5 Day Biochemical Oxygen Demand (BOD_5) Second Edition 1988

with Dissolved Oxygen in Waters, Amendments 1988

Methods for the Examination of Waters and Associated Materials

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The change in ATU concentration used in the inhibited version of this method between the first edition and this edition should not be taken as permission to change any consent or contract without prior written agreement by the parties concerned. If the presently agreed ATU concentration is still the 1980 value of 0.5 mg/L, use only 0.5 ml/L at step 8.5. However, parties to such an agreement should consider the data heregiven before their next agreement revision.

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5 Day Biochemical Oxygen Demand (BOD_5) Second Edition 1988

with Dissolved Oxygen in Waters, Amendments 1988

Methods for the Examination of Waters and Associated Materials

This booklet contains full instructions for the basic BOD_5 test and for the ATU (Allylthiourea) 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. As the amendments to the existing Dissolved Oxygen method are important, affect this method, but do not warrant a new edition, they are included here.

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About This Series

This booklet is part of a series intended to provide both recommended methods for the determination of water quality, and in addition, 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 These volumes inevitably took some years to prepare, so that they were often partially out of date before they appeared in print.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 series of booklets on single or related topics; 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 and notes 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 determined. It will be the responsibility of the users—the senior technical staff 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 a committee of the Department of the Environment set up in 1972. Currently 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 Microbiological methods
3.0 Empirical and physical methods
4.0 Metals and metalloids
5.0 General nonmetallic substances
6.0 Organic impurities
7.0 Biological monitoring
8.0 Sewage Works Control Methods
9.0 Radiochemical methods

The actual methods and reviews 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.

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 and minor additions to published booklets not warranting a new booklet in this series will be issued periodically as the need arises. 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 that booklet.

L R PITTWELL
Secretary and Chairman

11 August 1988

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 for one's self, one's colleagues, those outside the laboratory or work place, or subsequently for maintenance or waste disposal workers. 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. 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. Lone working, whether in the laboratory or field, should be discouraged.

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 check-list, 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. Hazardous reagents and solutions should always be stored in plain sight and below face level. Attention should also be given to potential vapour and fire risks. 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.

There are numerous handbooks on first aid and laboratory safety. Among such publications are: 'Guide to Safe Practices in 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.

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 radiochemical 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. If an ambulance is called or a hospital notified of an incoming patient, give information on the type of injury, especially if poisoning is suspected, as the patient may be taken directly to a specialised hospital.

Safety while Sampling

Prior consideration must be given, especially when sampling in confined spaces or where access is difficult, to guard against suffocation, drowning, falls, and poisoning or infection by ingestion, inhalation, or skin contact.

0 Preface

The 5 day BOD test using incubation at 20°C is an empirical bioassay with wide international agreement on the basic test procedure and test parameters. To accord with this situation and promote continuity changes to the first edition (1981) (Ref 1) have been mainly limited to matters of clarification and emphasis. These changes are identified below.

0.1 The most significant change is an increase in the concentration of allylthiourea (ATU) used in the BOD(ATU) procedure to restrict the oxygen demand during the incubation period to carbonaceous oxidation only. The concentration of 0.5 mg ATU/L in the incubated solution recommended in the first edition (1981) (Ref 1) is adequate for many samples. However, it has been shown to be inadequate in preventing nitrification in some samples, particularly those containing free ammonium ions together with significant numbers of nitrifying bacteria. (Refs 2 and 3). Based on its own and other published work (Refs 2, 4, 5 and 6) the working group recommends a level of 2.0 mg ATU/L in the incubated solution to ensure total suppression of nitrification.

A possibly wide-spread practice has been noted of adding the ATU to the dilution water prior to sample dilution instead of addition to the diluted sample, see step 8.4. This is unacceptable, as the ATU concentration in the incubated solution is then dependent on the dilution factor and could be reduced below the level required for effective supression of nitrification.

0.2 Another possibly wide-spread practice is use of the dilution water blank value without correction for the dilution factor as required in the calculation equation, step 8.14(b). The error is particularly significant whenever there is a combination of a high blank value for the dilution water and a low dilution factor, hence the proper calculation must be used. Attention is also drawn to the fact that no blank value is used in the calculation equation, step 8.14(a), for unseeded, undiluted samples.

0.3 No minimum figure has ever been given for the range of application, clause 1.4, in the table of performance characteristics, Section 1. Attention is drawn to the new clause 1.11 which gives qualified recommended minimum levels for undiluted samples. Tables 1 and 2 were published in the first edition (1981) (Ref 1) of the BOD test and have been retained with additions. Much of the performance statistics in these tables were derived for previous versions of the test, but are still regarded as relevant to the new procedure given in this booklet.

0.4 Section 5.2.7 has been extended to include an alternative procedure for preparing and storing the glucose/glutamic acid reagent so as to improve shelf life (Ref 3). Section 7 has been extended to include an alternative to low temperature storage of the original sample as received, when commencement of analysis is unavoidably delayed (Ref 3). Section 5.4 has been extended to refer to the use of seeding organisms in solid form (Ref 7). Section 6.2 has also been expanded to emphasise the importance of achieving an incubator temperature performance of 20°C±0.5°C, and that this must be confirmed by measurement, and not be assumed (ref: 3, 36, 37). It should be noted that the suggested alternative procedures or reagents have been reported as being successfully tested. However, there is as yet insufficient evidence available to recommend them as preferred options. Users with information pertinent to the suggested alternatives are requested to write to the Working Group at the address given for correspondence at the end of the booklet.

0.5 The Winkler titration is the reference procedure for measuring the concentration of dissolved oxygen in the BOD procedure (Ref 8). The alternative use of dissolved oxygen meter systems is widespread, especially for batch analysis, because of their convenience and speed. However, problems have been reported and failure to check routinely the correct functioning of the meter system could lead to significant errors. The major problems identified have been nonlinearity over the normal dissolved oxygen working range and loss of speed of response of the electrode. A new Section (6.4) has been included recommending a simple test procedure for routinely checking the performance of the dissolved oxygen meter system.

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 prediluted if necessary, and if appropriate 'seeded' with a suitable source of micro-organisms, is incubated in a completely filled and stoppered glass bottle 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) (Ref 8).
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 below section 1.11.
1.7	Sensitivity	For an undiluted sample a dissolved oxygen absorption 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 occurrence of bias may not readily be identified. Bias may arise on sample storage or from the presence of interferences (See also Sections 7 and 9).
1.9	Interference	Free chlorine. Substances toxic to aerobic bacteria including nitrifiers. 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.
1.11	Range of application, minimum values	

Limit of Detection

There is a wide variation in the estimates of limits of detection given in tables 1 and 2. They are a poor guide as to the minimum concentration which should be quoted for undiluted samples when routinely analysing only single test aliquots. The first edition (1981) (Ref 1) recommended a minimum oxygen depletion during incubation of 30% of the initial dissolved oxygen content. This is equivalent to about 2.7 mg/L of oxygen in air saturated water which for an undiluted sample is equal to a BOD of 2.7 mg/L. In practice this is considered too restrictive as a limiting concentration. Instead it is recommended that for routine work, using only a single determination per sample, the minimum reporting level should be 2.0 mg/L BOD on undiluted samples. Any result below 2 mg/L should be reported as less than 2 mg/L. For undiluted samples this is equal to a depletion during incubation of 2 mg oxygen/L or about 22% of the initial dissolved oxygen. This limiting value is pertinent to river waters in particular, which are frequently below 2 mg/L BOD. It is therefore recommended that analysts wishing to report figures of less than 2 mg/L should undertake replicate determinations on the

undiluted sample and additionally qualify the quoted result as to expected accuracy. Where duplicate determinations are made and results vary significantly, both results should be quoted. Where determinations are made in triplicate any outlying result should be discarded and the mean of the other two results quoted. Statistically the accuracy of results below 2 mg/L can be improved by increasing the number of replicates, but only for homogeneous samples. Sample inhomogeneity can make a nonsense of such data.

For diluted samples the quoted result should be based on the recommended 30% to 70% uptake of initial dissolved oxygen. In the case of diluted samples, where this minimal oxygen uptake is not achieved, the result should be qualified as to the expected accuracy. Alternatively a less than figure which is equivalent to the BOD which would have been given with a 30% uptake of the initial oxygen may be quoted.

The uptake range of 30 to 70% depletion of initial oxygen is an arbitarily selected range, selected so as to ensure that a realistic level of residual oxygen remains present together with a sufficient uptake of dissolved oxygen for the result achieved not to be seriously compromised. This is in line with usual practice with other oxygen demand tests which are also empirical. In practice, a range of glucose/glutamic acid standards of varying concentrations will give a linear relationship when plotted against oxygen uptake even for oxygen depletions exceeding 90% of the initial dissolved oxygen. This indicates that the dissolved oxygen uptake of 70% is not a limiting level.

Table 1 BOD (Total) (No ATU)

Sample	Concentration mg/L	Standard deviation mg/L	Degrees of freedom
Glucose/glutamic acid standard, nominal Concn. 1.1 mg/L[7]	0.95–1.15	0.05–0.44[1,2]	8–26
Glucose/glutamic acid standard, nominal Concn. 6.6 mg/L[7]	6.01–6.53	0.13–0.46[1,2]	8–26
Local river waters (clean)	1.23–3.23	0.04–0.27[1,3]	18
Local river waters (dirty)	2.60–6.42	0.06–0.40[1,3]	18
Trade effluent	348	23.1[1,4]	10
Industrial effluent	1342	53.3[1,4]	10
Chemical plant effluent	2453	67.1[1,5]	10
Methanol condensate	3349	192.7[1,5]	10
Blank Water		0.07–1.21[1,6]	18
Glucose/glutamic acid standard, nominal Concn. 220 mg/L	203.81	12.75[7]	17

Notes

(1) The data on the glucose/glutamic acid solutions, the river waters are from results for the Harmonised Monitoring Scheme of the Department of the Environment (Ref 9) using the DOE 1972 (Green Book) method (Ref 20). Better precision and interlaboratory compatibility of results than those shown in the table could be expected using the present 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 one 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 electrochemical sensor for dissolved oxygen measurements.

(5) Results 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. Calculated from actual standard deviations achieved and do not indicate the minimum value detectable, as the mean value was ignored in the calculation.

(7) Prepared by dilution from a nominal 220 mg/L standard solution (5.2.7). The revised procedure given in this booklet was used.

Table 2 BOD with ATU

Sample		ATU mg/L	Concentration mg/L	Standard deviation mg/L	Degrees of freedom
Glucose/glutamic acid standard, nominal concn. 1.1 mg/L		0.5	0.88–1.08	0.06–0.38[1,2]	8–26
Glucose/glutamic acid standard, nominal concn. 6.6 mg/L		0.5	5.41–6.72	0.16–0.74[1,2]	8–26
Local river waters (clean)		0.5	1.00–2.06	0.03–0.28[1,3]	18
Local river waters (dirty)		0.5	2.37–6.50	0.05–0.44[1,3]	18
Humus tank effluent		0.5	4.44	0.30[1,4]	6
Humus tank effluent		0.5	5.81	0.30[1,4]	10
Humus tank effluent		0.5	13.5	0.61[1,4]	10
Sewage works effluent		0.5	51.2	1.73[1,4]	6
Blank Water				0.10–0.66[1,5]	18
Glucose/glutamic acid standard, nominal Concn. 220 mg/L	(6,9)	0.5	207.04	16.82	17
	(7,9)	2.0	207.47	16.83	18
	(8,9)	5.0	210.21	15.51	18

Notes

(1) The data on the glucose/glutamic acid solutions, the river waters are from results obtained for the Harmonised Monitoring Scheme of the Department of the Environment using (Ref 9) the DOE 1972 (Green Book) method (Ref 20). Better precision and interlaboratory comparability of results than those shown in the table could be expected using the present 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 and 3 respectively used an electrochemical sensor for dissolved oxygen analysed without dilution or seeding and no blank correction was made.

(4) Results are 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. Calculated from actual standard deviations and does not indicate the minimum value detectable, as the mean value was ignored in the calculation.

(6) Methods as First Edition.

(7) Methods as this Edition.

(8) Method as this edition except for extra ATU.

(9) All but one set of data obtained using the dissolved oxygen probe.

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) 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 assessmen 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 (Refs 10 and 12), by comparing the value obtained for its BOD with, for example, its chemical oxygen demand (COD) (Ref 11).

3 Principle

3.1 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 allylthiourea is used to suppress nitrification during the course of the test the result if referred to as BOD (ATU).

The BOD test was originally envisaged by the Royal Commission on Sewage Disposal (Ref 13) 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 straightforward 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.

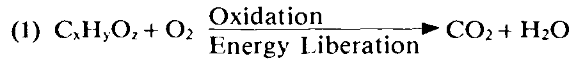
Stabilization in terms of oxygen uptake by a given sample may require a period of incubation longer than the 5-day period, consequently other periods of incubation are sometimes chosen. If periods of incubation other than the standard 5 days are used this MUST be indicated by a suffix denoting the period in days eg BOD_3, BOD_{20}. It should be noted that the quoted performance characteristics apply only to the standard 5-day period.

A number of substances and factors 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.

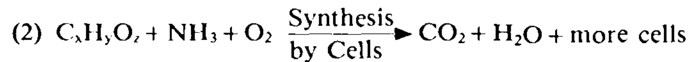
3.2 Mechanism

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;

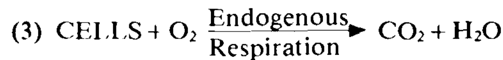


$$(1)\ C_xH_yO_z + O_2 \xrightarrow[\text{Energy Liberation}]{\text{Oxidation}} CO_2 + H_2O$$



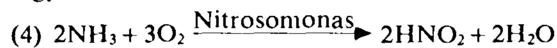
$$(2)\ C_xH_yO_z + NH_3 + O_2 \xrightarrow[\text{by Cells}]{\text{Synthesis}} CO_2 + H_2O + \text{more cells}$$

The 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:



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

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 low 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, particularly when the initial inoculum is large.

3.3 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 (Ref 13). 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 (Ref 14) 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 $0.3d^{-1}$ 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.

3.4 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 effluents discharged from sewage works which nitrify contain significant numbers of nitrifying bacteria).

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

The determination of BOD due to carbonaceous oxidation alone is common place especially 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 allylthiourea (Refs 15 and 16) and Montgomery and Borne (Ref 17) were able to show that this reagent did not influence the carbonaceous BOD value.

The 0.5 mg ATU/l concentration used in the first edition (1981) (Ref 1) was based on the recommendations of Montgomery and Bourne (Ref 17). Subsequent work has shown that this concentration is inadequate for samples containing free ammonium ions and significant numbers of nitrifying bacteria (Section 13). This sitution occurs commonly with effluents from activated sludge plants which are incompletely nitrified. The allyl thiourea is biodegraded during the incubation period which may exacerbate the situation by reducing the allyl thiourea concentration below that required to suppress nitrification effectively. Concentrations up to 5 mg/l have been recommended and have shown effective suppression for incubation periods exceeding 10 days with a nil or minimal increase in oxygen demand due to the added allyl thiourea. The recommended concentration in this booklet is 2 mg ATU/l in the incubated solution. The reagent 2-chloro-6- (trichlormethyl) pyridine (also known as TCMP, N-Serve and nitrapyrin) has also been recommended for suppression of nitrification (Ref 18). However, the use of ATU is favoured, and has also been recommended following a comparison assessment (Ref 2), because it is much simpler to use and has superior characteristics as a suppressant.

3.5 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 is within the range 30–70% 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 10).

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 19).

Curve 1

This may be considered to be a 'normal' oxygen curve for river waters, domestic waste water or a solution of a readily biodegraded compound with adequate micro-biological population (k value $0.30d^{-1}$). 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.

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 bacterial action. Measurements at increasing degrees of dilution may then lead to an apparently constant (asymptotic) value for the 5 day BOD (see Section 10).

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 10).

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 (Ref 8).

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 Ref 8 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 Specific health hazards have been reported for allylthiourea. Ref 39. Skin contact, inhalation and ingestion should 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 containing copper at concentrations greater than 0.01 mg/L is inhibitory and is therefore unsuitable. It is recommended that distillate from an all glass 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 and any other toxic or inhibitory materials.

Figure 1

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

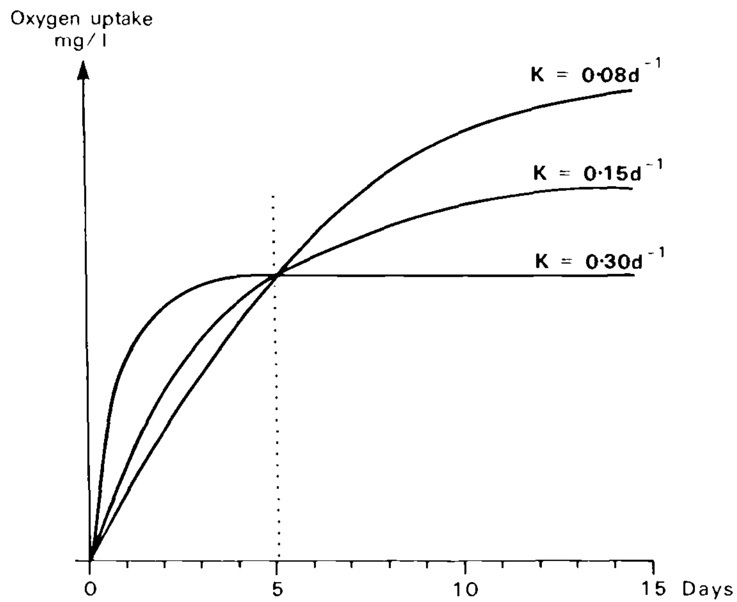
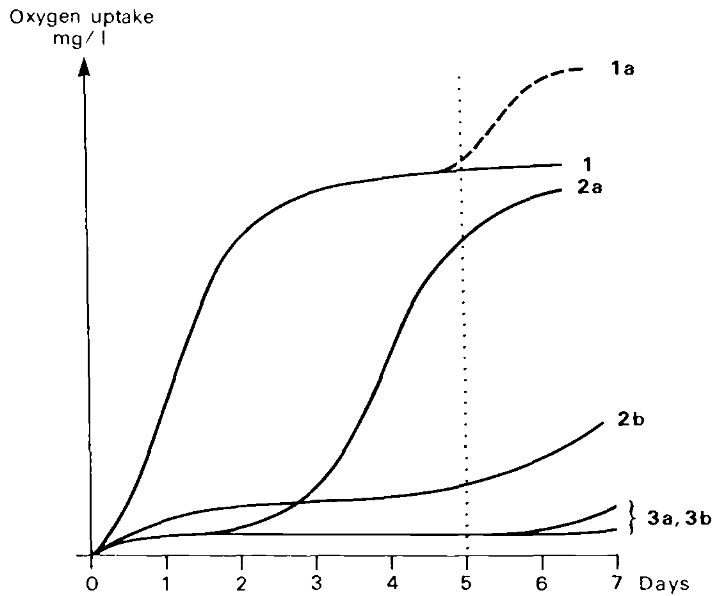


Figure 2

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 lending to 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.

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 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 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 52.5 ± 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 0.1% m/V Allyl thiourea solution

Dissolve 1.00 ± 0.02 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 2.00±0.01 litres of 1% m/V sulphuric acid. Shake to dissolve. Discard when the brown colour fades.

5.2.7 Standard solution of glucose/glutamic acid

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

Alternatively, dissolve 1.50 ± 0.01 g each of d.glucose and l.glutamic acid, both previously dried at 105°C, for 1 hour, in distilled water and dilute to 1 litre in a volumetric flask. Care must be taken to ensure complete solution of the two reagents. 25 mL aliquots are accurately pipetted into clean dry plastic storage vials. These are tightly capped or stoppered and placed in a freezer, or the freezer compartment of a refrigerator, where storage life is at least 3 months. Before use a storage vial is removed from the freezer and stood overnight at room temperature to thaw. The vial contents are quantitatively transferred to a 250 mL flask and diluted to the mark with water. Other aliquot volumes can be stored provided that a tenfold final dilution step is used to make the working solution. Automatic dispensing pipettes are recommended and the plastic vials can be reused (Ref 3).

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 1.00 ± 0.05 mL of each of the stock reagent solutions, ferric chloride, calcium chloride, magnesium sulphate and phosphate buffer in that order (so as to prevent precipitation).

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 a 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 3.5). 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 for 5 days without seeding under standard conditions, should not be depleted by more than 0.3 mg/L (step 8.12 (K)). This must be checked regularly. If this value is exceeded, the cause must be investigated and rectified. Higher values of oxygen depletion may sometimes be caused by the presence of water-soluble organic vapour which may be present in the laboratory atmosphere and be absorbed during the production of the 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 sewage works final effluent should be added to each litre of dilution water. If this seed solution is not clear, it should be allowed to settle for 30 minutes before use. The seed should be the final effluent from the sewage works in which the waste, represented by the sample, is a component of the feed to an aerobic process.

5.4.3 Commerically available powdered microbial cultures are available as seed inoculum produced specifically for the BOD test. The seed is supplied in capsules said to contain 100 mg of a range of micro-organisms commonly found in waste waters, and claimed to be sufficient for 500 determinations when added with the dilution water. For information on tests with this seed, see Ref 7. Concern remains as to the viability and the variability of the micro-organism concentrations between different batches of the seed inoculum. Potential users must satisfy themselves that such products will consistently provide an adequate population of active micro-organisms for the samples being analysed for BOD.

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 10 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 values as settled sewage. Add the diluted sample to primary settled sewage 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 vessels with freshly prepared sample/sewage mixture and again aerate. Repeat for three days and use the settled supernatant liquor, after the third day's 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. Also, the mould flashing on hollow plastic stoppers should be inspected periodically to ensure that a pin hole has not developed.

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 of the 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. Alternatively used bottles can be routinely cleaned using bottle washing machines which use hot water cleaning cycles capable of sterilising the bottles, followed by cold water rinsing. The appearance of high and variable blank values indicates incomplete sterilisation during the bottle-washing stage. This cleaning procedure also applies to new bottles. New bottles may initially give erratic results and should be precleaned by filling with acid iodine wash solution (5.2.6) and leaving to soak for 24th, then water washing as above.

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.

Reliable and accurate temperature control is essential for the BOD incubation step, since temperature differences of 2°C may cause BOD differences of c.10% (Ref 36) due to the influence of temperature on the microbiological reaction rates. Considerable concern has been expressed over the performance of commercial laboratory incubators (Ref 3, 36 and 37). The following problems have been reported as being widespread.

A. The setting and/or temperature indicator on the incubator can differ significantly from the true temperature within the incubator, eg when set to 20°C the temperature of BOD bottles full of water placed in thirteen different incubators equilibrated at temperature between 18°C and 23°C.

b. A significant temperature variation can occur within the incubation area with the greatest differences occurring horizontally between the centre of a shelf and the incubator walls. This variation can be as high as 5°C in extreme cases with 2°C being fairly common. It is believed that wall heated/cooled incubators will be subject to the largest variations.

C. Opening and closing an incubator, even briefly, will rapidly affect the air temperature within the incubator. The actual change being heavily influenced by the ambient temperature and by the packing level of samples within the incubator. Subsequently, within a few hours, the temperature fluctuations will be reflected within the sample bottles.

D. Different packing levels affect the rate at which the incubator and the BOD samples reach equilibrium temperature.

The following are suggested as good practice to ensure that an incubation temperature of 20°C ± 0.5°C is achieved.

A. The accuracy of the incubator temperature control should be checked regularly, using a high quality narrow range (< 50°C) calibration thermometer placed in a water filled BOD bottle placed centrally in the incubator.

B. Incubators should be checked for temperature uniformity after purchase, and then periodically thereafter, to confirm acceptable performance is being maintained. Do not measure the air temperature of the incubator which can fluctuate rapidly. Instead, site a number of BOD bottles filled with water across the internal area of the incubator and check their temperature after they have reached equilibrium.

C. Where practicable, incubators should be sited to minimise the difference between ambient and incubator temperatures.

D. Avoid overloading the incubator, and preferentially fill the central area. The extremities of the incubator should be avoided whenever possible, since these will show the greatest temperature variations.

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 entrainment of air bubbles.

Volumetric glassware should be of Class B or better

6.4 Dissolved oxygen meter system

Reference should be made to the manufacturers' instructions and to ref 8 when setting up and calibrating the meter system. Routine calibration usually involves setting the meter to zero using 'oxygen-free' water and then setting the maximum reading against air-saturated water whose concentration of oxygen has been determined titrimetrically by the Winkler procedure (ref 8). This procedure presumes that there is a linear response between the maximum and minimum points at all times. However, electrodes with a of non-linear response do occur. Non-linearity can even develop with use and together with other potential problems such as slow response of the electrode can generate erroneous readings and therefore cause biased results. Slow response can occur when successive samples differ widely in dissolved oxygen content. A mid calibration range check, using the same sample, to compare results from both the titrimetric and the instrumental methods should be carried out with each sample batch. Normally this would represent the potential maximum error for a non-linear response. This check is easily done by carrying out a Winkler titration on the glucose/glutamic acid standard sample (step 8.13) immediately after the instrumental measurement of the final dissolved oxygen (step 8.11). Typically, the standard will show about a 50% uptake of the initial dissolved oxygen during incubation. The two results should show consistent good agreement and, ideally, a control chart should be plotted. Any large and/or regular differences should be investigated by repeating the test for the full range of the calibration (Ref 8). A meter system which shows a linear response can still exhibit erratic agreement between the two methods where the electrode has (or develops) a slow response and an insufficient immersion period in each sample is allowed during routine analysis.

The following are typical of problems encountered with some types of oxygen electrodes. New electrodes should always be checked before use because:

(i) the initial output voltage of new batteries used in some DO probes can be greater than the quoted nominal output voltage (Ref 3). For example, silver oxide cells can be greater than 1.7 volts compared with their nominal output of 1.55 volts, and mercuric oxide cells can be greater than 1.5 volts compared with their nominal output of 1.35 volts. After c.12–24 hours the output voltages should have stabilised at the nominal values, and reliable calibration without the risk of rapid drift should be possible.

(ii) the battery polarity may be reversed from that stated.

(iii) some electrodes require exceedingly rapid stirring in order for the electrode to give a truely representative reading. Check for this and increase speed accordingly where applicable.

See also Dissolved Oxygen in Waters Amendments 1988 at the end of this booklet.

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, and that pin holes have not formed in the mould flashing on hollow plastic stoppers.

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. Alternatively steps 8.1 to 8.10 in the Procedure Section 8 are carried out, but instead of placing the bottle in an incubator, it is placed in a refrigerator at 2–4°C but not frozen. After refrigeration for the required period the sample bottle is transferred to an incubator for the 5 day incubation period at 20°C before determination of the final dissolved oxygen (step 8.11) (Ref 3). It is recommended that samples high in volatile matters such as lower molecular weight organic compounds should be quantitatively diluted prior to refrigeration to minimise any loss. Chemical preservatives must not be used. 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, final effluent, and on OECD synthetic sewage indicate that storage at 20°C may result in a 10–15% decrease in BOD value after 4–6 hours, 15–22% total decrease in 24-hours, and 16–39% decrease in 48 hours (Refs 3, 21 and 22).

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, and use of sampling machines with integral refrigeration is strongly recommended.

8 Procedure

Read Section 4 on hazards before starting this procedure. There are two methods for the determination of dissolved oxygen in water, either of which may be used in this procedure (see Ref 8). When using the instrumental method note the recommendations on check tests (Section 6.4).

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 value 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.
8.2	Select the volume of sample required V_1 ml (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.

Step	Procedure	Notes

Dilution

BOD Range	Vol Sample	Vol Dilutent	Type
Up to 6	1	Nil	Clean river
6–12	1	1	River
10–15	1	2	Good Sewage effluent
15–30	1	4	Sewage effluent
30–60	1	9	Poor effluent
60–120	1	19	Settled Sewage
120–240	1	39	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 recommended for high dilutions (see note d).

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 9.7).

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.

8.4 If no dilution is required proceed either to step 8.7 (No ATU) or to step 8.5 (with ATU).
If sample dilution is required, make up to the desired final volume V_2 mL (± 0.5%) by careful addition of dilution water (notes b, e and f). Then proceed either directly to step 8.6 or via step 8.5 if ATU addition is required.

(d) Ideally, the mixing vessel should be a stoppered measuring cylinder of appropriate volume. The capacity depends on the method employed in the measurement of dissolved oxygen. For the titrimetric a 1-litre cylinder may be used, but if measurement is by the instrumental method, 500 mL would be suitable. (See note g).

Where high dilution is used, requiring aliquots of less than 10 mL of sample, two stage serial dilution is recommended to avoid taking small aliquots, especially for samples with high solids content.

(e) The dilution water may be plain or seeded as appropriate (see Section 5.4).

8.5 If suppression of nitrification is desired, add 0.10% m/V allyl thiourea solution at the rate of 2.00 ± 0.02 mL/per litre of sample to be incubated. (note f) Proceed to step 8.6. **See also note on p 2.**

(f) The concentration of ATU in the sample to be incubated is 2.0 mg/L. Where stronger concentrations of ATU are required (see Section 13) the volume of ATU added (Reagent 5.2.5) is increased accordingly. The final result should then additionally report the actual concentration of ATU used.

8.6 Mix thoroughly but with a gentle action 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 siphon (note g).

(g) 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 will 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/L (Ref 8) (note h).	(h) 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. Bottles with funnel collars above the stopper are available commercially. 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 i).	(i) See Section 6.2. Incubation must be carried out in the dark. When sample preservation is required (Section 7), the labelled bottle is placed in a refrigerator for the required period before transferring to the incubator or water bath.
8.11	After 5 days determine the dissolved oxygen content of the sample (D_2 mg/L) (note j).	(j) If the instrumental method is used it is important to sterilize the bottles after use by washing with acidified iodine wash solution, followed by thorough rinsing with water. Alternatively wash in a bottle washing machine capable of sterilizing the bottles, followed by rinsing with 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/L) (note k).	(k) 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 cause investigation of the dilution water used. Blank values in excess of 0.5 mg/L can arise as a result of nitrification of the ammonium ion added as part of the phosphate buffer solution used to prepare the dilution water, when no ATU is added. For unseeded dilution water, the value should preferably not exceed 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, one sample be analysed in duplicate in each batch (note 1).	(1) Section 12 ('Checking the Accuracy of Analytical Results') gives further guidance on routine control procedures.

Step	Procedure	Notes
8.14	**Calculation** (Notes m and n). (a) Unseeded, undiluted sample $BOD = (D_1 - D_2)$ mg/L (b) Sample diluted with seeded or unseeded dilution water $BOD = f\,[(D_1 - D_2) - \frac{(f-1)}{f}(B_1 - B_2)]$ mg/L where f = dilution factor = $\frac{v_2}{v_1}$ where v_1 is the sample aliquot in ml (step 8.2) and v_2 is the volume in mL of the diluted sample (step 8.4).	(m) If allyl thiourea has been used in the test the result must be reported as BOD (ATU) (n) A graphical method of calculation has been reported (Ref 23)

9 Sample Pretreatment and Special Cases

9.1 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.

9.2 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-iodine 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 10).

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 several dilutions with seeded dilution water and proceed from step 8.5 in Section 8.

9.3 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 (Ref 8) will tolerate up to 1 mg/L of ferrous iron and recommends the 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 9.5 Immediate Oxygen Demand).

9.4 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 lead to negative readings, desensitization and/or slow response characteristics of the sensor.

Removal of hydrogen sulphide and sulphur dioxide is achieved (Ref 19 and 24) 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 significant changes in the sample volume result during these adjustments, the volumes of acid and alkali added should be noted and taken into account when calculating the dilution factor.

9.5 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. (See Sections 9.3 and 9.4).

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 iodene liberated in the acidification stage of the titrimetric method for dissolved oxygen. In such cases, since no actual immediate oxygen demand is exerted, use of the instrumental method is preferred.

9.6 High salinity

Concentrations of chloride greater than 10,000 mg/L can cause erratic results. It has been reported that as the 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 (Ref 25). This should be mentioned in the Test Report.

9.7 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: (Ref 38)

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 reproducible dilution and accurate representation of the original waste water sample.

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 be obtained in such cases only by provision of agitation in the bottle during incubation. However, the resultant turbulance may break up solid particules into a more readily biodegradable substrate with a consequent increase in measurable BOD content.

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. This may however result in a significant decrease in the BOD of the original sample. Comparison of this measurement with an attempted measurement on the whole sample may indicate the extent of any BOD reduction due to filtration. Any departure from the standard procedure specified in this method must be fully reported in the test report.

10 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 or does not contain the competent species).
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 note (e). Similarly the use of acclimatised 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 from 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 (Refs 10 and 12). 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, Refs 10 and 12).

11 Alternative incubation periods and temperatures

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

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

(see Section 3.3 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 (Ref 26) and this may be useful when considering the use of the BOD test in warmer cli-

mates, 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 tempratures (27° or 35°C respectively).

In some countries with very hot climates, the 3-day test may be a more practical 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. Commonly however, most countries with hot climates use the classic 5-day test, cooling the sample.

Previously 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 (Ref 27) show over 60 comparisons between the 3-day and 5-day tests. They rarely differed by more than ± 5%. Other authors have studied this subject, among them Tool (Ref 28), who gives rates curves showing correlation between 5-day tests at 20°C and 2.5 days at 35°C, Gotaas (Ref 29) and Flegal et al (Ref 30) who have studied the temperature effects on bio-oxidation, and Orford et al (Ref 31) who gives further comparisons of short term tests with the 5-day BOD.

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 used in Sweden for several years. A 7-day test at 18°C has also been studied.

An important consideration in all these modifications is the part played by nitrifying organisms. All the foregoing comparisons and correlations refer to total BOD results, without addition of ATU, and therefore no account was taken of the potential contribution of nitrification to the measured BOD when using alternative incubation periods and temperatures.

Both enhanced incubation periods and enhanced temperature will greatly increase the likelihood of nitrification occurring. This in turn leads to the likelihood of still greater concentrations of ATU being required to inhibit nitrification, possibly significantly greater than the concentration of 2.0 mg/L recommended in this procedure.

It is unlikely that universal conversion factors can be established for tests, using non-standard incubation periods and temperatures, to correlate them with standard BOD test results, particularly where a range of different sample types are analysed.

This situation is common to most empirical tests including the other common oxygen demand tests, COD (ref 11) and Permanganate value (ref 34). It may be possible to establish conversion factors for a very narrow band or type of sample. For example work at WRC (Stevenage) demonstrated that provided sufficient ATU was present to inhibit nitrification, 7-day BOD (ATU) for primary settled sewage and settled final effluents were higher by factors of 1.09 and 1.29 respectively (private communication to DOE).

Unless acceptable evidence can be established, it is recommended that the actual results found using alternative incubation periods and temperatures should be quoted, identifying the test conditions, without any attempt to convert them to 'standard test' BOD results. **MANDATORY AND LEGAL BOD TESTS MUST ALWAYS USE THE PROCEDURE SPECIFIED IN THE AGREEMENT.**

12 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 (Refs 32 and 33).

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 acid 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 (notwithstanding 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 '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 (Refs 1 and 20) cited a value of 220 mg O_2/L for the glucose/glutamic acid standard (corrected for the dilution). Examination of 1,970 results obtained for the same standard (5.2.7) (in a cooperative exercise by 11 laboratories in the Yorkshire, Severn-Trent and North West Water Authorities) using methods similar to that given here, has disclosed a somewhat lower overall mean, of 205.2 mg O_2/L. This value may be used to judge the conformity or otherwise of the result for the glucose/glutamic acid standard obtained in one laboratory with the mean result of many determinations by a group of UK laboratories having considerable experience of the method. Inter-laboratory studies in the United States of America gave a mean value of 199.4 mg/L BOD with a standard deviation of$\pm$37 mg/L for the glucose/glutamic acid standard which has been adopted in the USA as the acceptable working range (Ref 35). Larger differences from the 205 mg O_2/L value are a cause for concern, and a re-assessment should be made of all aspects of procedure and of the seed used. 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/L. Whether or not such differences from the latter figure are considered important must be decided by the user of the method, in the light of the purpose for which the BOD data is being obtained.

13 Background evidence for changes in the concentration of ATU used

Data have been produced to show that 0.5 mg/L ATU is, in some circumstances, insufficient fully to suppress nitrification. Because of this fact, it is now recommended that a concentration of 2.0 mg/L ATU should be routinely used, with the option of increasing this to 5.0 mg/L if the analyst has evidence that 2.0 mg/L is still insufficient.

The following data (tables 3, 4 and 5) provided by North West Water; Anglian Water, Severn Trent Water, Thames Water and Yorkshire Water, give comparative final effluent BOD values obtained using 0.5 and 2.0 mg/L ATU respectively. These data are a subset of all the data obtained in which the difference between the two comparative BOD ATU values was greater than 2 mg/L. In the remaining data (differences < 2.0 mg/L), the majority of the results still showed a decrease in BOD when the increased ATU concentration was used. The few cases with an increase were probably due to statistical error. (Ref 3).

Table 3 SCA (WG3) Survey (all results as mg/L)

Sewage Works Reference	Type of Aerobic Treatment*	BOD 0.5 mg/L ATU ($V_{0.5}$)	BOD 2.0 mg/L ATU ($V_{2.0}$)	Reduction in BOD (mg/L) ($V_{0.5}-V_{2.0}$)	% reduction in BOD $\frac{(V_{0.5}-V_{2.0})\times 100}{(V_{0.5})}$
A	AS + Nitrifying BB	8.9	5	3.9	-43
A	,,	11.1	4.1	7.0	-63
A	,,	14.6	8.3	6.3	-43
A	,,	16.2	8.7	7.5	-47
A	,,	10.2	5.1	5.1	-50
A	,,	7.4	5.3	2.1	-28
B	AS	25	23	2	- 8
B	,,	22	20	2	- 9
B	,,	27	25	2	- 7
C	AS + Nitrifying BB	12.5	8.6	3.9	-31
D	AS	21.6	19	2.6	-12
E	,,	156	93.6	42.4	-27
F	,,	6	4	2	-33
G	,,	7.5	5.1	2.4	-32
H	,,	13	7	5	-38
H	,,	8.5	4.5	4.0	-47
H	,,	6.5	4.5	2.0	-31
H	,,	6.3	3.6	2.7	-43
I	,,	9	4.5	4.5	-50
I	,,	13.5	2.5	11.0	-81
J	AS + BB	8	5.5	2.5	-31
K	AS	40.5	9.8	30.7	-75
K	,,	336	174	162	-48
K	,,	24.9	5.8	19.1	-77
K	,,	17	3	14	-82
K	,,	37.2	13.8	23.4	-63
L	,,	40.2	29.2	11	-27
L	,,	63	59	4	- 6
M	,,	12.3	1.4	10.9	-88
N	BB	22	16	6	-27
O	AS + BB	15.2	10	5.2	-34
O	,,	8.4	5.6	2.8	-33
P	AS	9.4	4.6	4.8	-51
Q	,,	7.2	4.3	2.9	-40

*AS = Activated Sludge System
BB = Bacterial Bed System (Percolating Filter)

Table 4 NWW Survey (mean results in mg/L for each works)

Sewage Works Reference †	Type of Aerobic Treatment*	BOD 0.5 mg/L ATU ($V_{0.5}$)	BOD 2.0 mg/L ATU ($V_{2.0}$)	Reduction in BOD (mg/L) ($V_{0.5}-V_{2.0}$)	% reduction in BOD $\frac{(V_{0.5}-V_{2.0})\times 100}{(V_{0.5})}$
A	AS	34.8	28.2	6.6	−19
B	AS	10.5	8.2	2.3	−22
C	AS	15	8.1	6.9	−46
D	AS	17	8.8	8.2	−48
E	BB	8.4	5.6	2.8	−33
F	BB	37.9	32.6	5.3	−14
G	BB	24.7	22.3	2.4	−10
H	,,	17.5	14	3.5	−17
I	,,	15	12	3.0	−20
J	,,	20.8	18.7	2.1	−10
K	,,	33	31	2.0	− 6
L	,,	14	12	2.0	−14
M	,,	54	44	10.0	−18
N	,,	11	9	2	−18

* AS and BB as in Table 3
†Not necessarily the same works as in Table 3

When insufficient ATU is used for suppression of nitrification, the BOD could be expected to vary with the dilution used, since this would alter the concentration of both ammonium ions and nitrifying bacteria present in the BOD bottles. The following data in Table 5 give the results for four final effluent samples taken from the same sewage works in NWW which support this view.

Table 5 Partially nitrifying AS plant effluent (all results in mg/L)

General analysis (other determinands)				BOD value with: ATU 0 mg/L		0.5 mg/L		2.0 mg/L	
COD	SS	Amm N	TON	Dil 1:5	1:10	1:5	1:10	1:5	1:10
65	7	17.5	7.5	41*	40	19	13	4	4
40	11	23.3	2.8	31	26	17	14	7	—
39	9	20.9	3.1	30	24	16	12	5	6
45	10	20.4	3.1	31	25	17	16	6	6

*Residual DO < 0.2 mg/L

Tables 6 and 7 list data produced by NWW which confirm that increased levels of ATU (up to c.10 mg/L) will not suppress or enhance carbonaceous BOD. The samples analysed were either crude/settled sewages, or the glucose/glutamic acid solution used for the AQC standard. These results confirm the work of Raff (Ref 2) who showed that there was a nil or minimal decrease or increase in BOD of real samples using an added ATU concentration of 5.0 mg/L.

Table 6 Glucose/Glutamic acid standard (all results as mg/L)

	BOD value with:										
Added ATU mg/L	0	0.5	2.0	3.0	4.0	5.0	10	20	25	30	50
Nominal 20 mg/L	18.8	19.5	—			19.3	—	—	—	—	17.5
Nominal 20 mg/L	18.8	18.3	18.2			18	18.4	—	16.8	—	17.3
Nominal 220 mg/L	224	228	218	227	227	227	—	—			
	210	215	210	224	216	217	—				
	200	202	203			199	195				
	187	192	197			201	191				
	210	202	195			207	197				
	197	195	190			187	190				
	—	211	202			203	205	202	—	197	196
	211	205	211			213					
	213	213	221			215					
	188	188	213			180					
	210	217	215			215					
	200	203	200			185					
	213	203.5	199.5			205.5					
	218.5	207.5	213			211					
	221	226.5	223.5			221.5					
	185	132	168			215	215				
	185	213	235			230					
	190	225	190			240					
	206	—	238			222					
Mean*	203.81	207.04	207.47			210.21					
SD*	12.75	16.82	16.63			15.51					
D of F*	17	17	18			18					

*Of nominal 220 mg/L standards only.

Comparison of the mean values in table 6 using the Student's 't' test, detected no significant differences.

Table 7 Crude/Settled Sewage (all results as mg/L)

Added ATU mg/L	BOD value with: 0	0.5	2.0	3.0	4.0	5.0	10	sample mean	(sample range) max–min
Sample									
1	245	229	230	221	220	235		230	245–220
2	156	160	159			157	159	158	160–156
3	149	152	149			155	153	152	155–149
4	260	250	250			250		252	260–250
5	142	136	136			136	138	138	142–136
6	182	182	186			172	178	180	186–172
7	175	168	187			165	167	172	187–165
8	167	169	164	166	160	169		166	169–160
9	76	76	75			74		75.2	76–74
10	233	246	235			245		240	246–233
11	258	267	277			259		265	277–258
12	540	548	541			519		537	548–519
13	196	200	204			180		195	204–180
14	215	232	220			210		219	232–210
15	273	252	280			273		269	280–252
16	275	255	265			—		265	275–255
17	172	147	167			173	167	165	173–147
18	350	340	330			300		330	350–300
19	328	344	368			344		346	368–328
20	400	370	370			370		377	400–370
21	310	380	340			388		354	388–310
22	218	—	214			199		210	218–199
23	155	—	159			191		168	191–155
Mean*	241.3	242.4	243.4			238.7			

*excludes samples 16, 22 and 23

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Dissolved Oxygen in Waters, Amendments 1988

A few important points worthy of publication have come to light since this booklet was approved in 1979.

Contents

1. Solubility of Oxygen in Waters (a revised table)
2. Titrimetric Method (a newly reported interference)
3. Electrode Method
4. Oxygen Content of Deep Water

1 Solubility of Oxygen in Waters

1.1 The Solubility of Oxygen table (Table 1) needs slight revision in the second column.

Table 1 Oxygen solubility (Revised)

Temperature °C	Solubility of oxygen in water equilibrium with air at 101.325 kPa mg/L	Correction to be subtracted for each degree of salinity (expressed as g total salts per kg water)	Correction to be subtracted for each increment of +500 mg/L of chloride
0	14.62	0.0875	0.0787
1	14.22	0.0843	0.0759
2	13.83	0.0818	0.0736
3	13.46	0.0789	0.0710
4	13.11	0.0760	0.0684
5	12.77	0.0739	0.0665
6	12.45	0.0714	0.0643
7	12.14	0.0693	0.0624
8	11.84	0.0671	0.0604
9	11.56	0.0650	0.0585
10	11.29	0.0632	0.0569
11	11.03	0.0614	0.0553
12	10.78	0.0593	0.0534
13	10.54	0.0582	0.0524
14	10.31	0.0561	0.0505
15	10.08	0.0546	0.0491
16	9.87	0.0532	0.0479
17	9.66	0.0514	0.0463
18	9.47	0.0500	0.0450
19	9.28	0.0489	0.0440
20	9.09	0.0475	0.0427
21	8.91	0.0464	0.0418
22	8.74	0.0453	0.0408
23	8.58	0.0443	0.0399
24	8.42	0.0432	0.0389
25	8.26	0.0421	0.0379
26	8.11	0.0407	0.0366
27	7.97	0.0400	0.0360
28	7.83	0.0389	0.0350
29	7.69	0.0382	0.0344
30	7.56	0.0371	0.0334

The corrections for salinity are only applicable to sea-water or estuarine waters. Where the salinity is due to other electrolytes it may be necessary to determine the correction factors experimentally using the titrimetric method.

1.2 If pressure corrections are applied, these should be measured at the site of sampling.

1.3 An additional reference for sea water solubility effects is Benson and Krause, Limnol. Oceanogr. 29 620–632, 1984.

2 Titrimetric (Winkler) Method

The method as printed is unchanged except for the addition of an interference.

2.1 Interferences. Allylthiourea interferes

If it is necessary to calibrate an electrode against this method, the calibration should be done on water to which ATU has not been added and the measurements in the presence of ATU made using an electrode.

3 Electrode Method (See also Section 6.4 of the BOD_5 Second Edition)

The method as printed is unchanged except for the addition of the following notes.

3.1 Variation in cell output

The mercury or silver cells used often change voltage slightly during the first few hours of their life and again just before they are exhausted. After changing one of these cells, calibrate until steady readings are obtained prior to putting the instrument into routine use. Recalibrate at regular intervals during the day. For routine use, sample analysis must be started within one hour of the instrument being calibrated.

3.2 Effect of sample stirring on calibration

The rate of sample stirring can affect the electrode reading. Using standard samples, measure the readings obtained at various stirrer rates until the optimum reading is obtained, taking care neither to entrain air nor to degas the sample in the process. Some electrodes require exceedingly vigorous stirring.

3.3 Time taken for Electrodes to come to Equilibrium with the sample

The time taken for the electrode to come to a steady reading in a new sample is proportional to the difference in concentration between the present sample and the preceeding sample. This is important if the equipment is automated.

3.4 Linearity of Electrode Responses

Not all electrodes have linear response. To check the calibration of an electrode prepare a fully air saturated water and several different dilutions of it with air free water. Note the electrode readings for each of these and determine the dissolved oxygen contents of each titrimetrically. Then plot the calibration curve.

Probes may lose their sensitivity after about three weeks use, hence regular checks on their calibration is essential. If such probes are soaked in clean water for several months, they may recover all or most of their sensitivity and be usable once more after recalibration.

4 Oxygen Content of Deep Waters

The solubility of oxygen in water increases with increase in pressure and also increases with reduction in temperature; hence the maximum solubility of oxygen in a cold bottom water from considerable depth in greater than for a surface water. However, as the main source of oxygen is surface air and plant photosynthesis which is light dependent, in practice, samples with elevated oxygen levels are almost never encountered. If one were to be met, it would gas-off (probably effervesce) on bringing to the surface. Electrodes calibrated in oxgen concentration are relatively reliable at depth, ones calibrated in degree (or percentage) of saturation should not be used. To check the reliability of an electrode used at depth, take a sample of water in a standard deep water sampler, seal at depth, bring to the surface and analyse the contents by titration. Should the sample show signs of degassing, resample and either quantitatively dilute without losing gas, or discharge under the Winkler oxygen aborbing Reagent.

5 The remainder of the original Dissolved Oxygen in Waters Booklet is unchanged

Addresses 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:

The Secretary
The Standing Committee of Analysts
The Department of the Environment
Romney House
43 Marsham Street
London SW1P 3PY
England

Department of the Environment

Standing Committee of Analysts

Members of the Committee responsible for this method and the amendment.

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First Edition

1 Members of the Standing Committee, 2 Members of the Working Group, 3 Members of the Panel.

Second Edition and Amendment

4 Members of the Standing Committee, 5 Members of the Working Group, 6 Members of the Panel. 7 Occasional.

Additional for Dissolved Oxygen Amendment 8.

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