982
4. http://en.wikipedia.org/wiki/Biochemical_oxygen_demand

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