BOD_5

(J. Kinross, March 2003: adapted from Standard Methods.)

This test was devised to ensure that organic wastes discharged to rivers would not seriously deplete the river of oxygen before discharging into the sea (on the basis that no river in the UK has a residence time of more than 5 days). The BOD_5 test is therefore a worst-case scenario. In practical terms, BOD_5 is defined as the amount of oxygen removed from solution by biological activity over 5 days incubation at 20°C in the dark. Dissolved oxygen (DO) can be measured titimetrically by the Winkler method, or using an oxygen electrode.

The constraints are:

a) The solubility of O2 from air in pure water is given by the equation:
$C_s = 475/(33.5 + t)$, Where C_s is the solubility of O_2 in mg l^{-1}; t is the temperature of water (°C)
Thus the higher the temperature, the lower the solubility. At 6°C Cs = 12 mgl^{-1} and at 20°C, Cs = 8.88mgl^{-1}.
The concentrations measured normally show deviations (lower values) from theoretically values, as natural waters are not pure and dissolved electrolytes affect solubility of O_2.

b) O_2 must be detectable in the sample at the end of the incubation to ensure the correct result, therefore the BOD_5 in each sample bottle must be less than about 7mg/l: dilution is necessary to ensure this

c) the microorganisms necessary to break down the organic material may not be present in the sample, though they could be in the receiving water. It is therefore necessary to seed the sample, either with a suspension of organisms from a sewage treatment works treating sewage of an appropriate nature (similar to the sample), or a standardized preserved inoculum (eg Polyseed).

d) the sample, or more likely the pure water used for dilution, may not contain adequate inorganic nutrients for the microbes to fully utilize the organics in the time. It is usual to supplement the dilution water.

e) Some wastes may be toxic or inhibitory: it is necessary to include positive controls to ensure that the seed is viable in case no uptake is found. A mixture of glucose and glutamic acid is used as a standard.

f) the pH of some wastes may need to be adjusted to 6.5-7.5, and samples containing chlorine may need to be treated by adding sodium sulphite.

g) oxidation of reduced forms of N eg ammonia, consumes oxygen also: if it is desired to exclude this uptake, you need to add a nitrification inhibitor (eg allylthiourea, 2-chloro-6-(trichloro methyl)pyridine)

A rough guide to dilutions:	Dilute 1 in:
River water	2, 4
STP effluent	2, 10
crude sewage	20, 50
settled sewage	15, 30
Industrial effluents	a range is necessary due to widely varying strengths and natures

Stock solutions for dilution water:				per litre:	per 100ml:	use rate:
A	phosphate buffer	:	KH_2PO_4	8.5g	0.85g	1ml/l
			K_2HPO_4	21.75g	2.175	
			$Na_2HPO_4.7H_2O$	33.40g	3.34g	
			NH_4Cl	1.70g	0.17g	
B	Magnesium sulphate		$MgSO_4.7H_2O$	22.50g	2.25g	1ml/l
C	Calcium chloride		$CaCl_2$	27.50g	2.75g	1ml/l
D	Ferric chloride		$FeCl_3.6H_2O$	0.25g	0.025g	1ml/l
E	Glucose-glutamic acid	Glucose		150mg	15mg	2% solution
			Glutamic acid	150mg		
	prepare fresh on day of use					

Preparation of dilution water:

Add solutions A-D to a suitable volume of water at 1ml/l. Place in 20°C incubator and aerate by means of an air pump and airstone.

Regenerate seed at the recommended rate: for Polyseed this is one pellet in 500ml : this stock is the used at the rate of 2ml per bottle.

BOD bottles: 250-300 ml glass bottles with ground openings taking glass or polythene stoppers: glass stoppers tend to leak air slightly more than polythene. Leakage can be prevented by inverting the bottles in a waterbath. At least 2 replicates (preferably 3) of each treatment need to be prepared. Label the bottles at the bottom, or the labels will come off in the water.

Controls:

1.	Dilution water control	fill one set of replicate bottles with the dilution water <u>without</u> seeding
2	Seed control	fill one set of bottles with the dilution water, seeding at different rates, eg 10, 15, 20, 25 ml <u>per bottle</u>
3	Standard	dilute the glucose-glutamic acid stock in the dilution water at the rate of 2% (20ml/l): fill one set of bottles and seed at 2ml/bottle

Treatment of samples: prepare dilutions in the dilution water and fill one set of bottles at each dilution: seed at 2ml/bottle.

Initial DO reading:

Make sure the O_2 probe on the YSI 59 has a working membrane fitted: the membrane and electrolyte require replacement every 2-4 weeks depending on the level of use. Switch on and allow to stabilize. Calibrate in water-saturated air at 20°C (see below). The probe has a self-contained stirrer and is small enough to be inserted into a BOD bottle. Insert into each bottle in turn, switch on the stirrer and take the DO reading when it stabilises. Switch off and remove the probe, top up with extra sample or dilution water and insert the stopper, excluding any bubbles of air. Place the bottles upside-down in a basin of water and place in a dark, 20°C incubator. Basins of water need to be covered to prevent evaporation and consequent cooling of the water and bottles - use another basin over the top or clingfilm. (bubbles appearing after incubation are more likely due to evolution of gas than leakage, if the above precautions are taken)
Incubate for 5 days, then repeat the readings. For each sample, use data from dilutions giving a residual DO reading of >1mg/l and an uptake of >2mg/l.

Calculations:

1 subtract the mean dilution water blank value from all other readings
2 calculate the DO usage of 2ml of seed as a proportion of one of the seed controls (eg as 10% of the 20ml control), and subtract this from all sample readings, and the glucose-glutamic acid ones.
3 the resulting values are the BOD of the sample dilutions: multiply by the dilution ratio used to obtain the BOD of the samples

quality controls, etc.: Blanks (dilution water only) should give < 0.1 mg/l to 0.2 mg/l maximum BOD
Standard glucose-glutamic acid (2% of stock) should give approximately 4 mg/l BOD
Samples should be read at a dilution which gives at least 2mg/l O_2 uptake in 5 days, and > 1 mg/l final DO

[see also: Logan, BE and Patnaik, R. 1997. A gas chromatographic-based headspace biochemical oxygen demand test. Water Environment Research. 69(2). 206-214]

Calibration of YSI 59 DO meter:
To calibrate in water-saturated air (recommended), place probe in a BOD bottle containing ~2cm water, within the BOD incubator if possible.
Obtain the current atmospheric pressure from a barometer, and read off the calibration value (%) against this on the back of the instrument.
Switch on and allow reading to stabilise. Turn knob to 'CALIBRATE'. Confirm 'calibrate in percent?'
Readout will say 'Enter cal value Last=116%'. Press 'Skip'. Reading will now say '100%'. Use the up or down arrows to set to the calibration value you want. Press 'CONFIRM'. Return the knob to 'O_2-TEMP'. Instrument is ready for use.