Biochemical Oxygen Demand 1981

Methods for the Examination of Waters and Associated Materials

This booklet contains full instructions for the basic BOD_5 test and for the ATU* nitrification suppression variant. It also contains a detailed discussion of the test, the problems that can occur and possible variants on the methods and their use.

*Allyl thiourea

Contents

London Her Majesty's Stationery Office

Warning to users

The analytical procedures given in this booklet should only be carried out by competent trained persons, with adequate supervision when necessary. Local Safety Regulations must be observed. Laboratory procedures should be carried out only in properly equipped laboratories. Field operations should be conducted with due regard to possible local hazards, and portable safety equipment should be carried. Care should be taken against creating hazards. Lone working, whether in the laboratory or field, should be discouraged. Reagents of adequate purity must be used, along with properly maintained apparatus and equipment of correct specifications. Specifications for reagents, apparatus and equipment are given in manufacturers' catalogues and various published standards. If contamination is suspected, reagent purity should be checked before use.

There are numerous handbooks on first aid and laboratory safety. Among such publications are: 'Code of Practice for Chemical Laboratories' and 'Hazards in the Chemical Laboratory' issued by the Royal Society of Chemistry, London; 'Safety in Biological Laboratories' (Editors Hartree and Booth), Biochemical Society Special Publication No 5, The Biochemical Society, London, which includes biological hazards; and 'The Prevention of Laboratory Acquired Infection,' Public Health Laboratory Service Monograph 6, HMSO, London.

Where the Committee have considered that a special unusual hazard exists, attention has been drawn to this in the text so that additional care might be taken beyond that which should be exercised at all times when carrying out analytical procedures. It cannot be too strongly emphasised that prompt first aid, decontamination, or administration of the correct antidote can save life; but that incorrect treatment can make matters worse. It is suggested that both supervisors and operators be familiar with emergency procedures before starting even a slightly hazardous operation, and that doctors consulted after any accident involving chemical contamination, ingestion, or inhalation, be made familiar with the chemical nature of the injury, as some chemical injuries require specialist treatment not normally encountered by most doctors. Similar warning should be given if a biological or radio-chemical injury is suspected. Some very unusual parasites, viruses and other micro-organisms are occasionally encountered in samples and when sampling in the field. In the latter case, all equipment including footwear should be disinfected by appropriate methods if contamination is suspected.

The best safeguard is a thorough consideration of hazards and the consequent safety precautions and remedies well in advance. Without intending to give a complete checklist, points that experience has shown are often forgotten include: laboratory tidiness, stray radiation leaks (including ultra violet), use of correct protective clothing and goggles, removal of toxic fumes and wastes, containment in the event of breakage, access to taps, escape routes, and the accessibility of the correct and properly maintained first-aid, fire-fighting, and rescue equipment. If in doubt, it is safer to assume that the hazard may exist and take reasonable precautions, rather than to assume that no hazard exists until proved otherwise.

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About this series

This booklet is part of a series intended to provide recommended methods for the determination of water quality. In addition, the series contains short reviews of the more important analytical techniques of interest to the water and sewage industries. In the past, the Department of the Environment and its predecessors, in collaboration with various learned societies, have issued volumes of methods for the analysis of water and sewage culminating in 'Analysis of Raw, Potable and Waste Waters'. These volumes inevitably took some years to prepare, so that they were often partially out of date before they appeared in print. The present series will be published as individual methods, thus allowing for the replacement or addition of methods as quickly as possible without need of waiting for the next edition. The rate of publication will also be related to the urgency of requirement for that particular method, tentative methods being issued when necessary. The aim is to provide as complete and up to date a collection of methods and reviews as is practicable, which will, as far as possible, take into account the analytical facilities available in different parts of the Kingdom, and the quality criteria of interest to those responsible for the various aspects of the water cycle. Because both needs and equipment vary widely, where necessary, a selection of methods may be recommended for a single determinand. It will be the responsibility of the users — the senior analytical chemist, biologist, bacteriologist etc, to decide which of these methods to use for the determination in hand. Whilst the attention of the user is drawn to any special known hazards which may occur with the use of any particular method, responsibility for proper supervision and the provision of safe working conditions must remain with the user.

The preparation of this series and its continuous revision is the responsibility of the Standing Committee of Analysts (to review Standard Methods for Quality Control of the Water Cycle). The Standing Committee of Analysts is one of the joint technical committees of the Department of the Environment and the National Water Council. It has nine Working Groups, each responsible for one section or aspect of water cycle quality analysis. They are as follows:

1.0 General principles of sampling and accuracy of results
2.0 Instrumentation and on-line analysis*
3.0 Empirical and physical methods
4.0 Metals and metalloids
5.0 General nonmetallic substances
6.0 Organic impurities
7.0 Biological methods
8.0 Sludge and other solids analysis*
9.0 Radiochemical methods.

The actual methods etc are produced by smaller panels of experts in the appropriate field, under the overall supervision of the appropriate working group and the main committee. The names of those associated with this method are listed inside the back cover.

Publication of new or revised methods will be notified to the technical press, whilst a list of Methods in Print is given in the current HMSO Sectional Publication List No 5, and the current status of publication and revision will be given in the biennial reports of the Standing Committee of Analysts.

Whilst an effort is made to prevent errors from occurring in the published text, a few errors have been found in booklets in this series. Correction notes for booklets in this series are given in the Reports of The Standing Committee of Analysts, published by the Department of the Environment but sold by the National Water Council, 1 Queen Anne's Gate, London SW1H 9BT. Should an error be found affecting the operation of a method, the true sense not being obvious, or an error in the printed text be discovered prior to sale, a separate correction note will be issued for inclusion in the booklet.

* These two working groups are in process of being wound up. Their tasks are being redistributed among the other Working Groups.

T A DICK
Chairman

L R PITTWELL
Secretary

25 September 1981

5-Day Biochemical Oxygen Demand (BOD).

1 Performance Characteristics

1.1	Parameter determined	Uptake of dissolved oxygen (mg/l) by the sample during 5 days at 20°C in the dark.
1.2	Type of sample	Natural and waste waters.
1.3	Basis of method	The air-saturated sample diluted and, if appropriate, "seeded" with a suitable source of microorganisms, is incubated in a full container for 5 days at 20°C (in the dark). The amount of oxygen absorbed is determined by means of appropriate dissolved oxygen measurements (volumetric or instrumental) (Reference 1).
1.4	Range of application	Up to 6.0 mg/l (without dilution). All larger values by appropriate dilution.
1.5	Standard deviation	See Tables 1 and 2.
1.6	Limit of detection	See Tables 1 and 2.
1.7	Sensitivity	For an undiluted sample a corrected dissolved oxygen absorbtion of 0.1 mg/l represents a BOD of 0.1 mg/l.
1.8	Bias	Since BOD is not an absolute, predictable quantity the occurence of bias may not readily be identified. Bias may arise as a result of prolonged sample storage or from the presence of interferences (See also Section 9).
1.9	Interference	Free chlorine. Substances toxic to aerobic or to nitrifying bacteria. Ammonia and organic nitrogen compounds may enhance the oxygen uptake by nitrification (see Section 3). An immediate oxygen demand may be exerted by ferrous iron, sulphite, sulphide or aldehydes.
1.10	Time for analysis	5 days for the incubation stage. Dilution of sample and analysis for dissolved oxygen could typically take 10–15 mins.

Table 1 **BOD (Total)**

Standard deviation[1]	Sample	Concentration mg/l	Standard deviation mg/l	Degrees of freedom
	Glucose/glutamic acid standard, nominal concn. 1.1 mg/l	0.95–1.15	0.05–0.44 [2]	8–26
	Glucose/glutamic acid standard, nominal concn. 6.6 mg/l	6.01–6.53	0.13–0.46 [2]	8–26
	Local river water (clean)	1.23–3.23	0.04–0.27 [3]	18
	Local river water (dirty)	2.60–6.42	0.06–0.40 [3]	18
	Trade effluent	348	23.1 [4]	10
	Industrial effluent	1342	53.3 [4]	10
	Chemical plant effluent	2453	67.1 [5]	10
	Methanol condensate	3349	192.7 [5]	10
Limit of detection[1]	0.07–1.21 mg/l (with 18 degrees of freedom) [6]			

(1) The data on the glucose/glutamic acid solutions, the river waters and on limit of detection is from results for the Harmonised Monitoring Scheme of the Department of the Environment using the DOE 1972 (Green Book) method (Reference 20). Better precision and interlaboratory compatability of results than those shown in the table could be expected using this method rather than the Green Book method.

(2) Results are for total standard deviation obtained by 9 laboratories, of which 4 used an electrochemical sensor for dissolved oxygen measurements and 5 used the Winkler titrimetric method. The standards were prepared in seeded dilution water and each result was corrected for a separate blank determination on seeded dilution water.

(3) Results are for within-batch standard deviation obtained by 5 laboratories, of which 1 used an electrochemical sensor for dissolved oxygen measurements and 4 used the Winkler titrimetric method. Samples were of local river waters and were analysed without dilution or seeding and no blank correction was made.

(4) Result is for within-batch standard deviation obtained by 1 laboratory using an elecrochemical sensor for dissolved oxygen measurements.

(5) Result is for within-batch standard deviation obtained by 1 laboratory using the Winkler titrimetric method for dissolved oxygen measurements.

(6) Results are from the within-batch standard deviation of seeded blank determinations obtained by 9 laboratories.

Table 2 **BOD (ATU)**

Standard deviation[1]	Sample	Concentration mg/l	Standard deviation mg/l	Degrees of freedom
	Glucose/glutamic acid standard, nominal concn. 1.1 mg/l	0.88– 1.08	0.06–0.38 [2]	8–26
	Glucose/glutamic acid standard, nominal concn. 6.6 mg/l	5.41– 6.72	0.16–0.74 [2]	8–26
	Local river water (clean)	1.00– 2.06	0.03–0.28 [3]	18
	Local river water (dirty)	2.37– 6.50	0.05–0.44 [3]	18
	Humus tank effluent	4.44	0.30 [4]	6
	Humus tank effluent	5.81	0.30 [4]	10
	Humus tank effluent	13.5	0.61 [4]	10
	Sewage works effluent	51.2	1.73 [4]	6
Limit of detection[1]	0.10–0.66 mg/l (with 18 degrees of freedom) [5]			

(1) The data on the glucose/glutamic acid solutions, the river waters and on limit of detection is from results obtained for the Harmonised Monitoring Scheme of the Department of the Environment using the DOE 1972 (Green Book) method (Reference 20). Better precision and interlaboratory comparability of results than those shown in the table could be expected using this method rather than the Green Book method.

(2) Results are for total standard deviation obtained by 11 laboratories, of which 4 used an electrochemical sensor for dissolved oxygen measurements and 7 used the Winkler titrimetric method. The standards were prepared in seeded dilution water and each result was corrected for a separate blank determination on seeded dilution water.

(3) Results are for within-batch standard deviation obtained by 11 laboratories (clean river water) and 9 laboratories (dirty river water), of which 4(3) used an electrochemical sensor for dissolved oxygen meaurements and 7(6) used the Winkler titrimetric method. Samples were of local river waters and were analysed without dilution or seeding and no blank correction was made.

(4) Result is for within-batch standard deviation obtained by 1 laboratory using an electrochemical sensor for dissolved oxygen measurements.

(5) Results are from the within-batch standard deviation of seeded blank determinations obtained by 11 laboratories.

2 Field of Application

In an aqueous environment biochemical oxidation is brought about by micro-organisms which utilise the available organic matter as sources of carbon and nitrogen while consuming the available oxygen. The action of these micro-organisms is the basis of the self purifying process which occurs when polluting organic matter is discharged to water courses, lakes and the sea. The rate of purification depends on many factors including the ambient temperature, sunlight levels, flow patterns, the nature of the polluting matter and the types of flora and fauna present.

It follows that a standard laboratory test cannot reproduce the complex natural variations found in streams and lakes. The Biochemical Oxygen Demand (BOD) determination described is an empirical test in which the experimental conditions must be carefully followed if valid comparisons of BOD values are to be made. Examples of situations in which the BOD test is useful are:

1 The assessment of the quality of river waters.

2 The determination of oxygen demand of waste waters, effluents and any other polluted waters.

3 The assessment of the effect of discharges to water courses.

4 The design and assessment of performance of sewage works and other waste water treatment plants.

5 As a guide to the biodegradability or treatability of a particular substance or effluent, by comparing the value obtained for its BOD with, for example, its chemical oxygen demand (COD) (Reference 21).

2.1 Definition of BOD

The BOD is defined as the mass of dissolved oxygen required by a specified volume of liquid for the process of biochemical oxidation under prescribed conditions over 5 days at 20°C, in the dark. The result is expressed as milligrams of oxygen per litre of sample.

When allyl thiourea is used to suppress nitrification during the course of the test the result is referred to as BOD (ATU)

The BOD test was orginally envisaged by the Royal Commission on Sewage Disposal (Reference 2) as a means of assessing the rate of biochemical oxidation that would occur in a stream to which a polluting effluent was discharged.

However, predictions of the effects of such a discharge on a stream are by no means straight forward and probably require the consideration of many factors not involved in the determination of the BOD. The laboratory environment does not reproduce the stream conditions particularly as related to temperature, sunlight, and the effects of bottom deposits, suspended matter, rooted plants, nitrification and planktonic algae.

Stabilization in terms of oxygen uptake by a given sample may require a period of incubation longer than the 5 day period, consequently other periods of incubation are sometimes chosen. If periods of incubation other than the standard 5 days are used this MUST be indicated by a suffix denoting the period in days eg. BOD_3, BOD_{20}.

A number of substances will influence the test either by inhibiting the activity of the micro-organisms or by causing an enhanced utilization of oxygen. In the former category are metals, free chlorine, high or low pH, cyanides, phenols, pesticides or any other substance toxic to micro-organisms. In the latter category, algae and nitrifying organisms will give high results. Some algae by dying in the dark and being metabolized by the bacteria present cause an oxygen demand. Nitrifying organisms, by oxidizing ammonia to nitrite and nitrate will cause an oxygen demand.

3 Principle

Biochemical oxidation of organic matter is primarily brought about by action of heterotrophic bacteria (ie. bacteria which utilize the organic matter present both for the production of energy and for growth). These processes can be represented by simplified equations:

1 $$C_x H_y O_z + O_2 \xrightarrow[\text{Energy Liberation}]{\text{Oxidation}} CO_2 + H_2O$$

2 $$C_x H_y O_z + NH_3 + O_2 \xrightarrow{\text{Synthesis}} \text{CELLS} + CO_2 + H_2O$$

This first phase of biochemical oxidation results in cell growth and depletion of the available organic matter, and is followed by a period of slower oxygen uptake usually referred to as endogenous respiration, during which time the cells produce energy by self-oxidation:

3 $$\text{CELLS} + O_2 \xrightarrow[\text{Respiration}]{\text{Endogenous}} CO_2 + H_2O$$

Nitrifying bacteria (Nitrosomonas and Nitrobacter) are usually present in small numbers initially and their numbers increase slowly during the time occupied by the oxidation stages represented by equations 1 and 2. These bacteria utilise CO_2 or bicarbonate for growth and oxidize inorganic nitrogen to nitrite and nitrate to acquire energy thus:

4 $$2NH_3 + 3\,O_2 \xrightarrow{\text{Nitrosomonas}} 2HNO_2 + 2H_2O$$

This step may be suppressed by allyl thiourea (ATU)

5 $$2HNO_2 + O_2 \xrightarrow{\text{Nitrobacter}} 2HNO_3$$

This step is not affected by ATU.

Because of the relatively slow growth rate of nitrifying compared with heterotrophic bacteria, nitrification usually becomes more significant during the later stages of biochemical oxidation in the BOD test although it is in fact occurring throughout.

3.1 Rate of biochemical oxidation

The use of a five day incubation period for the BOD test was an arbitrary choice made on the assumption that a large percentage of the ultimate oxygen demand would be satisfied in 5 days. (Reference 2). The rate of oxygen uptake will depend on a number of factors including type and concentration of micro-organisms present, type of organic material, presence of toxic materials and temperature.

Under the conditions of the BOD test, Phelps (Reference 3) has shown that the early stages of the oxygen uptake process (equations 1 and 2 above) may be represented by a first order reaction:

$$x = L\,(1 - 10^{-kt})$$

where x = BOD mg/l at time t, days

L = Ultimate BOD mg/l (at infinite time)

t = time, days

k = rate constant, day^{-1}

The rate constant may have values in the range $0.05-0.30d^{-1}$ depending on the nature of the organic material present, the temperature and the type and concentration of micro-organisms present.

Figure 1 shows, in a theoretical manner, the influence of k on the rate of oxygen uptake for a fixed value of the 5 day BOD. It can be seen that a sample exhibiting a k value of $0.30d^{-1}$ would have a 5 day BOD very close to the ultimate BOD value, whereas a sample having a k value of $0.08d^{-1}$ might show at 5 days only about half of the ultimate BOD value.

It must be realised that, even for easily oxidized substances, the theoretical BOD value for complete oxidation to CO_2 and water will not be obtained from measurement of the ultimate BOD value since stabilization by biochemical oxidation cannot be equated with complete chemical oxidation (as may often be achieved in the COD test) although the figures may closely relate.

3.2 Nitrification

Nitrification was originally considered not to exert an appreciable oxygen demand within 5 days and this situation is indicated in a theoretical manner in curve 1a of Figure 2. While this is true for many polluted waters it is not universally true (eg. secondary effluents from sewage works may contain significant numbers of nitrifying bacteria as received).

The occurrence of nitrification in such cases can lead to a measurable enhancement of the 5 day BOD value over that due to the carbonaceous matter present and this enhancement cannot be predicted with confidence from a knowledge of the concentration of nitrogenous matter present.

The determination of BOD due to carbonaceous oxidation alone is often desirable in samples where nitrification causes a significant and often variable oxygen uptake during the 5 day incubation period. Nitrification may be suppressed by specifically inhibiting the action of Nitrosomonas by addition of allyl thiourea and Montgomery and Borne (Reference 4) were able to show that this reagent did not influence the carbonaceous BOD value.

3.3 Interpretation of 5 day BOD value

Except for clean river waters, it is usually necessary to dilute test samples before carrying out the BOD measurement so that consumption of available oxygen during the test does not exceed 70% of that initially present. It is often possible, for a given sample, to measure significant BOD values at several dilutions and this is helpful in interpreting the result (see Section 9.3).

It is convenient again to refer to a set of idealized oxygen uptake curves (Figure 2) when discussing the interpretation of 5 day BOD measurements (Reference 5).

Curve 1

This may be considered to be a 'normal' oxygen uptake curve for river waters, domestic waste water or a solution of a readily biodegraded compound with adequate micro-biological population (k value $\sim 0.30d^{-1}$). It is of interest then to describe briefly four other types of oxygen uptake curves which may be commonly encountered and which will influence the value of the BOD measured after 5 days.

Figure 1

Effect of K on BOD (For given value of 5 day BOD)

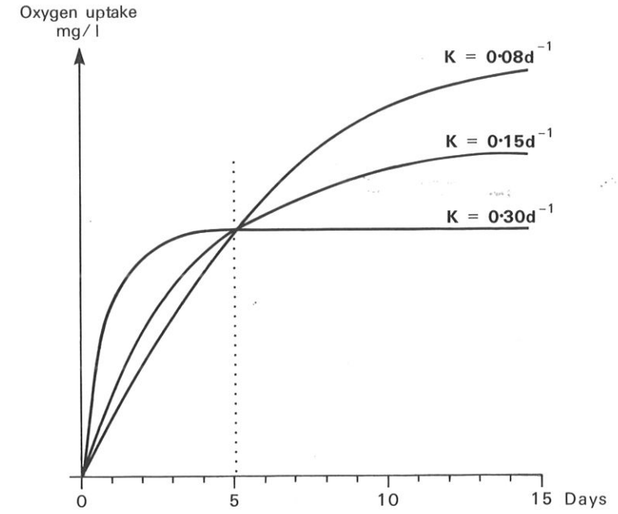
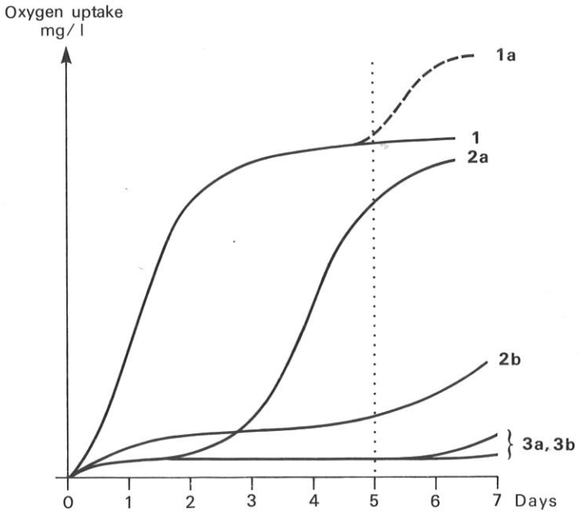


Figure 2

Factors affecting oxygen uptake at constant temperature and carbonaceous substrate concentration



1. Normal BOD curve .
1a. Added influence of nitrification.
2a. Insufficient bacteria leading to delay in oxygen consumption .
2b. Unacclimatised seed leading to delay in oxygen consumption .
3a. Toxic substances may grossly delay or totally inhibit oxygen consumption.
3b. Substances resistant to biodegradation may result in zero or very low oxygen uptake over and above the endogenous respiration rate.

Curve 2a

If the test solution does not contain sufficient micro-organisms initially, the oxygen uptake curve will be of the shape shown, with a slow initial uptake rate, reaching its plateau value at a time much greater than 5 days. In such cases seeding may be desirable (See Section 5.4).

Curve 2b:

If the test solution, or the seed, does not contain organisms already acclimatised to the substances present, the oxygen uptake curve will be as shown, being retarded by the need for acclimatisation and growth of a suitable population of micro-organisms. In such cases it may be desirable to carry out the BOD test using previously acclimatised seed (See Section 5.5).

Curve 3a

If the test solution contains substances toxic to micro-organisms (eg. certain metal ions, bactericides) then the biochemical oxidation may be almost entirely inhibited. In such cases, provided a fairly high organic concentration exists in the sample, it is often found that further increases in dilution actually result in an *increase* in the BOD value measured at 5 days because of the simultaneous dilution of the bactericidal action. Measurements at increasing degrees of dilution may then lead to an apparently constant (asymptotic) value for the 5 day BOD (See Section 9.3).

Curve 3b

If the test solution contains substances which are poorly degradable or even non-biodegradable then uptake curves of this form will be observed (See Section 9.3).

4 Hazards

4.1 This procedure makes use of two methods for the determination of dissolved oxygen which are published in detail in this series (Reference 1).

4.1.1 The titrimetric method involves the use of strong alkali, strong acid and sodium azide solution, all of which are considered to be hazardous. The user should consult Section A4 in Reference 1 for fuller details of these hazards and of the precautions to be taken in practice.

4.1.2 Iodine, used in reagent 5.2.6 should be regarded as a special hazard in the present procedure. The vapour irritates the respiratory system and the eyes; the solid burns the skin. Care must be taken to avoid inhaling the vapours and the solid should be kept out of contact with the skin.

4.2 No specific health hazards have been reported for allyl thiourea, even so, it is suggested that skin contact, inhalation and ingestion be avoided.

4.3 A few harmful species of micro-organisms can occur in natural waters and sediments. Care and cleanliness are therefore essential.

5 Reagents

5.1 Water

To ensure that BOD results are meaningful, and reproducible in different laboratories, it is essential that the water used for sample dilution is of a consistent, uniform, quality and composition. Standard dilution water is prepared by adding specified chemical reagents to good quality distilled or deionized water. These reagents provide osmotic balance, buffer the pH and provide essential nutrients (other than carbon) and trace metals.

Distilled water from copper stills can contain inhibitory concentrations of copper (greater than 0.01 mg/l) and is unsuitable. It is recommended that distillate from an all glass or block tin still be used.

If deionized water is used, regular checks should be made to ensure that a satisfactory blank value is obtained. This is particularly important when using water from either a new or an almost spent column, since resins may introduce, or fail to remove, undesirable organic matter.

Reagent water, whether distilled or deionized, must be free of chlorine, chloramines, caustic alkalinity, acids, ammonia, amines or any other toxic/inhibitory materials.

5.2 Preparation of stock reagent solutions

Analytical reagent grade chemicals should be used to prepare the following stock solutions. These solutions are stable for at least 1 month, unless otherwise indicated, and should be stored in glass in the dark and discarded at the first sign of precipitation or biological growth.

5.2.1 *0.0125% m/V Ferric chloride solution*

Dissolve 0.125 ± 0.005 g ferric chloride hexahydrate in water and dilute with water to 1 litre in a measuring cylinder.

5.2.2 *2.75% m/V Calcium chloride solution*

Dissolve 27.5 ± 0.5 g calcium chloride, (or equivalent if hydrated calcium chloride is used) in water and dilute with water to 1 litre in a measuring cylinder.

5.2.3 *2.5% m/V Magnesium sulphate solution*

Dissolve 25.0 ± 1.0 g magnesium sulphate heptahydrate in water and dilute with water to 1 litre in a measuring cylinder.

5.2.4 *Phosphate buffer solution (pH 7.2)*

Dissolve 42.5 ± 0.5 g potassium dihydrogen phosphate in 700 ± 10 ml water in a beaker and add 8.8 ± 0.1 g sodium hydroxide. Add 2.0 ± 0.1 g ammonium sulphate and when dissolved dilute with water to 1 litre in a calibrated flask.

5.2.5 *0.05% m/V Allyl thiourea solution*

Dissolve 0.50 ± 0.01 g allyl thiourea in water and dilute with water to 1 litre in a measuring cylinder. This solution is stable for at least 2 weeks.

5.2.6 *Wash solution for bottles*

Dissolve 5.0 ± 0.1 g iodine and 25 ± 1 g potassium iodide in 2 ± 0.01 litres of 1% m/V sulphuric acid. Shake to dissolve. Discard when brown colour fades.

5.2.7 *Standard solution of glucose/glutamic acid*

Dissolve 0.150 ± 0.001 g each of d –glucose and 1 –glutamic acid (both previously dried at 105°C for 1 hour) in water and dilute to 1 litre with water in a flask. Prepare freshly each day.

5.3 Preparation of dilution water

Transfer the required volume of freshly distilled or deionized water to a clean vessel which should be specially reserved for the preparation of dilution water. To each 1 litre ± 10 ml of water add 1 ± 0.05 ml of each of the stock reagent solutions, ferric chloride, calcium chloride, magnesium sulphate and phosphate buffer in that order.

Bring the prepared stock of dilution water to incubation temperature (20 ± 0.5°C) and maintain at that temperature. Saturate with oxygen by gently bubbling clean air, free of organic vapour, through the water from the fully immersed sintered glass diffuser (Porosity 4) for 1 hour ± 10 minutes.

If necessary add bacterial seed to the dilution water and use as soon as possible (see Section 3.3). Any unused dilution water should be discarded at the end of each day. Stocks of dilution water should never be 'topped up' with fresh solution. The vessel is cleaned daily by rinsing first with the wash solution and then thoroughly with potable supply water and finally with distilled or deionized water. Store the prepared dilution water out of direct sunlight. The dissolved oxygen concentration of a satisfactory dilution water, when incubated without seed under standard conditions, should not be depleted by more than 0.3 mg/l. Higher values for oxygen depletion may sometimes be caused by the presence of water-soluble organic vapour which may be present in the laboratory atmosphere and is absorbed during the production of distilled or deionized water, or during aeration of the prepared stock of dilution water.

5.4 **Seed**

5.4.1 Samples of raw or treated sewage and most river waters will contain adequate populations of active micro-organisms and additional inoculation of the dilution water should not be necessary.

5.4.2 Some samples, eg. certain industrial effluents, may be sterile, and will, therefore, need seeding with active micro-organisms. For this purpose 5 ml of a good quality settled sewage effluent should be added to each litre of dilution water. If possible the seed should be taken from the final settling tank of a sewage works in which the waste, represented by the sample, is a component of the feed to an aerobic process. If this seed solution is not clear it should be settled before use.

5.5 **Acclimatized seed**

If the BOD of a sample, measured using dilution water seeded as described in Section 5.4.2, is substantially less than its COD, then one or more of the following situations may exist:

(a) the seeding organisms may be of an unsuitable type or may require acclimatization.

(b) the organic matter present may be resistant to biodegradation,

(c) toxic or bacteriostatic substances may be present.

The identification of situations represented by (b) and (c) above is discussed more fully in Section 9.3 and in these cases the tedious preparation of acclimatized seed is generally unlikely to be rewarding. However, if it is considered that situation (a) exists, then acclimatized seed may be prepared as follows.

Adjust the pH of the sample to between 6.5 and 8.5 and dilute to approximately the same COD value as settled sewage. Add the diluted sample to primary settled sewage (see 5.4.2) in the ratio 1 to 9 and combine this mixture with an equal volume of activated sludge (of sewage works origin) to give a final mixed liquor suspended solids concentration of about 2,000 mg/l. Continuously aerate and agitate the mixture with diffused air. After 24 hours allow the sludge to settle and decant the supernatant (50% of total volume). Top up the aeration vessel with freshly prepared sample/sewage mixture and again aerate. Repeat for three days and use the settled supernatant liquor, after the third days' aeration, to seed dilution water as in Section 5.4.2 to determine the BOD of the sample. Continue the procedure, by doubling the proportion of the diluted sample present in the sample/sewage mixture at three day intervals, until a maximum BOD is reached when the seed is used in a subsequent test.

If a laboratory scale, continuously fed, activated sludge unit is available this can be used to produce an acclimatized seed.

6 Apparatus

6.1 Narrow-mouthed clear glass bottles, of nominal 250 ml capacity, should be used as standard. The bottles should have well fitting glass or plastic stoppers.

Plastic stoppers may be used provided tests show that the material is non-biodegradable, and does not interfere chemically with the procedure for determination of dissolved oxygen. All stoppers should be tapered so that they do not trap air bubbles when inserted into filled bottles.

It may be convenient where, for example, incubator space is restricted, to use bottles of a smaller capacity, eg. 125 ml or 175 ml. In such cases, comparative checks should be made to ensure that results are similar to those obtained when using standard bottles.

Cleanliness of the bottles, and indeed of all associated glassware, is of paramount importance. When using the Winkler procedure for determination of dissolved oxygen, cleanliness of the bottles is ensured by the action of the acidic iodine solution and no further treatment, other than rinsing with tap and distilled or deionized water, is normally necessary. However, when using the alternative instrumental procedure, the bottles should be rinsed, before re-use, using 5–10 ml of the wash solution (section 5.2.6) shaking well to coat the bottle walls. Stand for 15 minutes, pour off the solution and rinse thoroughly with water and finally distilled or deionized water. This latter cleaning procedure also applies to new bottles.

6.2 Samples should be incubated in a water bath, or air incubator equipped with fan assisted air circulation. Temperature should be thermostatically controlled at 20 ± 0.5°C and incubation must be carried out *in the dark* to prevent the formation of dissolved oxygen by algal activity. A cooling facility is normally required in order to achieve temperature control throughout the year.

6.3 All glassware used for the preparation of diluted samples should be of good quality and capable of being easily and thoroughly cleaned. Vessels from which diluted samples are transferred to bottles for incubation should preferably be of a tall cylindrical shape (eg. 500-ml cylinder), to facilitate mixing and transfer with minimum extrainment of air bubbles.

Volumetric glassware should be of Class B or better.

7 Sampling and Sample Preservation

A representative sample should be taken into a clean glass bottle fitted with a ground glass stopper. The bottle should be completely filled with sample and securely stoppered to minimize access of oxygen. A glass bottle with a plastic stopper may be used provided tests have shown that the material of the plastic does not affect the measured BOD.

Ideally, measurement should be commenced as soon as the sample has been taken since the BOD will change in an *unpredictable* manner on storage (usually the BOD value decreases on storage, but increases have been observed).

When delay is unavoidable the sample should be cooled immediately and placed in a refrigerator at 2–4°C (but not frozen) and held at this temperature until measured; the use of chemical preservatives is not recommended. If storage is necessary, special tests should be carried out for each type of sample to assess the effect on the BOD.

Limited tests carried out on settled domestic sewage, and on OECD synthetic sewage indicate that storage at 20°C may result in a 10–15% decrease in BOD value in 4–6 hours and a 15–22% total decrease in 24 hours. (References 18 and 19).

If a composite sampler is used, due consideration must be given to the effect on the measured BOD value of the prolonged storage of part of the sample.

8 Procedure

Read section on hazards before starting this procedure.
There are two methods for determination of dissolved oxygen in water, either of which may be used in this procedure (see Reference 1).

Step	Procedure	Notes
8.1	The sample should be brought to 20 ± 1°C and equilibrated with air by vigorous shaking in a partly filled, stoppered bottle (note (a)). If the pH of the sample is not between 6.5 and 8.5, add sufficient alkali or acid to bring it within that range; determine the amount of acid or alkali to be added by investigation of a separate sample. If the volume of acid or alkali added is significant, the measured BOD value may need appropriate correction.	(a) Refer here to Section 9 which may be relevant to the sample under test.

Step	Prodecure	Notes
8.2	Select the volume of sample required (note (b)).	(b) The dilution required depends on the expected BOD level. The table below is a guide, such that 30–70% of the initial dissolved oxygen concentration should be depleted over 5 days at 20°C.

Dilution

BOD Range	Vol. Sample	Vol. Diluent	Typical Sample
Up to 6	1	Nil	Clean river
6– 20	1	2	River
10– 30	1	4	Sewage effluent
20– 60	1	9	Poor effluent
40–120	1	19	Bad effluent
100–300	1	49	Raw sewage or industrial effluent.

The COD or TOC value of the sample, or other BOD-correlated parameter *can* be used, if known, as a guide for the dilution required. Serial dilution is required for dilutions >100:1.

In unknown cases, it may be necessary to prepare more than one dilution to cover the anticipated result.

The presence of suspended matter in the sample can cause difficulties (see Section 9.3.2).

8.3	Place the required volume of sample in a suitable mixing vessel (notes (c) and (d)).	(c) A measuring cylinder or pipette (with the fine end removed) can be used for dispensing the sample. Dilutions involving the measurement of less than 10 ml sample should be made by first diluting the sample in a calibrated flask and then using the appropriate volume of that dilution. (d) The mixing vessel should ideally be a stoppered measuring cylinder of appropriate volume. The capacity depends on the method employed in the measurement of dissolved oxygen. For the titrimetric method a 1-litre cylinder may be used, but if measurement is by the instrumental method 500 ml would be suitable.
8.4	If an unsuppressed BOD is required proceed to Step 8.5. If suppression of nitrification is desired add 0.05% m/V allyl thiourea solution at the rate of 1.00 ± 0.02 ml per litre of sample to be incubated.	
8.5	Make up to the desired final volume (± 0.5%) by careful addition of dilution water (note (e)).	(e) The dilution water may be plain or seeded as appropriate (see Section 5.4).
8.6	Mix thoroughly using a gentle rolling and inverting motion to avoid entrainment of air.	
8.7	Rinse out the bottle(s) with the prepared dilution and fill to overflowing either by careful pouring or by use of a siphon (note (f)).	(f) Two bottles are needed for the titrimetric method, one for the initial and one for the final dissolved oxygen measurement. When the instrumental method is used one bottle may suffice for both measurements.

Step	Procedure	Notes
8.8	Allow the bottle(s) to stand for 10 ± 5 minutes. Tap gently to remove any air bubbles.	
8.9	Determine the initial dissolved oxygen content of the sample in one of the two bottles (D_1 mg/1) (Reference 1) (note (g)).	(g) If the instrumental method is used it is found that some sensors cause a slight displacement of the sample during measurement. This problem may be overcome by the use of a displacement funnel in the neck of the bottle, or alternatively it may be necessary to top up the bottle contents after the initial measurement using some of the sample used to fill the bottle initially which has been kept for this purpose.
8.10	Stopper the second bottle (or the bottle used in Step 8.9 if the instrumental method is used for measuring the dissolved oxygen). Care must be taken to avoid the entrainment of air during the stoppering. Label the bottle and place in an incubator or water bath at 20.0 ± 0.5°C for 5 days ± 2 hours (note (h)).	(h) See Section 6.2. Incubation must be carried out in the dark.
8.11	After 5 days determine the dissolved oxygen content of the sample (D_2 mg/l) (note (i)).	(i) If the instrumental method is used it is important to clean the bottles after use by washing with acidified iodine wash solution followed by distilled water (see Section 6.1).
8.12	Blank Determination Treat the dilution water (seeded if appropriate) as sample and determine its dissolved oxygen content before (B_1 mg/l) and after 5 days incubation at 20.0 ± 0.5°C (B_2 mg/1) (note (j)).	(j) The test provides one check on the validity of the method and values of B_1–B_2 in excess of 0.5 mg/l (seeded) should be the cause of investigation of the dilution water used. For example, blank values in excess of 0.5 mg/l can arise as a result of nitrification of the ammonium ion added as phosphate buffer solution to the dilution water; in such a case it would be necessary to use ATU in the test procedure. For unseeded dilution water the value should lie in the range 0.1–0.3 mg/l.
8.13	Routine Analytical Quality Control. As a check on certain sources of error, it is recommended that a glucose/glutamic acid standard solution should be analysed, in the same way as samples, whenever the method is used. The control standard should be prepared by dilution of the glucose/glutamic acid solution, described in Section 5.2.7, using 1 volume of that solution to 49 volumes of seeded dilution water. It is also recommended that, to check precision, a sample be analysed in duplicate in each batch. Section 10 ('Checking the Accuracy of Analytical Results') gives further guidance on routine control procedures.	
8.14	Calculation (notes (k) and (m)). (a) Unseeded, undiluted sample $BOD = (D_1 - D_2)$ mg/l (b) Sample diluted with seeded or unseeded dilution water $BOD = f\left[(D_1 - D_2 - \frac{(f-1)}{f}(B_1-B_2)\right]$ mg/l where f = dilution factor = 1 + volume of dilution water added to unit volume of sample.	(k) If allyl thiourea has been used in the test the result must be reported as BOD (ATU). (m) A graphical method of calculation has been reported (Reference 17) but has not been fully evaluated by the Working Group.

9 Sample Pretreatment and Special Cases

9.1 Pretreatment

9.1.1 *Presence of algae*

Samples containing algae may initially be supersaturated with oxygen and can also give rise to high results due to the decay of algae using up dissolved oxygen during incubation. The algae may be removed by filtration or centrifugation. This will of course remove any suspended matter present and the resulting BOD value should be reported as being "filtered" or "centrifuged". It is advisable to seed such filtered samples when making the subsequent BOD determination.

9.1.2 *Presence of free chlorine or chloramines*

Samples containing free chlorine or compounds containing available chlorine, such as chloramines, require pretreatment prior to BOD determination because of the bactericidal effects and the errors in the dissolved oxygen measurement which would otherwise occur. Chlorine may be removed by reaction with sodium sulphite; however, in some samples, especially those containing industrial effluents, chlorine may have combined with organic compounds to produce substances which, though giving no chlorine reaction in the starch – iodide test, nevertheless inhibit biochemical oxidation or may even be bactericidal. In such cases the BOD value found will be lower than would be indicated by other determinations of organic matter content (see Section 9.3).

If, despite this uncertainty in the interpretation of results, a BOD value is required, the procedure outlined below may be used.

Adjust the pH of a suitable aliquot of the sample, to 4 ± 0.5 with 0.5 M sulphuric acid. Add 0.1 ± 0.01 g potassium iodide per 100 ml of solution and determine the amount of sodium sulphite required to remove the chlorine by titration with a freshly-prepared 0.01 M solution of sodium sulphite to the starch-iodide end point. Take a second aliquot of the sample, to be used for the BOD test, adjust the pH as above and add the requisite amount of freshly prepared sodium sulphite solution as determined by the titration. Mix thoroughly and allow to stand for 10–20 minutes, then check the absence of chlorine by testing a small portion of the solution with neutral starch-iodide. Re-adjust the pH to 7.5 ± 1.0 with 1 M sodium hydroxide solution, make up dilutions with seeded dilution water and proceed as for unchlorinated samples.

9.1.3 *Presence of ferrous iron*

Ferrous iron causes two problems in BOD determinations. Firstly, it interferes in the titrimetric determination of dissolved oxygen by the Winkler method by reducing liberated iodine back to iodide. The method used for the dissolved oxygen determinations must therefore be chosen with care. The titrimetric method published in this series (Reference 1) will tolerate up to 1 mg/litre of ferrous iron and recommends an instrumental procedure for higher concentrations.

The second problem caused by ferrous iron occurs when the sample is diluted to bring the oxygen demand into the measurable range. In these cases the ferrous iron reacts rapidly with the oxygen in the dilution water and gives rise to a falsely high biochemical oxygen demand. The oxygen demand of the ferrous iron (and of other reducing species) can be separately assessed and reported (see Section 9.2.1 Immediate Oxygen Demand).

9.1.4 *Presence of hydrogen sulphide and/or sulphur dioxide*

Compounds such as sulphur dioxide and hydrogen sulphide consume oxygen and should therefore be removed prior to BOD measurement. Hydrogen sulphide can also interfere in instrumental oxygen measurements resulting in a reduction of the observed dissolved oxygen value for a water and possibly leading to negative readings, desensitization and/or slow response characteristics of the sensor.

Removal of hydrogen sulphide and sulphur dioxide is achieved (References 5 and 6) by adjusting the pH of the water to about 3.0 and passing nitrogen through the acidified sample for 30 minutes. The pH is then readjusted to 6.5–8.5. Volatile organic compounds will be lost during this procedure resulting in a low value of the BOD.

Sulphuric acid and sodium hydroxide solutions of suitable strength are used for the pH adjustments and if changes in the sample volume result during these adjustments the volume of acid/alkali added should be noted and taken into account when calculating the dilution factor.

9.2 Special cases

9.2.1 *Immediate oxygen demand*

Owing to the presence of reducing agents such as sulphite, ferrous iron etc., some samples, on dilution will exhibit an immediate oxygen demand.

Whether determined by titrimetric or instrumental procedures, such samples may exhibit low initial dissolved oxygen concentrations at the start of the 5 day incubation period. In such cases the sample requires further shaking until the sample to be incubated is fully equilibrated with air.

In some cases, for example in the presence of the thiosulphate, an apparent immediate oxygen demand is caused by reduction of iodine liberated in the acidification stage of the titrimetric method for dissolved oxygen.

In such cases, and since no actual immediate oxygen demand is exerted, use of the instrumental method is preferred.

9.2.2 *High salinity*

Concentrations of chloride greater than 10,000 mg/l can cause erratic results. It has been reported that as salt concentration increases, the BOD value decreases.

In such cases, salt tolerant micro-organisms should be used in place of the normal seed and saline diluent water may be preferred (Reference 7).

9.2.3 *Alternative incubation periods and temperatures*

The rate of carbonaceous oxidation during the first stage of the BOD test may be expressed by Phelps Law:

$$\text{Log}_{10} \frac{L}{L - x} = kt$$

(see Section 3.1 for definition of the parameters).

For a given type of organic matter and microbial seed, the effect of temperature on the rate constant k and on the value of L can be predicted to a first approximation (Reference 8) and this may be useful when considering the use of the BOD test in warmer climates, or in studies of long rivers which traverse a number of climatic regions. Such relationships must, however, be used with caution.

The standard BOD result is obtained after 5-day incubation at 20°C. Over the years a vast amount of data has been accumulated and because of this, other more rapid tests which have been developed to measure organic pollution are usually correlated with the 5-day BOD.

One of the drawbacks associated with the test has always been the 5-day delay before a result is obtained. Attempts have been made to produce the same results within a shorter period (3 or 2½ days) by using higher temperatures (27° or 35°C respectively).

In countries with very hot climates the 3-day test is practically standard procedure, not to save time, but because ambient temperatures are so much higher and the various micro-organisms responsible for degradation and oxidation of organic matter are exposed and acclimatised to temperatures between 25°C and 30°C.

The 3-day test has been used by many workers in this country and numerous comparisons and correlations with the 5-day test have been compiled. Using various types of sewage and effluent Halliwell's tables (Reference 9) show over 60 comparisons between the 3-day and 5-day tests. They rarely differed by more than ± 5%. Many other authors have studied this subject, among them Tool (Reference 10), who gives rate curves showing correlation between 5-day tests at 20°C and 2.5 days at 35°C, Gotaas (Reference 11) and Flegal et al (Reference 12) who have studied the temperature effects on bio-oxidation, and Orford et al (Reference 13) who gives further comparisons of short term tests with the 5-day BOD.

In this country the 3-day BOD test has been used, not only to obtain a speedier result, but also to solve the problem of week-end attendances resulting from completion of analysis of samples taken on Mondays and Tuesdays. A 3-day BOD result may not exactly match a 5-day BOD, but it is preferable to keeping a sample 2 or 3 days before commencing the analysis.

Another approach has been to introduce a 7-day BOD at the standard temperature of 20°C. No change is thus required in the procedure and samples necessarily requiring the standard 5-day test can be analysed at the same time. Correlations between the two tests are also easier to obtain. The 7-day test has been standard in Sweden for several years.

An important consideration in all these modifications is the part played by nitrifying organisms. Nitrification in the BOD test can be sporadic in occurrence and extremely uncertain in interpretation. Increasing the temperature or extending the period of incubation could greatly increase the likelihood of nitrification occurring. Certainly for 7-day tests at 20°C it is recommended that suppression of nitrification should be practised. This topic is more fully discussed elsewhere eg. WPRL (Reference 14), Montgomery et al (Reference 4) and Wood et al (Reference 15). A private communication to DOE from WRC (Stevenage) demonstrated that if sufficient ATU was present to inhibit nitrification, the 7-day BOD results for primary settled sewage samples are higher than the 5-day results by a factor of 1.09 and by 1.29 for sewage final effluent samples (after settlement).

As yet there is insufficient information on oxygen up-take curves for "carbonaceous" BOD after the 5-day period for any recommendations to be made. Nor is there enough known about nitrification during the 5 or 7 day period, especially the part played by nitrites which are unaffected by ATU.

A further possibility, which preliminary results have shown to be encouraging, is a 7-day test at 18°C.

9.3 Trade effluents

THIS SECTION SHOULD BE READ IN CONJUNCTION WITH SECTION 3.

For the following reasons, difficulties may be experienced or the results may be misleading when applying the BOD test to trade effluents.

1 The sample may be sterile.

2 Such micro-organisms as are present may be unable to oxidize the organic matter present (ie. the micro-organism population is not acclimatized).

3 The sample may contain organic compounds which are resistant to biochemical breakdown (or biodegradation).

4 Toxic or bacteriostatic compounds are present which inhibit bio-oxidation under conditions of the test.

The seeding of industrial effluents as part of the BOD measurement has been discussed in Section 5.4.2 and is referred to again in Section 8.5 note (e). Similarly the use of acclimatized seed is referred to fully in Section 5.5

Before embarking on the tedious and sometimes unrewarding task of preparing an acclimatized seed inoculum, other tests should be run on the sample which will give an indication of its organic content (some or all of which may be biodegradable). Such tests include the measurement of Chemical Oxygen Demand (COD), Total Organic Carbon (TOC) content and measurement of the concentration of specific organic compounds believed to be present in the sample. The results of such tests, when compared with the results of a conventionally seeded BOD test can indicate whether cases 2, 3 or 4 above may apply to the sample.

If the measured BOD value still appears very low compared with the known organic content of the sample then the presence of toxic substances (eg. heavy metals) or of bacteriostatic substances may be suspected. Such substances give rise to the phenomenon of "sliding" BOD values. That is to say, as the sample is progressively diluted, the apparent BOD value of the sample does not remain constant, but actually increases due to dilution of the toxic effect. In some cases it is possible to dilute the sample until a constant value of the BOD is obtained, but the dilution required to achieve this may be so great that the carbonaceous oxygen demand in the diluted sample is too small to measure with confidence. In such cases, the only useful

information that may then be obtained is an assessment of the degree of sample dilution required to eliminate the toxic effect; this may be measured in a separate test in which standard solutions of glucose/glutamic acid are diluted with a fixed volume of water containing various concentrations of the initial sample. BOD measurements are carried out on these dilutions until the known BOD value of the glucose/glutamic acid solution is obtained (Reference 16). Seeding will be necessary in all such tests.

If toxic substances are not present, or their effect is readily overcome by dilution, then the sample should be examined using an acclimatised seed (Section 5.5).

If cases 1, 2 and 4 above have been eliminated, yet the BOD value is still low compared with the known organic content of the sample, then the presence of compounds resistant to biodegradation must be suspected.

The detailed interpretation of the oxygen demand exerted by trade effluent during treatment or when diluted in receiving waters is outside the scope of this test and would involve respirometric measurements of oxygen uptake rate, treatability studies etc., such tests usually being carried out at higher concentrations of substrate and of biomass than in the simple BOD test. (Suitable methods for these measurements are available in other publications in this series, Reference 16).

9.4 Suspended solids

Industrial effluents and some river waters may contain significant amounts of dense suspended matter. This can cause difficulties in the BOD test for the following reasons:

a) part of the suspended solid may be biodegradable organic matter, and if the sample has to be extensively diluted it may be difficult to achieve a reproduceable dilution.

b) the suspended matter may adsorb much of the micro-organism population, leading to stratification of dissolved oxygen concentration in the bottle during the incubation period.

Satisfactory results can only be obtained in such cases by provision of agitation in the bottle during incubation.

In some cases it may be appropriate to remove the suspended solids (making some separate estimate of their organic content) and to measure the BOD on the filtered solution. Comparison of this measurement with an attempted measurement on the whole sample may be informative.

10 Checking the Accuracy of Analytical Results

It has been recommended (Step 8.13) that duplicate analyses of a real sample, and an analysis of a seeded glucose/glutamic acid solution, be made in each batch of analyses, for routine control purposes. The results of such control analyses may be plotted on quality control charts to facilitate detection of a deterioration of performance. The principles, construction and use of such charts are described elsewhere. (Reference 22 and 23).

The use of duplicate analyses of a real sample permits detection of a deterioration of precision, and is straightforward. However, in the case of the glucose/glutamic standard, the assignment of an 'expected value' for control chart purposes is less simple than in the case of specific determinands, and merits further consideration.

Because the BOD result for the standard may be affected by the particular seed being used (nothwithstanding procurement and use of the latter in strict accordance with the procedures described), a single, fixed 'expected value' cannot be assigned in the usual way. The use, as the 'expected value', of the mean result obtained for the standard in preliminary tests to establish the method should permit subsequent changes in performance to be identified. However, such an approach will not facilitate comparison of the results obtained for the control standard with those typically observed by other laboratories. To allow such comparison, the forerunner of the present method (Reference 20) cited a value of 220 mg O_2/l for the glucose/glutamic standard (corrected for the dilution). Examination of 1,970 results obtained for the

same standard,* using methods similar to that given here, has recently disclosed a somewhat lower overall mean, of 205 mg O_2/l. This value may be used to judge the conformity or otherwise of the result for the glucose/glutamic standard obtained in one laboratory with the mean result of many determinations by a group of UK laboratories having considerable experience of the method. Although large differences from the 205 mg O_2/1 value may be a cause for concern, and prompt re-assessment of all aspects of procedure and of the seed used, it should also be noted that it is not impossible that the seed employed could be appropriate to the samples of interest, yet produce a mean result for the glucose/glutamic standard solution differing appreciably from 205 mg O_2/1. Whether or not such differences from the latter figure are considerd important must be decided by the user of the method, in the light of the purpose for which the BOD data is being obtained.

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* The BOD value for the standard glucose/glutamic acid solution is based on recent measurements by Yorkshire Water Authority, Severn Trent Water Authority and North West Water Authority, (11 Laboratories). In all 1970 measurements were submitted which gave a grand mean BOD value of 205.2 mg/l.

Address for Correspondence

However thoroughly a method may be tested, there is always the possibility of a user discovering a hitherto unknown problem. Users with information on this method are requested to write to:

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The Standing Committee of Analysts
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