Methods for Assessing the Treatability of Chemicals and Industrial Waste Waters and their Toxicity to Sewage Treatment Processes 1982

Methods for the Examination of Waters and Associated Materials

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Contents

Warning to Users

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

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

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

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.

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

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About this series

This booklet is part of a series intended to provide 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 individual methods, thus allowing for the replacement or addition of methods as quickly as possible without need of waiting for the next edition. The rate of publication will also be related to the urgency of requirement for that particular method, tentative methods being issued when necessary. The aim is to provide as complete and up to date a collection of methods and reviews as is practicable, which will, as far as possible, take into account the analytical facilities available in different parts of the Kingdom, and the quality criteria of interest to those responsible for the various aspects of the water cycle. Because both needs and equipment vary widely, where necessary, a selection of methods may be recommended for a single determinand. It will be the responsibility of the users — the senior analytical chemist, biologist, bacteriologist etc. to decide which of these methods to use for the determination in hand. Whilst the attention of the user is drawn to any special known hazards which may occur with the use of any particular method, responsibility for proper supervision and the provision of safe working conditions must remain with the user.

The preparation of this series and its continuous revision is the responsibility of the Standing Committee of Analysts (to review Standard Methods for Quality Control of the Water Cycle). The Standing Committee of Analysts is a committee of the Department of the Environment set up in 1972. Currently it has seven 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
3.0 Empirical and physical methods
4.0 Metals and metalloids
5.0 General non-metallic substances
6.0 Organic impurities
7.0 Biological 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

31 October 1983

Methods for Assessing the Treatability and Toxicity of Chemicals and Industrial Waste Waters and their Toxicity to Sewage Treatment Processes 1982

Introduction

Industrial waste waters may, like sewage, produce harmful effects in the aquatic environment should they be discharged without treatment. At best some depletion of dissolved oxygen may occur, while at worst the receiving water may become turbid with bacterial growth, followed by death of fish, onset of anaerobic zones giving foul odours and disappearance of plant life. Some wastes could have direct toxic effects on aquatic life. It is essential, therefore, that such wastes be treated before discharge to rivers and other inland waters. Since waste waters sometimes contain substances which have detrimental effects on sewage treatment, the topics of treatability and toxicity can be conveniently examined together. Indeed, in general it has been found to be good practice to treat admixtures of sewage and industrial waste waters because of the benefits derived from the dilution by sewage of any toxic substances and from the correction of any nutrient deficiencies (N,P,K) in some industrial wastes.

In order to ascertain whether, and at what concentration, a waste water can be treated and whether it affects normal sewage treatment, tests simulating the activated sludge process and the biological filter are often applied both to the waste itself (or diluted with water) and to admixtures with sewage. Two such methods (E and F) are described in this booklet. For wastes to be discharged to the sewer, admixtures with the appropriate sewage containing 5–10 times the expected ratio of industrial waste to sewage are tested to allow a safety margin.

The main object of study in treatability trials is the removal of organic matter and ammonia. In toxicity experiments the effects on these processes are assessed. However, treatability strictly covers a wider range of factors, such as the production of harmful vapours or gases in the sewer and deleterious effects on sludge settlement and dewaterability. The latter properties can be studied, using the methods for SSVI and CST given in The Conditionability, Filterability, Settleability and Solids Content of Sludge 1983 in this series[1]; but preferably on pilot plant, rather than the laboratory-scale apparatus described here (methods E and F) because of the need for larger volumes of samples and possible effects of size of plant on the properties.

Methods simulating sewage treatment are costly and time-consuming so that cheaper, shorter methods are preferentially employed, which will give an indication of the treatability and toxicity of industrial waste waters and of individual compounds. Such methods, called screening or indicative methods (B, C and D) are based on the measurement of the rate and amount of oxygen uptake, similar in principle to bio-degradability tests, on the assumption that oxygen uptake in the presence of a waste water is proportional to substrate removal. Tests based on effects on growth of micro-organisms take more time and effort than respirometric methods. Whereas bio-degradability is more of a theoretical concept and implies no time limit (although in practical tests limits have to be applied), treatability implies that substances present in a waste water are bio-degraded within the retention period of the sewage in the treatment (or simulation) plant. Thus, conditions under which indicative tests are carried out must be such that the results obtained are relevant to full-scale treatment. Some waste waters or chemical substances will require a period of acclimatization, before yielding to oxidation, which cannot be accommodated in the indicative tests unless pre-acclimatized populations of micro-organisms are used.

Toxicity, especially of individual substances, presents a different problem. It is likely that the inhibitory effect of at least some substances is dependent on the concentration of micro-organism present, that is, on the substrate: micro-organism ratio (F/M). It is thus instructive to carry out tests at various concentrations of micro-organisms; Method B provides 10^2/ml, Methods C and D 10^6–10^8/ml. The use of various concentrations of inoculum is also necessary to assess the impact of chemicals on different parts of the

aquatic environment. It is convenient to quantify the toxic effect as EC_{50} — the concentration of substance giving 50% inhibition of the oxygen uptake of the control — though it is often sufficient to report the EC_{50} as being <0.1, <1, <10 or <100 mg/l. These values are also helpful in deciding the concentration of substance to use in bio-degradability tests.

Another criterion is the maximum concentration of the substance giving "no-effect" on the respiration rate. The direct measurement of this "no-effect" concentration is laborious, involving many determinations, and cannot be readily determined, while extrapolated values cannot easily be assessed. In the absence of a "no-effect" concentration, that concentration giving 20% inhibition is often quoted, although an inhibition of only 3% is sometimes used.

Toxic effects are estimated from the oxygen uptake of a suitable population of bacteria in the presence of a standard synthetic sewage (OECD/EEC)[2] or, for particular cases, the sewage into which the substance is to be discharged.

As with bio-degradability tests, the indicative tests can give misleading results both for treatability and toxicity. Industrial waste waters, as mentioned earlier, may appear to be untreatable in an indicative test merely because pre-acclimatization is required. With toxicity, the predictive EC_{50} may or may not be correct. It is not easy to assess the accuracy of predicted values, since insufficient data and experience have so far been accumulated. Substances (eg metals), which precipitate or adsorb on to the sludge or biological film are likely to be more toxic in simulation tests (and in practice) than in indicative tests. Substances, which cause the development of a tolerant bacterial population and/or which are bio-degraded only after acclimatization of the micro-organisms, will exhibit a lower toxicity in simulation tests than in indicative tests.

A strategy for testing potentially harmful materials is given in the accompanying diagram (Fig. 1). It is convenient to divide the waste waters into those being discharged to a natural water-course and those to a foul sewer. Thereafter, one or other of the indicative toxicity tests (Methods B, C or D) is carried out if the EC_{20} value is not known, followed by a treatability test (Methods E or F) or not, according to the EC_{20} and the tonnage or volume of the potentially harmful material. The actions "stop and report" indicate that further testing is not required and that the results already obtained are sufficient for a conclusion to be reached — either that it is safe to discharge the material or that some action must be taken to prevent, or reduce, its discharge, or to treat it at source.

Application of Toxicity and Treatability to Full Scale Plant Operation

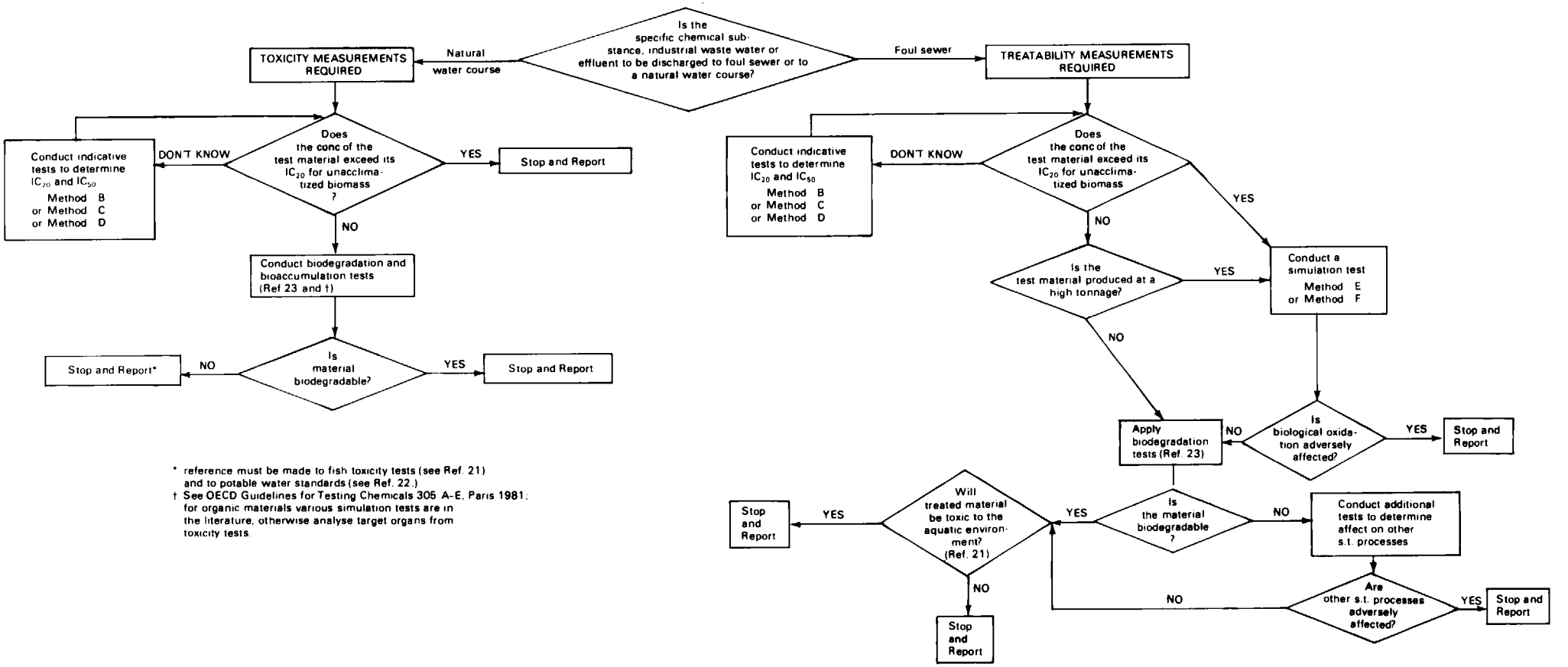
In most of the simulation tests described in this compendium experimental conditions will not exactly reflect conditions existing in large scale treatment plants. As a consequence, the results of laboratory tests may not truly describe the impact of the test substances on full scale operations. It is, therefore, important to appreciate the potential sources of divergence between experimental laboratory conditions and those pertaining to full scale operations. In the light of these considerations it may be necessary to modify standard procedures. Further, an understanding of how the standard methods described in this document can be used to quantify full scale plant operation will emerge: an example of one such application is described in the sub-section which follows this one.

In the indicative tests (Methods B, C and D) experimental conditions differ considerably from those in full scale plants. The tests are batch processes in which the progress of a biochemical reaction is observed over a period extending from hours to days on a sample to which no further additions are made. The simulation is therefore for wholly plug-flow dispersion and mixing characteristics. In full scale continuous-flow plants the mixing regime seldom approaches wholly plug flow and exhibits either completely mixed or dispersed plug-flow character.

Results from non-continuous laboratory toxicity tests will not reflect this ameliorating affect and will thus overestimate the short-term impact of the toxic substance on the full scale process.

A second important difference arises from the batch character of laboratory tests and the limited time scale of experiments. In full scale continuous-flow plants biomass is retained

Fig. 1 Testing Strategy for potentially harmful materials



* reference must be made to fish toxicity tests (see Ref. 21) and to potable water standards (see Ref. 22.)

† See OECD Guidelines for Testing Chemicals 305 A-E, Paris 1981; for organic materials various simulation tests are in the literature, otherwise analyse target organs from toxicity tests.

IC_{20} = EC_{20} = that concentration inhibiting bacterial action by 20%

in the system and this enables it to become acclimatized to the feed material. Retention may also permit the accumulation in the biomass of potentially toxic components of the feed material. The indicative tests described in this document do not necessarily allow these processes to develop to the same extent that they might in normal treatment practice. Thus unless a preacclimatized seed is used, the test may indicate that a substance will exhibit a greater toxicity than will be experienced in practice. Similarly, the failure of a short term test to model bio-accumulation may indicate a lower toxicity than is actually found in full scale plant.

A further important consideration arises from differing terminology used to express organic loading. For full scale activated sludge processes organic loading is inaccurately defined as the weight of substance (traditionally BOD) entering the aeration unit per day divided by the weight of micro-organisms (MLSS) under aeration. For a biological filter organic loading is defined as the weight of substrate administered per day to unit volume of filter medium. On the other hand, in those indicative laboratory tests which involve the loading parameter, the latter is calculated by dividing the concentration of substrate by the concentration of biological solids (F/M).

Problems of interpretation can also arise with the simulation tests (Methods E and F). In the simulation of the activated sludge process (Method E) the loading is calculated in the same manner as for a full scale process, but care has still to be taken when using the results of the test because treatment in the laboratory under constant and controlled conditions may often proceed more efficiently than on the full scale. In the rotating tube simulation of the biological filter process (Method F) the biomass is held differently to that in the full scale and this, together with closely controlled conditions, makes impossible direct comparisons of loadings. Based on the limited information currently available, an arbitrary value for the flow of synthetic or actual sewage is used in the proposed test method, but if it is considered necessary to specify the loading more closely the following procedure simulating a specific full scale plant is suggested.

Using primary settlement tank effluent as feedstock, a series of tests with different loadings is carried out and that load which simulates the actual plant performance is calculated. This loading can than be used with either synthetic or actual sewage for comparative treatability and toxicity tests.

The conditions in standard tests are so arranged that the results throw light directly on either the toxicity or the treatability of the test substance. Test substances may, however, exert purely physical effects which can influence the operation of large scale plants so as to indicate apparent toxicity or lack of treatability when none would be expected from the results of laboratory investigations. The presence of surface-active substances in fine-bubble activated sludge plants provides an illustration of this point: the influx of demonstrably non-toxic and treatable detergent can significantly reduce oxygen transfer and may thereby cause deterioration in plant performance. A false impression of toxicity or poor treatability may thereby be gained.

Substances diagnosed as non-toxic and treatable in standard tests can exhibit other deleterious effects on full scale operation. Whereas the degradation of test substances does not require that the micro-organisms should be in any particular physical state, satisfactory operation of full scale biological plants requires that the biomass should be flocculated to permit separation of supernatant from solids. Some substances have the ability to deflocculate even though they do not inhibit respiratory activity. Other materials may encourage the growth of filamentous micro-organisms thereby adversely affecting the settleability of the sludge and the efficiency of treatment.

Clearly the interpretation of standard laboratory toxicity and treatability tests requires a detailed appreciation of the process which is to be exposed to the test substances.

Use of Respirometry in the Design and Optimization of Activated Sludge Plants

Much general information is available in the literature on the oxygen requirements of wastewater treated in the activated sludge process and on the oxygen supply characteristics of aerators. Nevertheless, situations often arise when it is necessary to

determine the oxygen demand of the process with a particular sewage or the supply characteristics of aerators in individual aeration plants particularly if they exhibit poor performance or if they are to be subjected to future increases in load. In such cases, respirometric techniques are extremely useful.

Conventionally, in the design of activated sludge plants, oxygen demand is directly estimated from BOD data. However, the BOD test does not accurately model the treatment process and, depending on the nature of the wastewater and mode of operation of the process, more or less than one kg of oxygen may be required to remove one kg of BOD.

Normally, oxygenation capacities of aeration devices are determined under standard conditions in clean water. Oxygen transfer in activated sludge mixed liquor can differ significantly from that taking place in clean water, and the ratio of oxygen transfer in sewage to that in clean water is termed the α-factor.

Because of such uncertainties in design data, actual plant operation may differ significantly from design intentions. The extent of under- or over-provision of oxygen can be determined using respirometry (see methods AC and AD in part A).

The rate of oxygen supply to mixed liquor, oxygenation capacity or OC, is given by the expression

$$OC = \alpha\, K_L\, a\, (\beta C_s - C)\, V/10^3 \text{ kg/h}$$

where
- $K_L a$ is the overall oxygen mass transfer coefficient (h^{-1})
- α is the ratio of $K_L a$ in mixed liquor to $K_L a$ in clean water
- β is the ratio of the oxygen saturation concentration in sewage or mixed liquor to that in water
- C_s is the saturation concentration of oxygen in clean water at the relevant temperature and pressure (mg/l)
- C is the actual dissolved oxygen (DO) concentration (mg/l) in the aeration tank
- V is the aeration tank volume (m^3)

At any instant in time there must be a balance between oxygen input and consumption in the process such that

Rate of oxygen input	=	Rate of oxygen uptake by MLSS (Respiration rate)	+	Rate of increase of DO in the mixed liquor

$$\alpha\, K_L\, a\, (\beta C_s - C)\, V = R\, V + \frac{dC}{dt} V$$

where R is respiration rate (mg/lh)

$$\alpha\, K_L\, a = \frac{R + \frac{dC}{dt}}{\beta C_s - C}$$

If the respiration measurements are made when load to the aeration plant is fairly constant, the dissolved oxygen concentration will approximate to an equilibrium value and so $\frac{dC}{dt} \simeq 0$, when the above equation reduces to

$$\alpha\, K_L\, a = \frac{R}{\beta C_s - C}$$

From a study of the change in dissolved oxygen concentration and the respiration rate of the mixed liquor, the overall absorption coefficient can be determined. Respiration studies which are for aerator sizing purposes must be carried out during the diurnal peak loading period of the aeration plant. For most works with fairly local catchments, this period tends to be between noon and midnight, but for works serving more extended

drainage areas, the peak of the load tends to be less marked and extends further into the night.

The oxygen requirement can be compared with the BOD load treated to estimate an OC-load factor. By determining the variation in α K_La with power input to the aeration plant, it is then possible to predict the ultimate load which the plant will be capable of treating, or alternatively to predict what extension or uprating is necessary to treat the intended load.

For such respirometric determinations samples can be taken from aeration tanks and measured as in Method AC or direct measurements made in the aeration tanks as in Method AD. It should be noted that if the DO in the aeration pocket is rate limiting, ie less than 2 mg/l for nitrifying plants, and less than 0.5 mg/l for non-nitrifying plants, the respiration rate obtained in Method AC, after aeration of the sample, may exceed the existing rate in the plant. Such a measurement is therefore of value in determining the oxygen requirement at that point in the aeration plant but it cannot be used to estimate the actual oxygenation efficiency of the aerators. Method AD should be used only where the DO concentration in the aeration plant is sufficiently high for 1 mg/l DO to be left in the trapped sample at the end of the respiration determination. Otherwise, non-linear respirometric traces result and it is difficult to estimate the true non-oxygen-limited respiration rate.

Care must be taken to ensure that the sample tested is representative of the contents of the aeration pocket as a whole, bearing in mind that the mixing characteristics in the aeration pocket can lead to gradients in dissolved oxygen concentrations and non-homogeneity[3].

Unlike standard chemical analyses, respirometric determinations on a full scale aeration plant incur all the problems associated with variable seed quality, sewage nature, flow rates of sewage and re-cycled sludge and temperature. A sufficient number of determinations must be made to ensure that the data collected are sufficiently typical for the intended use of the results. For example a rise in temperature of 10°C increases the aerobic respiration rate by a factor of about 2. Therefore, respirometry measurements carried out on an aeration plant in January at 8°C should not be used directly to size aerators since good plant performance is required at peak load under the highest temperatures normally reached during the summer, possibly 15 to 20°C.

Glossary of Terms

1. Acclimatization

The processes, including selection and adaptation, by which a mixed population of micro-organisms develops the ability to degrade a substance, or develops a tolerance to it.

2. Activated sludge

A flocculated mixture of micro-organisms, other organic and inorganic material produced by the aeration of sewage and/or waste water.

3. Adsorption

The adherence of a substance to a surface (organic or inorganic) by physico-chemical processes.

4. Analogue Metabolism (a special case of co-metabolism)

The process by which a normally non-biodegradable compound is biodegraded in the presence of a structurally similar compound, which can induce the necessary enzymes.

5. Bio-accumulation

The ability of a micro-organism to assimilate and retain a chemical unaltered by normal physiological processes.

6. Biodegradability

The ability of a substance to undergo microbial attack.

7. Biodegradation

The breakdown of a substance by micro-organisms. This may be either:—

a. Primary — the alteration of the chemical structure of a substance, resulting in loss of a specific property of the substance, or

b. Ultimate — the complete breakdown of a substance to either fully oxidized or reduced, simple molecules (eg CO_2, H_2O, NO_3^-, NH_3, CH_4) and formation of new cells.

8. Bio-elimination

The removal, from the liquid phase, of a test substance in the presence of living micro-organisms by physico-chemical as well as biological processes.

9. BOD (Biochemical oxygen demand)

The amount of oxygen consumed by micro-organisms when metabolizing a compound under standard conditions of temperature and duration, normally 20°C for 5 days.

10. COD (chemical oxygen demand)

The amount of oxygen consumed during oxidation of a compound with hot acid dichromate under standard conditions; it provides an estimate of the oxidizable matter present.

11. Co-metabolism

The process by which a normally non-biodegradable substance is biodegraded only in the presence of an additional carbon source. (see also analogue metabolism).

12. A Completely Mixed System

A system in which the feed is rapidly mixed with the contents of the aeration tank so that no significant concentration gradients exist between inlet and outlet.

13. DOC (Dissolved organic carbon)

The amount of carbon present in organic compounds in aqueous solution.

14. EC_{50}

The concentration of test substance (specific compound or waste) which inhibits the measured microbial function by 50%. This parameter may be time-dependent, either increasing or decreasing with time of exposure to the test substance.

15. Inhibition

The effect(s) of a substance or its metabolites on micro-organisms which may be manifested as a reduction in oxygen uptake, substrate degradation, gas evolution or growth.

16. Inoculation

The process of adding micro-organisms to a test medium.

17. Inoculum

Micro-organisms added to a test medium.

18. Loading Factors

a. sludge loading

This term relates to the operation of activated sludge plants and is the weight of BOD applied to the plant per day per unit weight of activated sludge under aeration. This is usually expressed as kg BOD/kg MLSS.d.

b. Initial Sludge Loading

This term relates to the operation of batch processes, and is the weight of BOD added at the start of the test per unit weight of micro-organisms. This is usually expressed as g BOD/g MLSS.

c. F/M

(Food: Micro-organism) This is a somewhat confusing term, which may represent either of the above loading situations. In this document, it is used in the sense of initial sludge loading.

19. Maximum 'no-effect' concentration

The maximum concentration of a test substance at which no significant inhibition of the measured microbial function is observed (often called the minimum inhibitory concentration (MIC), or the threshold concentration).

20. Mixed liquor

The mixture of feed sewage and returned activated sludge under aeration in an activated sludge tank.

21. Mixed Liquor suspended solids (MLSS)

The concentration of activated sludge solids in the aeration tank of a treatment plant, expressed as mg dry solids/l.

22. Nitrification

The sequential oxidation of ammonium salts to nitrite and nitrate by micro-organisms.

23. Plug-flow system

A system in which the feed to an activated sludge plant passes along the length of the aeration tanks with little or no longitudinal mixing, thereby giving concentration gradients between inlet and outlet.

24. Respiration Rate

The weight of oxygen taken up in unit time by unit volume of sample (mg/lh). The specific respiration rate of a sample is the weight of oxygen taken up in unit time by unit weight of activated sludge, usually expressed as mg/g h.

25. Screening (indicative) tests

Relatively simple, cheap and rapid batch tests which may be used for preliminary assessment of the biodegradability, treatability or toxicity of a substance.

26. Simulation Tests

Tests designed to predict the effect of a substance on micro-organisms using a set of conditions relevant to a particular environment e.g. activated sludge treatment or biological filtration.

27. TOC (Total Organic Carbon)

The total amount of organic carbon in an aqueous solution/suspension.

28. TOD (Theoretical oxygen demand)

The total amount of oxygen required to oxidize a compound completely to mineral products, calculated from its formula.

29. Toxicity

The extent to which a substance adversely affects micro-organisms.

30. Treatability

The amenability of substances to removal during biological treatment without adversely affecting the normally operation of the treatment process. Adverse effects are usually: reduced removal of COD and/or DOC, inhibition of nitrification and/or anaerobic sludge digestion etc.

A. Respirometry: Basic Equipment and Techniques

Introduction

The degree to which oxygen is taken up by an industrial wastewtaer in the presence of activated sludge alone, or with sewage, is a measure of the treatability of the waste water. Also, the extent to which a waste water or a chemical depresses the respiration rate of an activated sludge-sewage mixture is a measure of the inhibitory effect of the test material.

Respiration rate measurements can be made in two distinct ways. In the first, the sample absorbs oxygen from a confined atmosphere; either the atmosphere is recharged with oxygen to maintain a constant volume (Method AA — electrolytic respirometer) or the change in volume is measured by means of a calibrated scale (Method AB — Hach respirometer). In the second method, the decrease is observed of the concentration of dissolved oxygen in the sample, which is confined so that no further replenishment of the dissolved oxygen can take place (Method AC — oxygen electrode respirometer; Method AD — as AC, with a submersible system).

It should be noted that none of these methods distinguishes between chemical and biological oxidation. If such distinctions are required, it will be necessary to devise suitable experiments. With the exception of a few chemicals and some trade wastes, the vast majority of oxygen demand is biological in nature and oxygen uptake rates are thus referred to as rates of respiration. Oxygen uptake due to nitrification and carbonaceous oxidation may be distinguished by means of a specific inhibitor of nitrification (see Method CD).

Method AA Electrolytic Respirometer

AA.1. Performance Characteristics

AA.1.1.	Property determined	Oxygen uptake (respiration) rate
AA.1.2.	Types of sample	Specific chemicals, trade waste waters.
AA.1.3.	Basis of method	Replenishment, by controlled electrolysis, of oxygen consumed by a confined, inoculated sample and air.
AA.1.4.	Range of application	0–200 mg O_2/l h
AA.1.5.	Standard deviation	No data
AA.1.6.	Limit of detection	1 mg/l h
AA.1.7.	Interferences	See Section AA.3
AA.1.8.	Time required	Usually 1–5 days Operator time: 10 h per run up to 6 units

AA.2. Principle

A measured volume of test material is stirred in a closed flask and the consumption of oxygen is determined by measuring the quantity of oxygen required to maintain constant volume in the respirometer flask. A magnetic stirrer is used to mix the contents of the flask and form a vortex which permits rapid transfer of oxygen to the sample; carbon dioxide is formed, but mostly remains in solution as carbonate or bicarbonate. Gas-phase carbon dioxide is absorbed in potassium hydroxide solution, and the fall in pressure due to this and the uptake of oxygen as a result of microbial respiration causes the interruption of an A/C circuit in a manometer — electrolytic cell device attached to the respirometer. This in turn switches a preselected stabilized D/C current through the electrolytic cell releasing oxygen at its anode which replenishes oxygen consumed in the respirometer.

AA.3. Interferences

AA.3.1. Toxic materials may cause inhibition and care should be taken to ensure that they do not enter the system except by design. Examples of materials which may be present adventitiously are chlorine from tap water and chromate from glass cleaning operations. No special precautions, other than rinsing with distilled water, are needed to ensure removal of these substances.

AA.3.2. If silicone grease is used on the ground glass joints, care should be taken to avoid its entering the main body of the flask as it will cause interference by reducing the rate of oxygen transfer.

AA.4. Hazards

Hazards associated with handling sewages and industrial wastes may be encountered, and appropriate precautions should be taken.

AA.5. Reagents

AA.5.1. **Water.** Distilled or deionized water is normally used, although in some experiments it may be necessary to use the public supply because of quality considerations, e.g. hardness.

AA.5.2. Activated sludge

The source of the activated sludge used will depend on the aim of the experiment. If the object is to test the treatability or inhibitory effect of trade wastes or chemical substances, sludge from a plant treating domestic sewage is ideal. Clearly, if the focus of attention is on nitrifying activity a nitrifying activated sludge will be required.

Thickened sludges (e.g. containing approximately 12 000 mg/l MLSS) are required. This may be achieved by settlement or slow centrifugation. For some applications it may be necessary to wash the sludge by resuspension in an isotonic solution and to settle/centrifuge.

Sludge should be aerated continuously before use to prevent the onset of anaerobic conditions, and should be kept in a water bath maintained at the temperature of the experiment.

AA.5.3. Reference Sewage

In treatability or toxicity testing a reference sewage is required. This may be domestic sewage, or a synthetic sewage such as OECD synthetic sewage[4]. Sewages of domestic origin with a BOD of 125–250 mg/l have been used successfully. For treatability and oxygen demand measurements relating to a particular plant, settle sewage from that plant will be required.

AA.5.4. Allyl thiourea solution

Inhibition of nitrification in activated sludges which have not previously been exposed to the inhibitor can be achieved by adding allyl thiourea solution (5 ml of a 2.5 g/litre solution to each litre of test sample) so as to give a concentration of inhibitor of 12.5 mg/litre.

AA.5.5. 8% Sodium hydroxide solution W/V.

AA.5.6. 20% Potassium hydroxide solution W/V.

AA.5.7. Buffer solution, pH 7.0

AA.5.7.1. *Sodium dihydrogen phosphate 128.7 g/l.*

AA.5.7.2. *Dipotassium hydrogen phosphate 330.8 g/l.*

AA.6. Apparatus

The apparatus is that developed by the former Water Pollution Research Laboratory in 1968 and originally intended for the determination of rapid respiration rates[5].

The equipment is in two parts, (i) a constant temperature water bath (180 cm × 30 cm × 17 cm) in which are immersed the six reaction flasks and, (ii) a central console (56 cm × 30 cm × 75 cm).

AA.6.1. Reaction flask

Two suitable flask systems are described

AA.6.1.1 An example of a suitable reaction flask is as follows:—

A flat-flanged reaction flask of 500 ml nominal volume (actual volume about 720 ml) and its electrolytic cell are shown in Fig. 2. The flask has a 5-necked demountable lid. The central neck is closed by a suitable screw thread cone adaptor containing a glass rod supporting a glass dish to contain the CO_2 absorbent. The other necks contain:

(i) the manometer-electrolytic cell

(ii) a stopcock

(iii) a stopper, and

(iv) optionally a dissolved oxygen (DO) probe, which is otherwise replaced by a similar stopper to (iii).

The stoppers are extended to reduce the volume of the gas phase.

PTFE sleeves and gaskets should be used to give an air tight seal at the ground glass joints. This avoids the use of silicone grease which can cause interference (see Section AA.3).

AA.6.1.2. A simpler two or three necked non-flanged flask has also been successfully used. Use a standard two or three necked flask fitted with ground glass joints preferably with the central neck wide for cleaning. One neck carries the cell system (II in Figure 2). The glass dish on (III) is hung below the stopper (I) which also carries the tap (V). If an indicator marker with electrode is used, the third side neck is used; this may also carry the tap (V) instead of it being mounted on the stopper.

AA.6.2. Electrolytic — Manometer Cell

The electrolytic cell is shown in detail in Fig. 2. Three platinum electrodes are incorporated in the cell; one acts as a level detector whilst the other two comprise the anode and cathode of the electrolytic cell. The cell electrolyte is 8% w/v sodium hydroxide.

AA.6.3. Stirrers

The contents of the reaction flasks are stirred by PTFE coated slave magnets ("Followers"). The stirrer drive motors are placed beneath the water bath and thus must be capable of effective magnetic coupling not only through the flask wall, but through the water bath as well. Not all shapes of slave magnet are suitable: an egg-shaped spinbar and 32 mm "circulus" have been used successfully in the round-bottomed flasks.

Very rapid stirring (at rates of the order of 1000 rev/min) is needed to achieve the necessary rates of oxygen transfer with the more actively respiring samples. Two types of magnetic stirring motor are available, one electrical and one driven by compressed air. Experience has shown that the air driven motor may be more reliable than the electric motor, and that both should be fitted flush with the bottom of the bath. The bottom of the water bath is "dished", both to locate the reaction flasks centrally over the motors, and to minimize the distance between the master and slave magnets.

AA.6.4. Control Console

The control console contains all the necessary power supplies, circuitry, controls, and printing counters. As each channel is independent, a single timer cam could be used to operate all channels simultaneously but in order to reduce power requirements individual cams, arranged in sequence, are provided.

The solid state logic and printing counter drive circuits are assembled on three plug-in printed circuit boards for each channel, the total of eighteen cards being contained in a card frame at the top of the unit. Commercially available items have been used throughout and for ease of assembly no chassis is employed, all components being mounted on the base and panels.

Details of design and construction of the control console are available from the Water Research Centre on request.

AA.6.5. Basis of Operation

The consumption of oxygen by the sample causes a reduction in the volume of the gas phase and the level of electrolyte falls in the outer annulus of the cell. Ultimately the level falls below that of the side contact and a small AC current flowing between the contact and the cathode ceases. A standard cam timer (synchronous motor driven) allows a logic circuit to interrogate the condition of the AC "probe" circuit once every minute and on the next operation after the AC current has ceased, the logic circuit causes the electrolytic current to be switched on at a pre-set value, and the time, in minutes from the start of the experiment, to be printed out by an electro-mechanical printer. If the oxygen supplied by electrolysis is sufficient to re-make the AC circuit, the electrolytic current ceases at the end of 1 min., otherwise electrolysis continues for further consecutive periods of 1 min. until the AC circuit is re-made, the printer operating at the beginning of each minute of electrolysis.

The results are obtained as a series of printed times, each corresponding to a known increment of oxygen.

Step	Procedure	Notes
AA.7.1.	**Preparation of Apparatus**	
	Assemble the six reaction flasks and place them in the constant temperature water bath. Add 8% sodium hydroxide solution to the electrolysis cells to just below the level of the sensing probes. Switch the power on and adjust the individual current for each channel (note a). Allow 1–2 mg oxygen to be supplied to each vessel by suitable adjustment of the level of the electrolyte. Leave the flasks for a period e.g. overnight and, if the flasks are airtight, no further evolution of oxygen should take place. Although one stopper on each flask will have to be moved again, this procedure considerably reduces the possibility of overlooking leaks.	(a) The current normally used is 402 mA which corresponds to the evolution of 2 mg of oxygen in 1 min, or 201 mA, 1 mg O_2 in 1 min.
AA.7.2.	**Preparation of Activated Sludge**	
	Collect the mixed liquor on the day of the test and prepare as described in section AA.5.2.	(b) The inoculum should be a small proportion of the total volume and therefore high suspended solids are required e.g. 12 000 mg/l.
	Determine the suspended solids (or volatile suspended solids) of the thickened activated sludge (see method already described in this Series Ref 8). Place the thickened activated sludge in the water bath and aerate until use (note b).	
AA.7.3.	**Characterization of Test Materials**	
	Determine the BOD and COD of the test and reference materials by standard methods in this series[10, 24].	
AA.7.4.	**Preparation of Flasks**	
	Calculate (note c) the required volumes of reference sewage, test material, ATU (if needed) and water and add to the flasks (see Tables in Method D). Add 10 ml of each buffer (AA.5.7.1. and AA.5.7.2. — note d). The final volume in the flask should be such as to leave a gas space of not less than 100 ml nor more than 170 ml; 550–620 ml of liquid is usually suitable (note e).	(c) The volume calculated should provide a constant organic (COD) load in each flask. (d) 10 ml of the buffer solutions at the given strength will give concentrations of 0.015M sodium dihydrogen phosphate and 0.025M dipotassium hydrogen phosphate if the final volume is 550 ml.
	Start the magnetic stirrers and adjust so that a smooth rapid stirring in the flask is obtained. Switch on the electrolysis cells and allow to run for 15 minutes at 201 mA or 7 min at 402 mA. During this period add a volume of inoculum to give the required concentration of MLSS. Add 5 ml of 20% potassium hydroxide solution (AA.5.6.) to the glass dish using a syringe.	(e) Increasing the volume of liquid reduces errors but also reduces the rate of solution of oxygen. Work with the minimum gas space for measuring respiration rates at the lower end of the range, and vice versa.
AA.7.5.	**Measurement of Oxygen Uptake**	
	Insert the stoppers and close the stopcocks. Finally, press the 'reset' button on the printers and allow the apparatus to run.	
	Make good any evaporative losses from the electrolytic cell by the addition of water. If the	

Step	Procedure	Notes
	oxygen uptake exceeds 250 mg, put fresh potassium hydroxide solution into the carbon dioxide absorber. Usually no further attention is required other than occasional checks to see that adequate stirring is maintained.	
AA.7.6.	From the print-out of times of oxygen production (2 mg if 402 mA for 1 min and pro rata), plot a respiration curve.	

AA.8. Sources of error

AA8.1 Barometric pressure

An increase in barometric pressure reduces the volume of the gas phase and also causes more air to dissolve, causing the electrolytic cell to supply oxygen to restore the original volume of gas. Similarly, a decrease in pressure retards the operation of the cell. A correction may be made graphically, using the results of a control experiment, or by calculation. A correction of 1 mg oxygen is necessary for a change in pressure of 5 mm Hg with 620 ml liquid and 110 ml of gas, while 550 ml liquid and 180 ml gas the correction is 1 mg per 3 mm Hg. It is imperative to make these corrections or to allow for them in a control experiment using the same gas/liquid ratio as in the test.

AA.8.2. Oxygen deficit

Errors due to the development of an oxygen deficit in the liquid phase become evident only when the respiration rate is high.

AA.8.3. Time scale

Because the sample should reach equilibrium in the reaction flask before readings are started an uncertainty may arise in the time scale. This would be small for biodegradability assessments but can be almost certainly eliminated by previously equilibrating the buffered samples with air at the temperature of the experiment.

Method AB. Manometric Respirometer (Hach)

AB.1. Performance Characteristics

AB.1.1.	Property determined	Oxygen uptake (respiration) rate.
AB.1.2.	Types of sample	Specific chemicals, trade waste waters.
AB.1.3.	Basis of method	Oxygen consumption of a confined inoculated sample plus air is measured by means of a calibrated closed-end manometer.
AB.1.4.	Range of application	Up to 700 mg oxygen/l in total.
AB.1.5.	Standard deviation	Not available.
AB.1.6.	Limit of detection	Depends on sample volume and scale chosen eg. 416 ml, scale 0–35 limit = 0.5 mg/l; 240 ml, scale 0–350 limit = 5 mg/l; 93 ml, scale 0–700 limit = 10 mg/l.
AB.1.7.	Interferences	See section AB.3.
AB.1.8.	Time required	Operator time: 10 h per run up to 6 units

AB.2. Principle

A measured volume of test material is stirred in a partially filled bottle which is connected to a closed-end mercury manometer. Oxygen consumption is measured by observing the change in level of the mercury column in the manometer. Carbon dioxide evolved into the bottle atmosphere is absorbed in alkali held in a small cup within the bottle cap. Temperature control is normally achieved by placing the equipment in an incubator.

AB.3. Interferences

AB.3.1. Toxic materials may cause inhibition and care should be taken to ensure that they do not enter the system except by design. Examples of materials which may be present adventitiously are chlorine from tap water and chromate from glass cleaning operations. No special precautions, other than rinsing with distilled water, are needed to ensure removal of these substances.

AB.3.2. If silicone grease is used on the rims of the bottles, care should be taken to avoid its entering the main body of the flask as it will cause interference by reducing the rate of oxygen transfer.

AB.4. Hazards

Hazards associated with handling sewages and industrial wastes may be encountered, and appropriate precautions should be taken.

AB.5. Reagents

AB.5.1. Water

Distilled or deionized water is normally used, although in some experiments it may be necessary to use the public supply because of quality considerations, e.g. hardness.

AB.5.2. Activated sludge

The source of the activated sludge used will depend on the aim of the experiment. If the objective is to test the treatability or inhibitory effect of trade wastes or chemical substances, sludge from a plant treating domestic sewage is ideal. Clearly, if the focus of attention is on nitrifying activity a nitrifying activated sludge will be required.

Sludges containing approximately 3000 mg/l MLSS are required. This may be achieved by settlement or slow centrifugation. For some applications it may be necessary to wash the sludge by resuspension in an isotonic solution and settle/centrifuge.

Sludge should be aerated continuously before use to prevent the onset of anaerobic conditions, and should be kept in a water bath maintained at the temperature of the experiment.

AB.5.3. Allyl thiourea solution

Inhibition of nitrification in activated sludges which have not previously been exposed to the inhibitor can be achieved by adding allyl thiourea solution (5 ml, 2.5 g/litre) to each litre of test sample so as to give a concentration of inhibitor of 12.5 mg/litre.

AB.5.4. 20% Potassium hydroxide solution W/V.

AB.5.5. Dilution water

AB.5.5.1. *Solution A:*

Potassium dihydrogen phosphate, 8.5 g; di-potassium hydrogen phosphate, 21.75 g; disodium hydrogen phosphate dihydrate, 33.4 g; ammonium chloride, 20.0 g; dissolved in distilled water and made up to 1000 ml. The pH value should be 7.2.

AB.5.5.2. *Solution B:*

Magnesium sulphate heptahydrate, 22.5 g dissolved in distilled water and made up to 1000 ml.

AB.5.5.3. *Solution C:*

Calcium chloride dihydrate, 36.4 g dissolved in distilled water and made up to 1000 ml.

AB.5.5.4. *Solution D:*

Ferric chloride hexahydrate, 0.25 g dissolved in distilled water and made up to 1000 ml.

The dilution water is made up to contain 3 ml of each of solutions A–D per litre of distilled water.

AB.5.6. Reference sewage

A reference sewage is required which may be of domestic origin or a synthetic sewage, e.g. OECD synthetic sewage[4]. To study the effect of the test material on a particular plant sewage supplied to that plant should be used.

AB.6. Apparatus

AB.6.1. The apparatus used for these studies is a constant volume respirometer, measuring oxygen uptake manometrically. Each individual unit (Fig. 3) consists of a dark glass bottle connected to a closed-end mercury manometer. Carbon dioxide produced during respiration is absorbed by potassium hydroxide placed in the seal cup and the oxygen taken up is measured as a decrease in pressure by reading directly the BOD (mg/l) on a scale*. The contents of each bottle are individually stirred by a PTFE covered stirrer-bar driven by magnets attached to a motor.

A commercially available form of the apparatus is manufactured by the Hach Chemical Co., Ames, Iowa, US, whose agents in the UK are Camlab Ltd, Cambridge.

AB.6.2. Basis of Operation

The appropriate volume of sample material (industrial waste water, sewage etc) is introduced into the bottle, buffer, dilution water and inoculum are added as required, and connection made to the manometer. Thermal equilibrium is allowed to become established, then the manometer is sealed, and readings are commenced. The equipment would normally be placed in an incubator to ensure that measurements are made at a defined temperature.

*Mathematically calibrated by the method given in Section AB.9.

AB.6.3. Seed concentration

The concentration of seed micro-organisms is normally 1500 mg/l. The MLSS of the activated sludge is determined on the day of use and volume required per litre of dilution water is calculated as follows:

$$V = \frac{X \times 1000}{y}$$

where V = volume of seed ml per litre of dilution water
X = Desired seed concentration (normally $\geq$ 1500 mg/l)
y = MLSS of activated sludge (mg/l)

AB.6.4. Volume of test medium

The volume of the test medium and hence the amount of test compound that must be dispensed to each bottle depends on the anticipated BOD of the sample under test. The theoretical oxygen demand can usually be calculated for pure compounds or formulated products of known composition or the chemical oxygen demand (COD) may be measured if no information is available. Table 1 indicates the appropriate volume — scale relationship.

AB.7. Procedure

Step	Procedure	Notes
	Effluents and water soluble compounds	
AB.7.1.	For each sample and reference sewage to be tested, calculate the total volume of dilution water required (note a) and half fill a measuring cylinder of appropriate volume with aerated distilled water.	(a) The volume required depends on the anticipated BOD (See section AB.6.4). The greater the oxygen uptake expected, the larger the volume of air required to satisfy the demand and hence the smaller the volume of solution to be tested in each bottle.
AB.7.2.	Add to this measuring cylinder 3 ml per litre (final volume) of each of the solutions 5.5.1–5.5.4. followed by the appropriate volume of activated sludge and as calculated above (AB.6.3) (note b)	(b) The activated sludge can be varied from the level stated. Also the seed can be obtained from other sources (e.g. laboratory units acclimatized to the test compound) depending on the objectives of the experiment.
AB.7.3.	Finally add the appropriate volume of sample and/or reference sewage (note c) and make the total volume in the measuring cylinder up to the required level with distilled water.	(c) The volume calculated should provide a constant organic load (COD) in each flask.
AB.7.4.	Mix thoroughly and dispense the required volume into each of three Hach bottles (note d).	(d) Normally three replicate bottles are used to ensure reproducibility. If equipment availability is limited, fewer bottles can be used.
AB.7.5.	Set up the blank Hach bottles (seed and dilution water for treatability and additionally reference sewage for toxicity). The blank volume added to each bottle is normally 416 ml and the control volume, 157 ml.	
AB.7.6.	Place a magnetic follower in each bottle and then apply a thin smear of grease around the rim of the bottles (note e)	(e) Ensure that the grease does not come into contact with the test solution where it could interfere.
AB.7.7.	Place a sealed cup to which has been added 2 drops of 20% w/v KOH (note f) in the mouth of each bottle and then appropriately position the bottles on the Hach stirring apparatus.	(f) The potassium hydroxide absorbs any carbon dioxide gas produced.

Step	Procedure	Notes
AB.7.8.	Apply a further smear of grease to the top of the seal cup and then switch on the stirrer motor checking that each bottle is correctly agitated.	
AB.7.9.	Open the manometer caps, check that each manometer contains 4–5 drops of water and then lightly grease each manometer cap seal before loosely replacing on the manometer body (note g).	(g) The closed-end manometers compensate for changes in atmospheric pressure.
AB.7.10.	Screw down the bottle caps loosely and then leave the whole apparatus to reach thermal equilibrium (30–60 minutes) (note h).	(h) This time can be shortened by bringing all solutions to the operating temperature before filling the bottles.
AB.7.11.	After this time period, tighten both bottle and manometer caps, loosen the manometer scale screws and set the zero mark on the scale level with the top of the mercury column. Tighten scale screws.	
AB.7.12.	Record time and date. After a further 30 minutes check the reading on the manometer. If either a negative or a positive reading has resulted in any of the manometers briefly loosen both manometer and bottle caps then reclose. Repeat AB7.11.	
AB.7.13.	Continue to record manometer values daily until the end of the experiment and plot the respiration curve.	
	Viscous or solid samples (not water soluble)	
AB.7.14.	Follow steps AB.7.1. to AB.7.2.	(i) In most cases the volume of compound to be added is an insignificant proportion of the seeded dilution water and need not be taken into account.
AB.7.15.	Make up the volume of dilution water to the appropriate level with distilled water and/or reference sewage (note i).	
AB.7.16.	Place viscous samples directly into the Hach bottles then add the required volume of dilution water.	
AB.7.17.	For solid samples, place the required volume of dilution water into a clean wash bottle and then wash the sample into the appropriate Hach bottle.	
AB.7.18.	Follow steps AB.7.5 to AB.7.13.	

AB.8. Sources of Error

AB.8.1. Barometric pressure

Unlike most manometric respirometers, this version is not subject to errors due to changes in barometric pressure because of the closed-end manometer.

AB.9. Calibration of the manometer scale on the Hach respirometer

For a scale reading change of x cm during the experiment the weight (mg) of oxygen absorbed by the volume of liquid, v, is calculated as follows:—

BOD (wt in mg of O_2 absorbed by volume v) =

$$\frac{32\ T_N \cdot x}{22.4.\ T_1 \cdot P_N}\ \left[V_1 + P_1\ \frac{\pi d^2}{4} - \frac{\pi d^2\ x\ .}{4}\right]$$

where T_N = standard temperature (°K)
T_1 = temperature of experiment (°K)
P_N = standard pressure (cm of mercury)
P_1 = atmospheric presure (cm of mercury)
d = diameter of sight glass tubing
V_1 = volume of air in vessel A plus the length (L) of flexible tubing, at the start of the experiment.

$$V_1 = V - v + \frac{\pi D^2 L}{4}$$

where V = volume of glass vessel A
v = volume of liquid in glass vessel
D = diameter of flexible tubing
L = length of flexible tubing

AB.9.1. Calibration procedure

The manometer sight glass tube is of constant diameter (d = 0.15 cm) and a calibration scale is provided by the manufacturer (20 divisions = 5.1 cm). The length of the 'Tygon' tubing is constant (25 cm), as is its diameter (0.65 cm). The volume of the glass bottle is constant (500 ml) and the volume of the liquid sample (v) is known. From these data the BOD corresponding to a scale division can be calculated at the start of the experiment for a given temperature and pressure.

Normally the temperature is kept constant throughout the experiment and the apparatus and the test solutions are brought up to this temperature at the start. Theoretically corrections should be made to the calibration for the atmospheric pressure prevailing at t = 0. In practice this is not done and a change in pressure (ΔP) from the assumed norm (76 cm Hg) will cause an error in BOD reading of about

$$\frac{\Delta P}{(P_1 + \frac{4\ V_1}{\pi\ d^2})}\ \times 100\%$$

For tubing of 0.15 cm diameter this will always be negligible.

Once the apparatus is in operation correction for changes in barometric pressure do not need to be made to the daily readings.

Table 1: Volume — scale relationships and conversion factors for Hach BOD bottles

Sample volume (ml)	Selected[a] scale	Conversion[b] factor	Expected BOD[c] (mg/l)
416	0 - 35	1.0	0 - 35
416	0 - 350	0.1	0 - 35
352	0 - 70	1.0	0 - 70
352	0 - 350	0.2	0 - 70
305	0 - 350	0.3	0 - 100
293	0 - 350	0.33	0 - 150
240	0 - 350	0.5	0 - 175
190	0 - 350	0.75	0 - 250
157	0 - 350	1.0	0 - 350
123	0 - 350	1.4	0 - 500
123	0 - 700	0.7	0 - 500
93	0 - 350	2.0	0 - 700
93	0 - 700	1.0	0 - 700

Notes

(a) The Model 2173 apparatus only has a scale from 0–350. The Model 2173 A scale can be varied as follows: 0–35; 0–70; 0–350; 0–700.

(b) The averaged results must be multiplied by this correction factor before blank correction.

(c) Where there is an alternative; it is advantageous to take the largest volume possible with the smallest scale range. This makes scale reading easier and minimizes errors in multiplication.

Method AC. Oxygen electrode

AC.1. Performance Characteristics

AC.1.1.	Property determined	Oxygen uptake (respiration) rate.
AC.1.2.	Types of sample	Specific chemicals, trade waste waters.
AC.1.3.	Basis of method	Decrease in dissolved oxygen concentration with time of a well aerated, confined inoculated sample, excluding gaseous phase, is measured using an oxygen electrode.
AC.1.4.	Range of application	0–4 mg O_2/l min; 0–240 mg/gh for sludge containing 1,000 mg MLSS/l.

AC.1.5. Standard Deviation

	Sample	Respiration rate mg/gh mean	SD	n
1.	Sludge alone (5250 mg MLSS/l)	8.0	0.2	4
2.	(1) Washed and centrifuged four times	16.5	2.4	4
3.	(2) Plus sewage	44.0	2.8	4

AC.1.6.	Limit of detection	0.05 mg O_2/l min
AC.1.7.	Interferences	See Section AC.3.
AC.1.8.	Time required	Operator time: 15 min for one determination

AC.2. Principle

Suitably aerated samples are introduced into a sample vessel containing an electrode which detects the decrease in DO concentration which is recorded as a function of time. The slope of the resulting line is the respiration rate in mg O_2/litre per unit time. By measuring the mixed liquor suspended solids (MLSS) or volatile solids (MLVSS) of the samples under test[1,8], it is possible to express respiration as specific oxygen uptake rate, mg O_2/g ML(V)SS hour.

AC.3. Interferences

AC.3.1. Toxic materials may cause inhibition and care should be taken to ensure that they do not enter the system except by design. Examples of materials which may be present adventitiously are chlorine from tap water and chromate from glass cleaning operations. No special precautions, other than rinsing with distilled water, are needed to ensure removal of these substances.

AC.3.2. Some substances may affect the calibration of the electrode; this may be checked, if suspected, by appropriate experiments in the absence of bacteria.

AC.4. Hazards

Hazards associated with handling sewages and industrial wastes may be encountered, and appropriate precautions should be taken.

AC.5. Reagents

AC.5.1. Water. Distilled or deionized water is normally used, although in some experiments it may be necessary to use the public supply because of quality considerations, e.g. hardness, in which case test for residual disinfectant[6] and remove if necessary.

AC.5.2. Activated sludge

The source of the activated sludge used will depend on the aim of the experiment. If the object is to test the treatability or inhibitory effect of trade wastes or chemical substances,

sludge from a plant treating domestic sewage is ideal. Clearly, if the focus of attention is on nitrifying activity a nitrifying activated sludge will be required.

For general use, sludges containing approximately 3000 mg/l MLSS are required. This may be achieved by settlement or slow centrifugation. For some applications it may be necessary to wash the sludge by resuspension in an isotonic solution and settle/centrifuge. Sludge should be aerated continuously before use to prevent the onset of anaerobic conditions, and should be kept in a water bath maintained at the temperature of the experiment.

AC.5.3. Reference sewage

A reference sewage is required which should be of domestic origin, but OECD/EEC[4] synthetic sewage may also be used. To study the effect of the test material on a particular plant, the sewage supplied to that plant should be used.

AC.5.4. Allyl thiourea solution

Inhibition of nitrification in activated sludges which have not previously been exposed to the inhibitor can be achieved by adding allyl thiourea solution (5 ml, 2.5 g/litre) to each litre of test sample so as to give a concentration of inhibitor of 12.5 mg/litre

AC.5.5. Dissolved oxygen electrode zero calibration solution. Prepare and use a solution of the composition recommended by the electrode manufacturer.

AC.6. Apparatus

AC.6.1 Equipment is required to confine the sample so that it is not in contact with air, to stir the sample, and to measure the decrease in concentration of dissolved oxygen within it. This can be assembled from general purpose laboratory equipment or, alternatively, proprietary equipment can be purchased and suitably adapted.

One type of proprietary device, which is readily available, is the Rank Respirometer, and this can be obtained in cell capacities ranging from 7 ml to 50 ml.

A suitable system can also be constructed from standard laboratory glassware, such as a conical flask or BOD type bottle, incorporating a DO probe, such as is available from Yellow Springs Instruments (YSI), Electronic Instruments Ltd (EIL), or International Biophysics Corporation (IBC). Typical arrangements are shown in Figures 5 and 6.

The Rank electrode requires a suitable operating circuit, as shown in Figure 4 and any chart recorder with a sensitivity in the range 1–20 mV full scale deflection (fsd).

The YSI and IBC probes require their associated DO meters but the EIL probe can be connected to any suitable pH or millivolt (mV) meter. The YSI and IBC instruments should be connected to a suitable chart recorder, preferably having 100–500 mV fsd sensitivity range, using the circuit shown in Figure 5. The EIL probe should be used with a meter which has an output signal compatible with the sensitivity range of the available chart recorder.

For consistent results, it is important to maintain the test vessel and contents at a constant, known temperature since slight temperature variations may lead to significant changes in concentration of dissolved oxygen, and to a lesser extent in respiration rate.

AC.6.2. Basis of operation

The cell or bottle is filled with the sample under test, the DO probe and/or stopper is inserted, and the rate of decrease of dissolved oxygen concentration is measured, ideally by recording on a suitable chart recorder.

Step	Procedure	Notes
	Set up the oxygen electrode according to the manufacturers' instructions.	
	Calibration of Respirometer	
AC.7.1.	Zero calibration	
AC.7.1.1.	*Rank type* Fill the cell with the zero DO calibration solution (AC.5.5), insert the magnet and stopper and start the stirrer motor. Adjust the recorder zero offset control so that the recorder pen is set at scale zero.	
AC.7.1.2.	*YSI and IBC types* Fill the bottle or flask with the zero DO calibration solution, insert the probe, and start the stirrer motor. Move the function switch to DO 10 mg/l range. The meter needle should fall to not more than 0.2 mg/l. If it does not, the electrode requires servicing (note a). Adjust the recorder zero offset control so that the recorder pen is set at scale zero.	(a) If the needle does not indicate zero, do not adjust the zero control to make it indicate zero. Refer to the manufacturer's handbook on electrode servicing.
AC.7.1.3.	*EIL type* Fill the bottle or flask with the zero DO calibration solution, insert the probe and start the magnetic stirrer. Select the pH range and adjust the buffer control until the meter needle indicates zero (note b). Adjust the recorder zero offset control so that the recorder pen is set at scale zero.	(b) If there is insufficient adjustment on the buffer control to permit this, as is likely to be the case with pH/mV meters other than EIL models 7010/7020, 7030 or 7050, the mV range should be used instead.
AC.7.2.	Air-saturated water calibration Prepare a sample of clean air-saturated water at the required temperature. Determine the dissolved oxygen concentration in the sample by the method described in this series (note c). a) *Rank type* Thoroughly rinse the cell and then fill with the air-saturated water, insert the magnet and stopper and start the stirrer motor (note d). Adjust the circuit potentiometer 'fine' control until the recorder pen indicates the correct value as determined for DO concentration.	(c) See Ref 7. (d) Sensor response varies with stirring speed below a minimum critical value. This value should be determined by increasing the stirrer speed until no increase in meter reading occurs and the reading is steady.

Step	Procedure	Notes
	b) *YSI and IBC types* Rinse the bottle or flask well and then fill it with clean air-saturated water. Insert the DO probe and start the stirrer. With the function switch on the appropriate DO concentration range, adjust the meter reading using the DO calibration control until the meter needle indicates the correct DO concentration (note e).	(e) The stirrer speed is not adjustable but the standard speed is sufficient to give an optimum response.
	YSI Adjust the circuit potentiometer until the chart recorder indicates the correct DO concentration also.	
	IBC The recorder should give the same readings as the DO meter, if not, the electrode requires servicing.	
	c) EIL Type Rinse the bottle or flask thoroughly and then fill it with clean air-saturated water. Insert the DO probe and start the stirrer motor (note f).	(f) If stirrer speed is too slow, a bumpy recorder trace will be obtained during a respirometric run. Ideally, the trace should be smooth and straight.
	Turn the scale length control on top of the probe fully anti-clockwise and allow the meter reading to stabilize for about five minutes. When a steady reading is obtained, slowly turn the scale length control clockwise until the meter reading corresponds to the dissolved oxygen concentration present in the sample (note g). The normal scale calibration range is 0–14 mg/l. The chart recorder should now also read the correct value without further adjustment.	(g) If it does not, the recorder input does not match the output signal from pH/mV meter. In this case the recorder sensitivity range should be changed.
	The pH/mV meter should now read the correct value for DO concentration (note h). The chart recorder should also read the correct value for DO concentration.	(h) This will be the case with EIL meters such as models 7030/7050. Meters with separate slope/offset controls will require further adjustment. In this case, the slope control should be adjusted until the meter needle indicates the correct value of DO concentration in the air-saturated water.
	Preparation of Test Samples	
AC.7.3.	Bring the aerated sludge, reference sewage and test solutions to the required temperature and then mix them in the necessary proportions to yield the test samples.	
	Measurement of Respiration Rate	
AC.7.4.	Transfer sufficient sample completely to fill the cell, bottle or flask. (note i).	(i) The mixed samples should contain at least 5 mg/l of dissolved oxygen and if necessary should briefly be vigorously aerated prior to transfer to the respirometer.
	a) *Rank type* Insert the stirrer magnet and the stopper (note j).	(j) In all cases, a small quantity of sample will be displaced from the respirometer vessel so that air is effectively excluded from the test sample.
	b) YSI and IBC Insert the DO probe.	

Step	Procedure	Notes
	c) EIL type Insert the stirrer magnet and the DO probe.	
	Switch on the stirrer, and the recorder chart drive. Allow the test to proceed until a sufficient chart trace has been obtained to allow the gradient of the line to be determined accurately (note k).	(k) For typical sewage and/or activated sludge mixtures, a suitable chart drive speed is 10 mm/min. An adequate recorder trace is normally obtained within 10 minutes. See also note h.
	Caution should be exercised when the uptake exceeds 2 mg/l min, since the response time of the electrode may mask changes in the actual DO, and lead to an underestimate of the actual respiration rate.	
	Stop the recorder chart drive. Thoroughly rinse the cell, bottle or flask. (note l).	(l) Otherwise cross contamination may occur and cause erroneous results.
	Determination of MLSS	
AC.7.5.	Determine the MLSS of the relevant samples by the method described in this series (notes m and n).	(m)See Refs. 1 and 8. (n) Care should be taken to ensure that the specimens withdrawn for solids determination are drawn from well-shaken liquors.
	Calculation of Results	
AC.7.6.	Measure the slope of the recorded respiration trace and calculate the result as mg O_2/l h. Divide this result by the MLSS concentration (g/l) to obtain the specific respiration rate as mg O_2/g h (note o).	(o) The recorder trace may not be linear during the initial period of a respiration determination. Also if a determination continues until the DO concentration falls below 1 mg/l and becomes rate limiting, the slope again deviates from linearity. The central linear portion (7–2 mg O_2/l) of the trace should be used for the calculation of the respiration rate.

Method AD. Submersible Electrode Respirometer

AD.1. Performance Characteristics

AD.1.1.	Property determined	Oxygen uptake rate (respiration rate).
AD.1.2.	Type of sample	Activated sludge mixed liquor.
AD.1.3.	Basis of method	Decrease in dissolved oxygen concentration with time is measured using an oxygen electrode, of a confined sample of mixed liquor in situ in an aeration tank of an activated sludge plant.
AD.1.4.	Range of application	No data.
AD.1.5.	Standard deviation	
AD.1.6.	Limit of detection	
AD.1.7.	Interferences	See AD.3.
AD.1.8.	Time required	About 5 min per test.

AD.2. Principle

The respirometer is lowered horizontally into the liquid under examination. The sample is admitted to the test chamber by moving a Perspex sleeve pneumatically, the sleeve then being moved back to its original "closed" position, thereby confining the sample. The test chamber contains a stirrer and DO electrode, and is thus in principle very similar to the equipment of Method AC. The measurement of solids content and method of expression are also similar to Method AC.

AD.3. Interferences

Air may be entrapped which would give false results.

AD.4. Hazards

AD.4.1. Hazards associated with handling sewages and industrial wastes may be encountered and appropriate precautions should be taken.

AD.4.2. Fouling of the connecting tubes and leads, between the submersed electrode cell and the meter and recorder, with aerators, etc must be avoided.

AD.4.3. Take care not to fall in the tank.

AD.5. Reagents

AD.5.1. Distilled Water. Saturated with air at the temperature of the experiment.

AD.5.2. DO electrode zero calibration solution. Prepare and use a solution of the composition recommended by the electrode manufacturer.

AD.6. Apparatus

AD.6.1. The WRC submersible respirometer is very similar in many ways to the system described under Method AC. However, it is designed specifically to be used *in situ* for measurements on, for example, activated sludge plants, at the ambient temperature of the sample. Provision has been made to confine the sample out of contact with air and the surrounding liquid, and to stir the sample, but no provision has been made for sample aeration. DO measurements are made with an electrode system, and the mechanical operations required to take and confine the sample are carried out below water level by use of a pneumatic system. A diagram of the apparatus, which is used with the major axis horizontal, is given in Figure 7.

The test chamber has a volume of approximately 250 ml, and contains a DO electrode, and stirrer. It consists of a cylindrical Perspex shield which can be made to slide back and forth pneumatically, together with two end plates.

AD.6.2. Basis of operation

Sample is admitted to the test chamber by withdrawing the Perspex shield. At the same time as the shield is withdrawn, the DO electrode (EIL type 1521 or YSI 5739) is caused to be moved past the seal in the end plate which carries the electrode, thereby cleaning the membrane surface. When the shield is restored to its original position, the sample is confined out of contact with air and the surrounding liquid. The integral magnetic stirring system ensures that liquid velocities in the cell are sufficiently high for the DO probe to give true indications, assuming that respiration rates are not too high. Readings from the probe are taken with an EIL Model 1520 portable DO meter.

AD.7. Procedure

Step	Experimental Procedure	Notes
	Standardization of Respirometer	
	Zeroing the DO Probe	
AD.7.1.	Fill a suitable container with zero DO calibration solution, immerse the respirometer, and fill the sample chamber with the solution. Start the magnetic stirrer, allow the DO meter reading to become steady and adjust the meter according to the maker's instructions. Empty the sample chamber and thoroughly wash the equipment with clean water.	
	Air-saturated water calibration	
AD.7.2.	Fill a suitable container with clean air-saturated water, preferably at the temperature of the liquid to be sampled. Immerse the respirometer, fill the sample chamber, start the stirrer and allow the DO meter reading to become steady. Adjust the meter to read 100% saturation for EIL electrodes and the appropriate concentration for YSI electrodes (note a).	(a) Because there are no provisions made in the equipment for aeration of the sample, the 0–50% saturation scale, or 0–5 mg/l scale, may be the most useful. Make all adjustments according to the maker's instructions.
	The DO level in the saturated water may be calculated from measurements of its temperature and the ambient pressure, but for preference, should be established by chemical means (note b). This value will be required for the calculation of respiration rates in terms of mass concentration.	(b) See Ref. 7.
AD.7.3.	*In-situ* measurements should not be made using this respirometer if the sample DO level is less than 3 mg/l if the EIL 1521 probe is in use because its response time is such that non-linear respiration traces may result. Levels down to 1.5 mg/l ambient DO are acceptable if the YSI 5739 probe is in use. It should be noted that at DO concentrations below 0.5 mg/l, respiration rate is reduced because the DO concentration becomes rate-limiting and this therefore sets the useful end-point of the respiration run. If satisfactory DO concentrations cannot be achieved then a sub-sample should be removed and rapidly aerated in a suitable container. The respiration rate should then be measured immediately using either the submersible respirometer, or Method C. Measurements of DO should be taken until the concentration has fallen below that found in the activated sludge plant. The respiration rate in the plant may then be obtained by interpolation to the appropriate DO.	

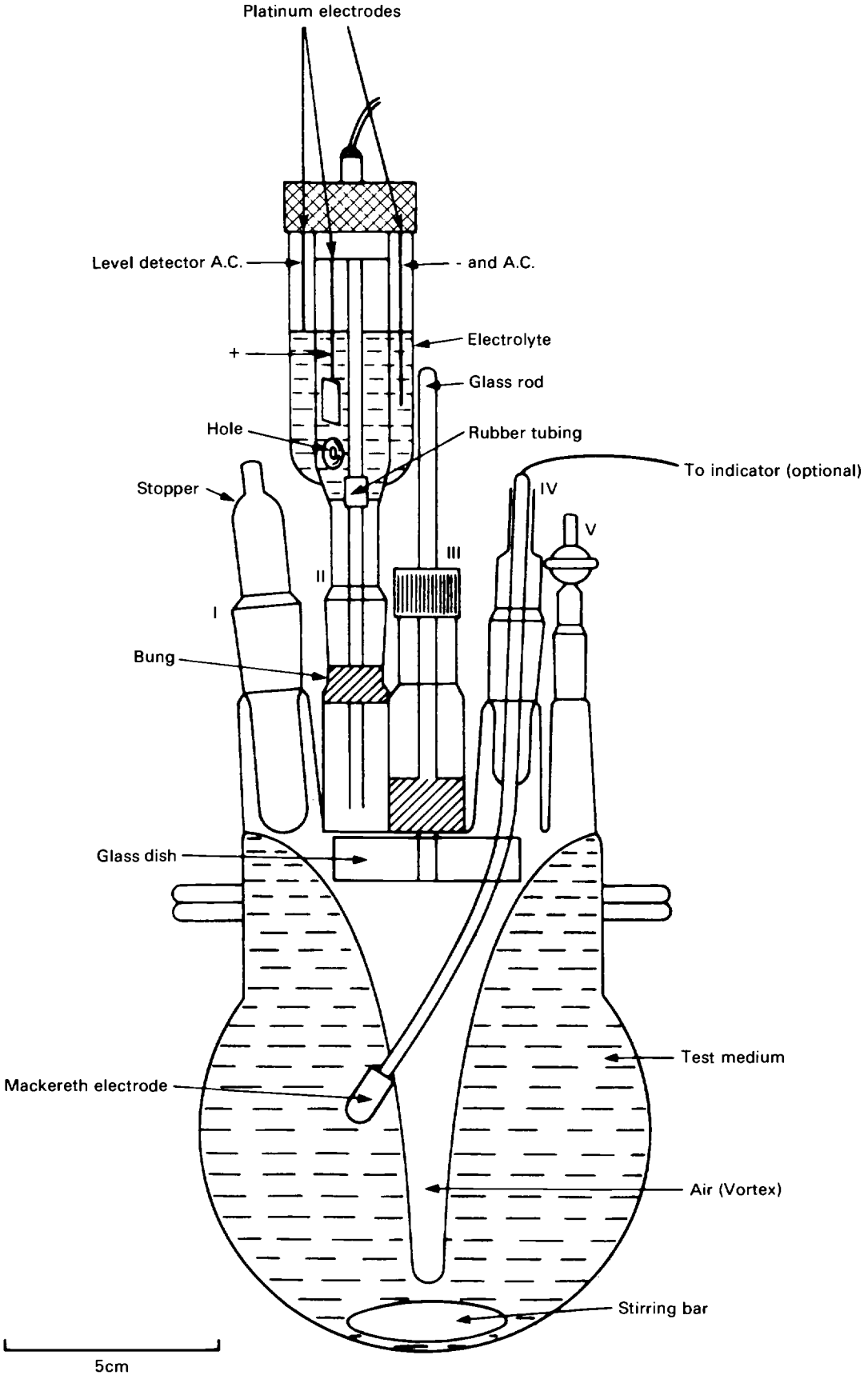


FIGURE 2 AUTOMATIC ELECTROLYTIC RESPIROMETER

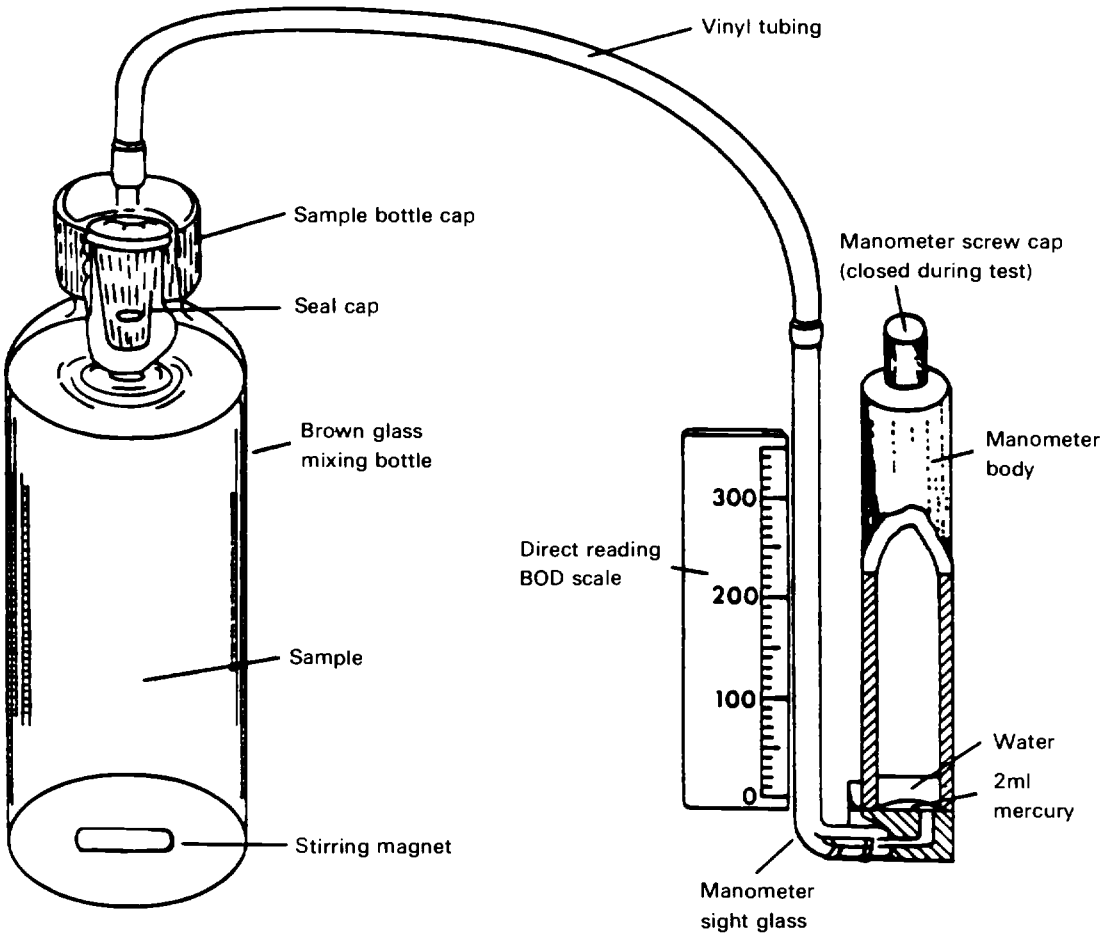


FIGURE 3 HACH-TYPE MANOMETRIC APPARATUS SHOWING ONE CELL

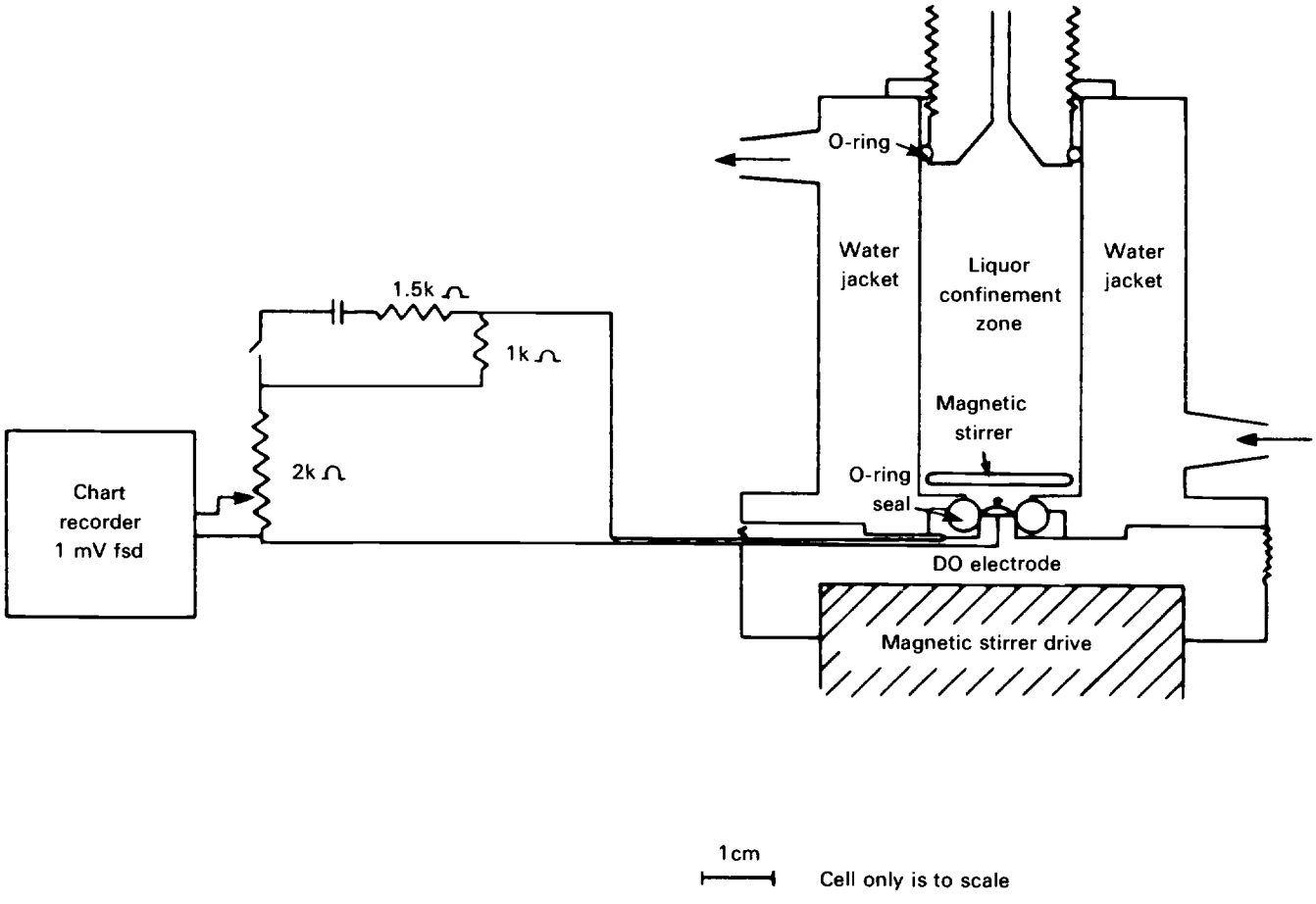


FIGURE 4 RANK TYPE RESPIROMETER

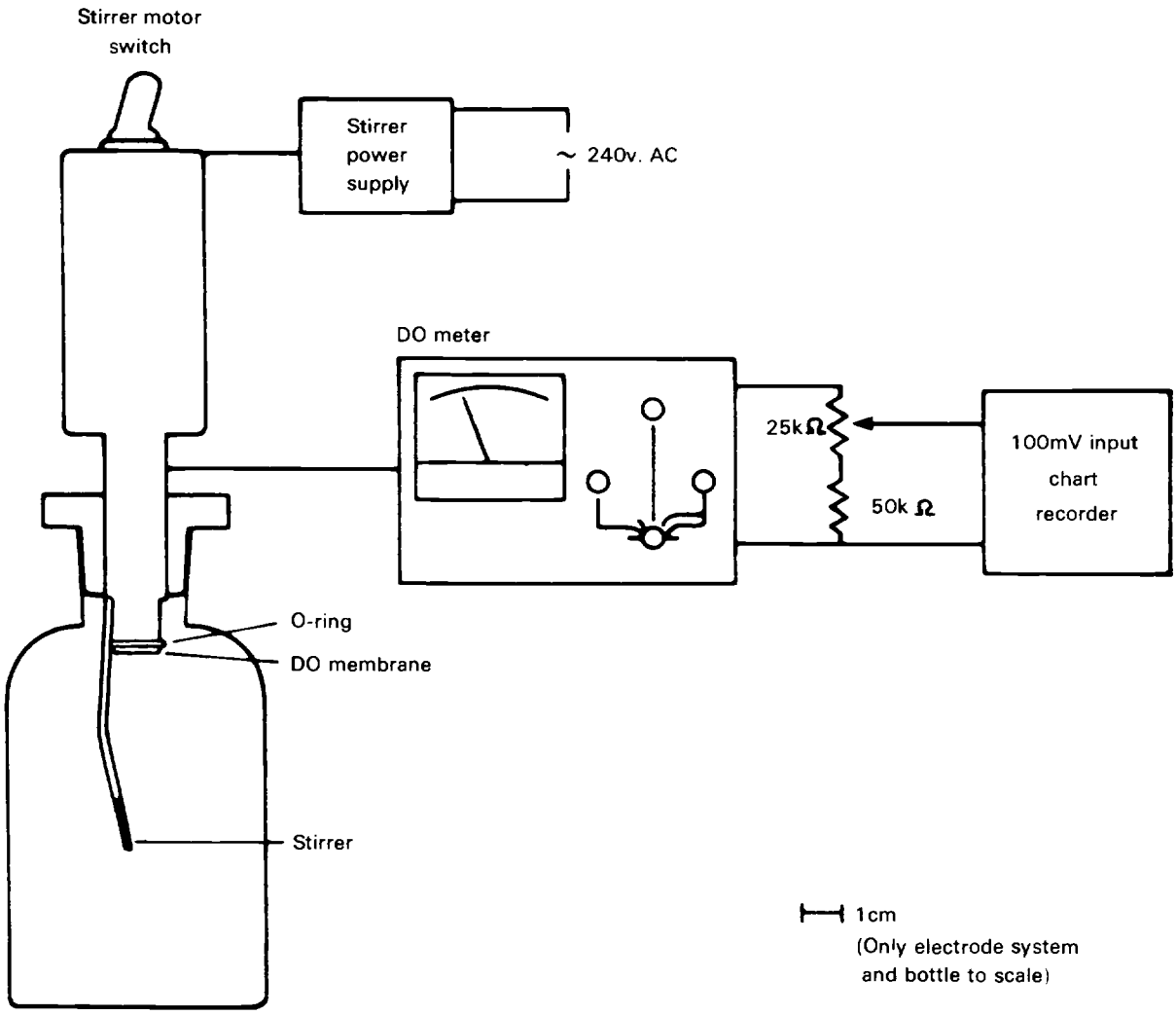


FIGURE 5 TYPICAL ARRANGEMENT USING YSI BOD-TYPE DO METER

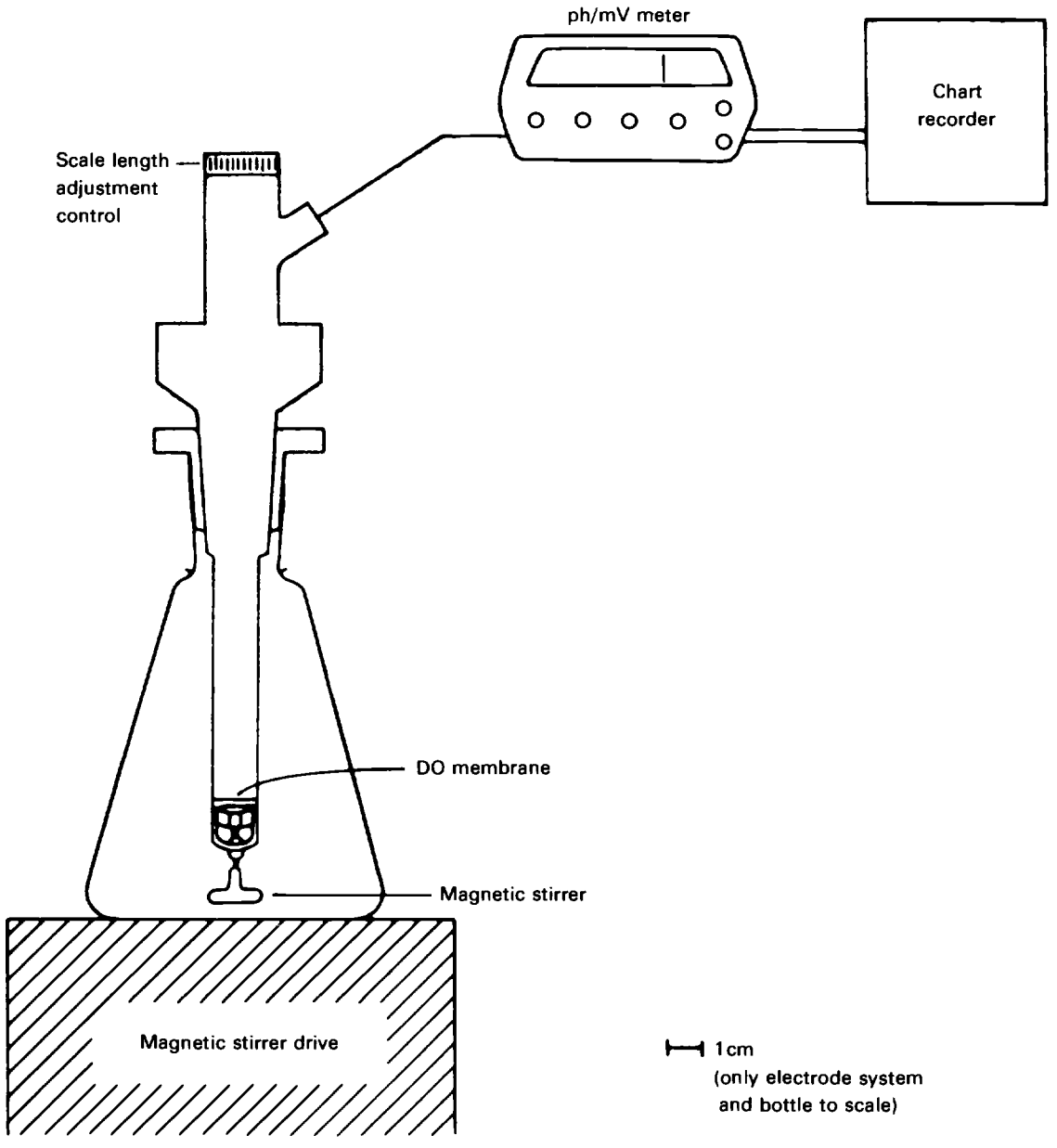
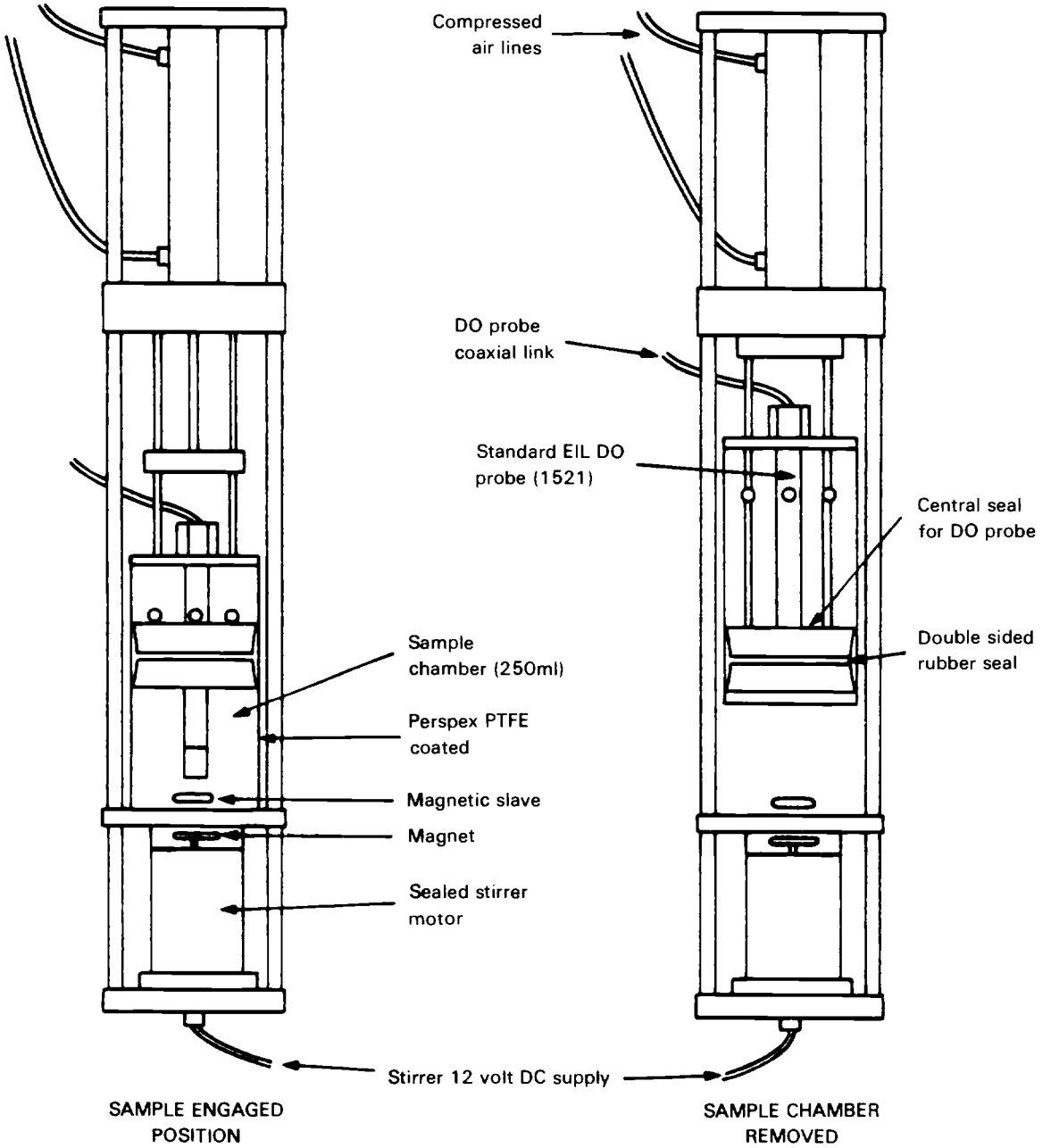


FIGURE 6 TYPICAL ARRANGEMENT USING EIL TYPE ELECTRODE



Diagrams are to scale :- Overall length 53.5cm
Maximum diameter 10cm

FIGURE 7 WRC SUBMERSIBLE RESPIROMETER

B. Method for assessing the toxicity to micro-organisms of a chemical or industrial waste water using the BOD method

Introduction

One of the simplest ways of assessing the toxicity of a chemical or industrial waste is to ascertain its effect on the uptake of oxygen by micro-organisms growing on a known, readily degraded substance or mixture as measured in the standard 5-day BOD test. A number of standard substrates have been suggested: in this method a mixture of glucose and glutamic acid is used.

By extending the test period (5–30 d) more can be ascertained about the nature of any toxicity and by using the technique of "sequential seeding"[9] (see section B5.4) evidence may be collected on whether acclimitization to the toxic substance occurs.

B1. Performance Characteristics

	Characteristics	Notes
B1.1.	Property determined	Effect of test substance (usually expressed as EC_{50}) on the uptake of dissolved oxygen by micro-organisms in the presence of a biodegradable standard.
B1.2.	Type of sample	Single substance, a mixture or an industrial waste water.
B1.3.	Basis of method	The test substance and biodegradable standard substances are dissolved in BOD dilution water. The mixture is saturated with air, seeded and placed in a closed bottle which is incubated in the dark for 5 days at 20°C. The amount of oxygen taken up is determined by appropriate measurements of dissolved oxygen and compared to the uptake by the standard substances alone.
B1.4	Range of application	0–100% inhibition.
B1.5.	Standard Deviation	For 3,5-dichlorophenol (often used as a typical inhibitor). EC_{50} = 16 mg/l ± 9.5 (n = 5)
B1.6.	Interferences	Any substance which interferes in the method for determination of dissolved oxygen (see Section B3).
B1.7.	Time for analysis	Test time: 5 days Operator time: 15 min per sample for a large number tested together — longer for individual samples.

B2. Principle

The uptake of dissolved oxygen of a solution of glucose and glutamic acid is determined by the standard BOD_5 or BOD(ATU) method[10]. A series of bottles is also prepared each containing the same concentration of the glucose/glutamic acid solution but with a range of concentrations of the test substance, giving a set of BOD_5 values from which the EC_{50} of the test substance can be deduced. More detailed information may be obtained by ascertaining the effects on oxygen uptake after various periods of incubation.

B3. Interference and sources of error

The test substance may be inhibitory at higher concentrations but biodegradable at lower concentrations, while mixtures and industrial waste water may contain biodegradable and toxic components. In these circumstances it is advisable to determine the BOD(ATU) of the test material at various concentrations in the absence of the standard solution of glucose-glutamic acid.

Also, in rare cases the test material may react with ATU and thus interfere with the suppression of nitrification.

B4. Hazards

This procedure makes use of two methods for the determination of dissolved oxygen which are published in detail in this series[7].

The titrimetric method involves the use of strong alkali, strong acid and sodium azide solution, all of which are potentially hazardous. The user should consult Section A4 in Reference 2 for fuller details of these hazards and of the precautions to be taken in practice.

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 vapour and the solid should be kept out of contact with the skin.

If the properties of the test chemicals are unknown, or an industrial waste water with unknown components is tested, these substances should be treated as potentially harmful, handled with care and protective clothing worn if appropriate.

B5. Reagents

B5.1. 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 and uniform quality and composition. Standard dilution water is prepared by adding specified chemical reagents to good quality distilled or deionized water. These reagents provide osmotic balance, buffer the pH and provide essential nutrients (other than carbon) and trace metals.

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

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

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

B5.2. 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 and should be stored in glass in the dark and discarded at the first sign of precipitation or biological growth.

B5.2.1. *Ferric chloride solution*

Dissolve 0.125 (± 0.005) g ferric chloride hexahydrate in 1 litre of water.

B5.2.2. *Calcium chloride solution*

Dissolve 27.5 (± 0.5) g calcium chloride, (or equivalent if hydrated calcium chloride is used) in 1 litre of water.

B5.2.3. Magnesium sulphate solution

Dissolve 25.0 (± 0.5) g magnesium sulphate heptahydrate in 1 litre of water.

B5.2.4. Phosphate buffer solution

Dissolve 42.5 (± 0.5) g potassium dihydrogen phosphate, in 700 (± 10) ml water and add 8.8 (± 0.1) g sodium hydroxide. Add 2 (± 0.1) g ammonium sulphate and dilute to 1 litre.

B5.2.5 Allyl thiourea solution

Dissolve 0.50 (± 0.01) g allyl thiourea in 1 litre of water.

B5.2.6. Washing solution for bottles

To 2 litres of 1% m/v sulphuric acid add 5 g iodine and 25 g potassium iodide. Shake to dissolve. Discard when brown colour fades.

B5.2.7. Standard solution of glucose/glutamic acid

Dissolve 0.150 ± 0.0001 g each of D-glucose and L-glutamic acid (both previously dried at 105°C for 1 hour) in 1 litre of water in a calibrated flask. Prepare freshly each day.

B5.3. Preparation of dilution water

Transfer the required volume of freshly distilled or de-ionized water to a clean vessel which should be specially reserved for the preparation of dilution water. To each litre of water add 1 ml of each of the stock reagent solutions (B5.2.1 to B5.2.5).

Bring the prepared stock of dilution water to the 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. Add bacterial seed (see B5.4) to the dilution water and use as soon as possible. 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 washing solution B5.2.6 and then thoroughly with tap water and finally with distilled or deionized water. Store the prepared dilution water out of direct sunlight. The dissolved oxygen concentration of a satisfactory dilution water, when incubated without seed under standard conditions, should not be depleted by more than 0.3 mg/l. Higher values for oxygen depletion may sometimes be caused by the presence of water-soluble organic vapour which may be present in the laboratory atmosphere and is absorbed during the production of distilled or deionized water, or during aeration of the prepared stock of dilution water.

B5.4 Seed

Add 5 ml of fresh, good-quality sewage works effluent, taken from the final settlement tank, to each litre of dilution water.

It is preferable that the substance under test is not present in the sewage from which the seed is derived, otherwise the bacterial population may have become acclimatized to the substance.

It may be necessary to know whether bacteria become acclimatized to the test compound, in which case the test should be repeated using as seed the contents of previously incubated (preferably for 30 d) BOD bottles containing the substance. Alternatively, a model filter (rotating tube) or activated sludge plant should be fed with sewage containing the substance; after a suitable period, eg 1–3 weeks, effluent from the model treatment plant can be used as seed.

B5.5. Stock solution of test substance

Stock solutions of test substance should be made in distilled water e.g. 0.1 g/l, 1 g/l or 10 g/l. If the pH value of the solution is not between 6.5 and 8.5 add sufficient alkali or acid to bring it within this range.

B6. Apparatus

B6.1. Narrow-mouthed clear glass bottles, of nominal 250 ml capacity, should be used as standard. The bottles should have well fitting glass or plastic stoppers.

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

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

Cleanliness of the bottles, and indeed of all associated glassware, is of paramount importance. When using the Winkler procedure for determination of dissolved oxygen, cleanliness of the bottles is ensured by the action of the acidic iodine solution and no further treatment, other than rinsing with tap and distilled/deionized water, is normally necessary. However, when using the alternative instrumental procedure, the bottles should be rinsed, before re-use, using 5–10 ml reagent B5.2.6, shaking well to coat the bottle walls. Stand for 15 minutes, pour off the solution and rinse thoroughly with water and finally distilled or deionized water. This latter cleaning procedure also applies to new bottles.

B6.2. Samples should be incubated in a circulatory 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.

B6.3. 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 (e.g. 500 ml cylinder), to facilitate mixing and transfer with minimum entrainment of air bubbles.

Volumetric glassware should be of Class B or better.

B7. Procedure

Read section on hazards before starting this procedure. There are two methods for determination of dissolved oxygen in water, either of which may be used in this procedure (see Reference 7).

The following method assesses the inhibitory effect on BOD in which nitrification has been suppressed. If the effect on unsuppressed BOD is required, omit solution B5.2.5. (allylthiourea) from the dilution water (B5.3).

Step	Procedure	Notes
B7.1.	Prepare a dilution of the glucose-glutamic acid standard solution (B5.2.7) using 1 volume to 49 volumes of seeded dilution water (B5.3) in a suitable mixing vessel (note a). (The corrected BOD value should lie in the range 220 ± 20 mg/l).	(a) Use a pipette to dispense the solution into, ideally, a stoppered measuring cylinder. The capacity depends on the method employed in the measurement of dissolved oxygen. For the titrimetric method, a 1 litre cylinder may be used, but if an electrode is used 500 ml would be suitable. Larger volumes will be required if additional bottles are to be incubated for periods other than 5 days.
B7.2.	Prepare five further batches of the diluted standard but add a different concentration of test substance to each. The concentration should increase in a logarithmic manner (note b).	(b) Ratio of Volumes Glucose/glutamic acid : Suitable stock soln of test substance : Seeded dilution water 1 : 0.5 : 48.5 1 : 1.0 : 48 1 : 2.0 : 47 1 : 4.0 : 45 1 : 8.0 : 41 It will probably be necessary to carry out a preliminary test to determine the range over which the substance is inhibitory, e.g. at 1, 10 and 100 mg/l.
B7.3.	Make up to the desired final volume (± 0.5%) by careful addition of dilution water.	
B7.4.	Mix thoroughly using a gentle rolling and inverting motion to avoid entrainment of air.	
B7.5.	Rinse out the bottle(s) with the prepared dilution and fill to overflowing either by careful pouring or using a siphon (note c).	(c) For each period of incubation, two bottles are needed for the titrimetric method, one for the initial and one for the final dissolved oxygen measurement. When the instrumental method is used one bottle may suffice for both measurments.
B7.6.	Allow the bottle(s) to stand for 10 ± 5 minutes. Tap gently to remove any air bubbles. If the titrimetric method is used, stopper one of the duplicates carefully, but firmly, to avoid air entrainment.	

Step	Procedure	Notes
B7.7.	Determine the initial dissolved oxygen content of the diluted sample in the bottle (D_1 mg/l) (note d). If the electrode[7] method is used, stopper carefully, but firmly to avoid air entrainment. If the titrimetric method is used, discard the bottle.	(d) With sensors, slight displacement of the sample will occur during measurement; this problem may be eliminated by the use of a displacement funnel in the bottle neck, or alternatively it may be necessary to top up the bottle contents after the initial measurement using some previously reserved diluted sample.
B7.8.	Label the bottles to be incubated and place in an incubator or water bath at 20.0 ± 0.5°C for 5 days ± 2 hours (note e).	(e) Incubation must be in the dark.
B7.9.	After the required time, usually 5 days, determine (titrimetric) or re-determine (electrode) the dissolved oxygen content of the sample (note f). (D_2 mg/l).	(f) If the sensor method is used it is important to clean the bottles after use by washing with acidified iodine solution as described in Section B6.1.
B7.10.	**Blank determination.** Treat the seeded dilution water as sample and determine its dissolved oxygen content before (B_1 mg/l) and after (B_2 mg/l) 5 days incubation at 20.0 ± 0.5°C (note g).	(g) This test provides one check on the validity of the method and values of B_1–B_2 in excess of 0.5 mg/l should be the cause of investigation of the dilution water used.

B8. Calculation

The BOD is calculated from the following formula:

$$BOD = \left\{ (D_1 - D_2) - (B_1 - B_2) \frac{(f-1)}{f} \right\} f \quad mg/l$$

where D_1 is the initial concentration of dissolved oxygen (B7.7)
D_2 the concentration of d.o. after n days (B7.9)
B_1 the initial concentration of d.o. in the seeded dilution water (blank value) (B7.10)
B_2 the concentration of d.o. in the seeded dilution water after n days (B7.10)
f the dilution factor of the standard solution of glucose-glutamic acid = (1 + 49) = 50, in above scheme (B7.2).

Thus, for each concentration of test substance a BOD is calculated: BOD^1, BOD^2 etc. where BOD^1 is the value of BOD for the first concentration of substance tested, BOD^2 the BOD of the second concentration etc and BOD^s is the BOD of the standard glucose/glutamic acid solution.

The percentage inhibition at the first concentration is given by the formula:—

$$\frac{BOD^s - BOD^1}{BOD^s} \times 100\%$$

the percentage inhibition given by the other concentrations of test substance can be found by substituting the appropriate BOD value for BOD^1. Graphs are drawn plotting percentage inhibition against concentration, or logarithm of concentration of the test substance; one or other of these plots is usually a straight line and the concentration of substance giving 50% inhibition can then be interpolated.

The results should be accompanied by a statement of the source of the seed and whether it was acclimatized.

B9. Nitrification

If nitrification is suppressed in the BOD test[10] the effect of test substance on the carbonaceous respiration of the micro-organisms will be measured.

If nitrification is unsuppressed, spurious results may be obtained by varying amounts of nitrification occurring in test and control samples.

C. Method for the Determination of the Inhibitory Effects of Chemicals and Industrial Waste Waters on the Respiration and Nitrification of Activated Sludge

Introduction

Activated sludge in the presence of sewage (or synthetic sewage) will respire rapidly; addition of a toxic concentration of a chemical to this mixture will result in a decrease in the respiration rate proportional to the toxicity of the chemical.

The test can be carried out using activated sludge at a concentration of about either 100 or 1500 mg suspended solids/l depending on the purpose. The percentage inhibition at a particular concentration of test substance, or the concentration giving 50% inhibition can be estimated. The latter can be assessed roughly by a preliminary sighting experiment, in three ranges of concentration: 1–10 mg/l, 10–100 mg/l and greater than 100 mg/l, or more accurately by a definitive test.

However, since sludges from different sources may differ in activity, the results from any of the tests must be regarded only as guides to the likely toxicity of the chemical, since no laboratory test can truly simulate environmental conditions.

Contents

C1. Performance Characteristics

C1.1.	Property determined	Toxicity (EC_{50}) of chemicals and industrial waste waters to activated sludge bacteria.
C1.2.	Type of sample	Soluble or insoluble substances (see C5.4); mixtures; or industrial waste water.
C1.3.	Basis of method	Determination of the effect of test substance on the respiration rate of activated sludge or of the heterotrophs only.
C1.4.	Range of application	0–100% inhibition
C1.5.	Standard deviation	Method A: Mean 12.9 mg/l, SD 5.8, n 3 Method B: Mean 11.6 mg/l, SD 5.5, n 4 Method C: Mean 11.0 mg/l, SD 1.5, n 5 for 3,5-dichlorophenol
C1.6.	Interferences	Substances which may be auto-oxidized, or which affect the calibration of the oxygen electrode (see Section C3).
C1.7.	Time required for determination	Operator time:— Method A: 15 min Method D: 30 min Methods B, C, E and F: ~ 4 h

C2. Principle

The effect is determined of the addition of test substance or industrial waste on the respiration rate of activated sludge-synthetic sewage mixtures; the rates are measured using an oxygen-electrode.

Decreases in respiratory activity of sludge-sewage mixtures indicate that the test material is toxic to some or all of the species of micro-organisms present in the sludge. The EC_{50} (the concentration of test substance giving 50% inhibition of respiration) may be determined by assessing the inhibition caused by a suitable range of concentrations of test substance on activated sludge respiring in the presence of sewage or synthetic sewage.

The inhibition of heterotrophs and of nitrifying organisms can be separated by use of a specific inhibitor of *Nitrosomonas* (e.g. allyl thiourea[11]). Nitrification may lead to spurious results, so that it is advisable in any case to ascertain whether the test sludge nitrifies (see section C7.5) and to make allowances if it does (see Section C8.3).

C3. Interferences

C3.1. Some chemicals may be auto-oxidized and take up oxygen in the absence of micro-organisms or stimulate oxygen uptake eg by uncoupling of oxidative phosphorylation, thus appearing to be biodegradable. The presence of physico-chemical uptake of oxygen may be taken into account by inclusion of a control containing test substance, but no inoculum.

C3.2. Inhibitory substances may be present in the activated sludge collected from a sewage works receiving industrial discharges. If the object of the test is to investigate the effect of a chemical upon the activated sludge of a particular sewage works, then the sludge should not be pretreated. If more general studies are to be made, the sludge may be washed before use. Soluble substances can be removed by centrifugation and washing of the sludge as described in Section C5.1. Adsorbed substances are more difficult to remove and culturing the sludge with synthetic sewage for several days may be necessary.

Activated sludge may be diluted, with sewage effluent, to the suspended solids concentration required for testing, but if the effluent is known, or thought, to contain inhibitory substances, tap water may be used instead, assuming this is isotonic with the effluent.

C4. Hazards

C4.1. Hygiene

Sewages and derived activated sludges may contain potentially pathogenic organisms, therefore appropriate precautions should be taken when handling these materials.

Vessels containing aerated activated sludge should be loosely covered to avoid splashing and fine spray.

C4.2 Chemicals

Test chemicals, or components of the waste waters tested, may be toxic and should be regarded as potentially dangerous; precautions should be taken to avoid contact with skin and clothing, and to avoid breathing dust or vapour.

C5. Reagents

C5.1. Activated sludge

Activated sludge is preferably taken from the aeration tank of a plant treating domestic sewage or mixed industrial and domestic sewage or from a laboratory plant maintained for the purpose. It should be kept aerated before use and preferably used on the day of collection. If this is not possible, it should be fed daily with synthetic sewage. If it is required to ascertain effects of nitrification, a nitrifying sludge must be used. If no such effects are sought a non-nitrifying sludge will be suitable, but if the available sludges nitrify, allowance must be made for this in the experimentation (Sections C7.5–C7.6 and C7.7) and in the calculations (Section C8.3).

If coarse, extraneous particles are present, the activated sludge may be sieved to remove these.

If the activated sludge is obtained from a sewage works receiving industrial discharges, it may be necessary to centrifuge the sludge (after preliminary sieving if necessary) e.g. at

1100 g for 10 min, and wash it with tap water (or an isotonic solution) to remove any interfering substances that may be present in the industrial waste water. Washing is necessary to obtain information on the general inhibitory properties of the test substance, but if the effect of the test substance on that particular sludge is required washing is unnecessary. The washed sludge is recentrifuged, its dry weight determined, and then it is resuspended in synthetic sewage to give a suspended solids concentration of approximately 3.0 g/l and aerated overnight before use.

C5.2. Synthetic sewage

A 100-fold concentrated solution of OECD/EEC synthetic sewage is prepared by dissolving the following in 1 l of distilled water: 16 g peptone, 11 g meat extract, 3 g urea, 0.7 g sodium chloride, 0.4 g calcium chloride dihydrate, 0.2 g magnesium sulphate heptahydrate, 2.8 g dipotassium hydrogen phosphate. The pH of this solution should be 7.5. This concentration solution may be stored at 1°C for up to 1 week.

C5.3. Settled sewage

Although the methods described here make use of synthetic sewage the analyst may wish to use sewage relevant to his problem, in which case samples of settled sewage should be collected daily from the overflow of the appropriate settlement tank and be kept aerobic before use.

C5.4. Solution or suspension of test substance

Stock solutions of test chemicals are prepared in distilled water, e.g. 1 g/l or 10 g/l, as appropriate; saturated solutions or suspensions of less soluble compounds are prepared. The pH value should be between 6.5 and 8.5, if it is not, alkali or acid should be added to bring the pH value within this range.

C5.5. Industrial waste water

Fresh, representative samples of the industrial waste waters to be tested should be collected from the sites and, if necessary, stored at 1–4°C to avoid changes. The pH value of waste water should be brought to the range 6.5–8.5, if necessary.

C5.6. Standard substance

A stock solution of 1 g/l of an appropriate substance, e.g. 3,5-dichlorophenol, is prepared for use as a standard inhibitor of respiration.

C5.7. Distilled or deionized water

Distilled or deionized water free from toxic substances.

C5.8. Allyl Thiourea

A solution of 2.5 g/l is prepared, so that adding 0.5 ml of this to a 100 ml sample gives a final concentration of 12.5 mg allyl thiourea/l ($\simeq 10^{-4}$ M).

C6. Apparatus

C6.1. Oxygen electrode

Suitable electrodes are made by e.g. Electronic Instruments Ltd (EIL), Yellow Springs Instruments (YSI), International Biophysics Corporation (IBC) etc. and a respirometer cell by Rank Bros (see method A).

C6.2. BOD bottles of 300 ml capacity with stoppers. Note: Alternatively a Rank oxygen electrode cell may be used in method CA, CB, CD and CE.

C6.3. Funnel or suitable stopper for fitting the electrode probe into the BOD bottles.

C6.4. Magnetic stirrers, preferably air-driven (electrical stirrers tend to get hot with use), to give regular and identical stirring of each bottle.

C6.5. Circulatory water bath

Samples are kept immersed in the water bath before use to avoid temperature variations. The water bath is also required to pass water through the jacket of the Rank electrode cell (see method AC).

C6.6. Compressed air supply

Compressed air is passed through a suitable strainer (e.g. cotton wool) and wash-bottle of distilled water kept in a constant temperature water bath, and use for aerating activated sludge samples.

C6.7. Recorder

A suitable potentiometric recorder is required for continuous recording of the dissolved oxygen concentration.

C7. Procedure

Step	Procedure	Notes
C7.1.	Calibrate the oxygen electrode according to the manufacturer's instructions (see method AC).	

C7.2 Method CA. Determination of 'Immediate' Toxicity

C7.2.1. Determine the concentration of suspended solids on an aliquot of the activated sludge to be used (see Ref. 1 and 8).

Respiration Rate of Sludge and Sewage R_C (note a)

C7.2.2. Aerate a 250 ml sample of activated sludge (note b) with a suspended solids concentration of about 3 g/l.

C7.2.3. Dilute 32 ml of synthetic sewage concentrate (see 5.2) to 250 ml with tap water (note c), aerate and add this to the activated sludge.

C7.2.4. Fill the electrode vessel with the well-aerated mixture of sludge and sewage (note d). Close the vessel ensuring that no air bubbles are trapped.

Stir at the pre-set speed (note e) and record the decrease in concentration of dissolved oxygen at a suitable chart speed e.g. 600 mm/h for about 5 min, or until a suitable length of linear trace is obtained to measure the gradient.

For adequately-soluble chemicals proceed to C7.2.6.

(a) For explanation of symbols (see Sections C8.2 and C8.3).

(b) Prepared as described in Section 5.1. and diluted, with tap water or sewage effluent if necessary. If the effect of pH value is being investigated the sludge should be adjusted before-hand to the required value.

(c) All solutions used must be homogeneous and saturated with air at the required temperature e.g. 20°C. Samples may be kept in a constant temperature water bath before use. The temperature should not change during the test.

(d) If the mixture is not well aerated the dissolved oxygen concentration will soon reach zero.

(e) The speed of stirring affects the measurement of dissolved oxygen therefore the optimum speed should be determined beforehand, and should remain unchanged during the test.

Step	Procedure	Notes
	For less-soluble chemicals and industrial wastes, proceed to C7.2.5, and then to C7.2.8.	
C7.2.5.	Empty the electrode vessel, rinse with distilled water and repeat the procedure 7.2.3. 7.2.4. (note f).	(f) The procedure is repeated to obtain the required number of replicates (e.g. 6).
	Soluble Chemicals, R_T	
C7.2.6.	When the respiration rate of the sludge-sewage mixture has been measured and with the mixture still in the electrode vessel (note g) add the required volume of solution of the test substance (note h) allow to mix thoroughly by stirring, close and continue to measure the fall in concentration of dissolved oxygen for a suitable length of time.	(g) A syringe or pipette may be used to introduce the test substance into the mixture in the electrode vessel; allow a few seconds to ensure adequate mixing. Alternatively, the sludge and test substance may be mixed separately and a portion of this placed in the electrode vessel. (Steps C7.2.5, C7.2.8, C7.2.9)
		(h) The concentrations to be used in the mixture must be found by trial and error, but the range of respiration rates should span from zero to the uninhibited value; usually a ten-fold range of concentration is necessary. If the volume added is more than 10% of that of the cell, a control mixture with the same volume of water should be tested to take account of the effect of dilution.
	When a satisfactory trace has been obtained (note i) empty and rinse the electrode vessel.	(i) Any interference in the trace at the time of addition should be ignored.
C7.2.7.	Repeat, as required, 7.2.3–7.2.4–7.2.6 (note j).	(j) The procedure is repeated with freshly mixed sludge and synthetic sewage and the same concentration of test substance for replicates and subsequently at different concentrations to cover the required range.
	Industrial wastes and less-soluble chemicals, R_T	
C7.2.8.	Dilute the required volume (note k) of industrial waste, or sparingly soluble test substance, and 32 ml of synthetic sewage concentrate to 250 ml with tap water and aerate. Add to an equal volume of aerated activated sludge, with a concentration of about 3 g suspended solids/l. Mix thoroughly and aerate.	(k) A range of volumes must be used, see note h.
	Fill the electrode vessel with well aerated mixture and record the respiration rate, as described in 7.2.4. Empty and rinse the electrode vessel.	
C7.2.9.	Repeat C7.2.8. as required, (note l).	(l) The procedure is repeated with freshly mixed sludge, synthetic sewage and industrial waste, for replicates and subsequently at different concentrations of industrial waste (or test substance) to cover the required range. This gives R_{T1}, R_{T2}, etc.

Step	Procedure	Notes

C7.3 Method CB — Inhibition after 3 h (ETAD)[12] (note a)

C7.3.1. Put 16 ml synthetic sewage concentrate (note m) into a 500 ml measuring cylinder, make up to 250 ml with tap water, and add 250 ml of activated sludge, with a concentration of about 3 g suspended solids/l (note n).

Note the time of mixing, pour into a beaker and aerate by means of a Pasteur pipette (note o) for 3 h (note p).

(m) See Section C5.2.

(n) Or adjust the volumes of activated sludge and water to obtain about 1.5 g/l suspended solids in the test sample.

(o) Diffusers may tend to adsorb the test substance. The air flow should be such that the solids are in suspension and the sludge is fully aerated, since the respiration increases markedly on addition of sewage.

(p) 3 h was chosen arbitrarily; with a suspended solids concentration of 1.5 g/l, synthetic sewage will not normally have been fully metabolized during this period.

C7.3.2. Choose a suitable range of concentrations of test substance (note q). For a preliminary sighting run use three concentrations e.g. 1, 10 and 100 mg/l and include an inoculated control with no test substance and a physico-chemical control with 100 mg/l of test substance and no inoculum. For a definitive test use five concentrations selected from the results of the preliminary test, a control, and if necessary appropriate physico-chemical controls for each concentration of test substance.

(q) A suitable range of concentrations should be chosen to cover the range of respiration rates from zero to the uninhibited value. The range may be chosen from method CA, or from results of a preliminary test. It is helpful to use a logarithmic series of concentrations for plotting a graph to obtain the EC_{50} value. See Section C11 for an example.

C7.3.3. At 15 min intervals, prepare a series of test mixtures as described in C7.3.1. except that an appropriate volume of stock solution of the test substance or industrial waste is added to each before making up to 250 ml with water, if necessary, and before adding 250 ml activated sludge (note r).

Note the time of mixing and aerate for 3 h. Finally repeat the control mixture (C7.3.1) and aerate for 3 h (note s).

(r) Sludge should not be allowed to come into contact with the stock solution of the test substance or the undiluted industrial waste.

(s) To check that the activity of the sludge has not significantly altered over the test preparation period.

C7.3.4. After 3 h, measure the respiration rate of each of the mixtures in turn (note t). If the respiration rates of the two controls are not, within experimental error, identical, repeat the experiment with a fresh batch of sludge.

(t) This gives R_C and R_{T1}, R_{T2}, R_{T3} etc.

C7.4 Method CC — Inhibition over 3 h (AFNOR[13]) (note a)

C7.4.1. To a series of BOD bottles (see C7.3.2. and C12) add 9.6 ml of synthetic sewage concentrate (5.2), an appropriate volume of solution of test substance (note q) or industrial waste water, and half fill the bottles with distilled water (note u). Include

(u) All solutions should be saturated with air at the temperature of the test.

(v) The sludge should not be allowed to come into contact with undiluted test material.

Step	Procedure	Notes
	a control with no test substance and a physico-chemical control. To each bottle in turn (excluding the physico-chemical control) at convenient intervals of time (e.g. 5 min) add an equal aliquot of activated sludge seed (note v), such that the final concentration in each bottle is 100–200 mg suspended solids/l (note w). Fill the bottles with distilled water, stopper and start the stirrers.	(w) The concentration of activated sludge should be adjusted (after preliminary trials) to that which will result in a decrease in the dissolved oxygen concentration from~8 to~2 mg/l within the 3 h test.
C7.4.2.	After 30 min stop the stirrer in the first bottle, remove the stopper, insert the funnel and oxygen electrode probe, immediately restart the stirrer (note x) wait for equilibrium (note y) and measure the concentration of dissolved oxygen. Then stop the stirrer, remove the electrode probe, restopper the bottle (note z) and restart the stirrer.	(x) Rapid and even stirring is essential. The dissolved oxygen reading of the electrode will fall rapidly as soon as stirring ceases. (y) Equilibrium usually takes no more than a few minutes, the reading being taken when the trace has become steady. If equilibrium takes longer to reach than this, longer time intervals must be allowed between the initial additions of sludge inoculum.
C7.4.3.	Repeat the procedure for the rest of the bottles in turn after 30 min have elapsed from the start for each.	
C7.4.4.	Continue this process at 30 min intervals for 3 h, or until the concentration of dissolved oxygen in the control without test substance has reached 2 mg/l. Readings need to be taken only at the start and finish for the physico-chemical control.	(z) The use of a funnel allows the test solution expelled by the probe to be returned to the bottle. Care must be taken to avoid trapping air bubbles on re-stoppering.

C7.5 Method CD — as Method CA, allowing for Nitrification (note a)

C7.5.1.	Assessment of nitrification. Prepare a mixture of activated sludge and synthetic sewage (C7.2.3) and measure respiration rate (C7.2.4). This gives R_C (note aa).	(aa) If other sewages or media are used they must contain sufficient ammoniacal-N such that its concentration does not fall below 5 mg/l during the test.
C7.5.2.	Without emptying the electrode vessel, add an appropriate volume of a 2.5 g/l solution of allyl thiourea to the contents, allow to mix thoroughly (note ab) and continue to measure the respiration rate (note ac). This gives R_{ATU} . *For adequately soluble chemicals proceed to C7.5.3, C7.5.4. and C7.5.5.* *For less-soluble chemicals and industrial wastes, proceed to C7.5.4. then C.7.5.6.*	(ab) 0.5 ml of a 2.5 g/l solution of allyl thiourea is added per 100 ml sample to give a final concentration of about 10^{-4}M. (ac) The allyl thiourea completely inhibits nitrification; the remaining rate is due to carbonaceous oxidation (R_{ATU}). The difference $R_C - R_{ATU}$ is due to nitrification.
	Effect on carbonaceous oxidation for soluble chemicals	
C7.5.3.	Finally, add the test substance, at the required concentration, to the contents using a pipette or syringe and continue to measure the respiration rate.	

Step	Procedure	Notes
C7.5.4.	Empty the electrode vessel and rinse with distilled water.	(ad) The procedure is repeated with freshly mixed sludge and synthetic sewage, and the same concentration of test substance for replicates, and subsequently at different concentrations of test substance to cover the required range.
C7.5.5.	Repeat the procedure (C7.5.1, C7.5.2. and C7.5.3.) as required (note ad). This gives R_{ATU+T_1}, R_{ATU+T_2} etc.	
	For Industrial Wastes and Less-Soluble Chemicals	
C7.5.6.	Measure the respiration rate of a mixture of activated sludge, synthetic sewage and industrial waste (or sparingly soluble substance, C7.2.8.) but use sludge to which has been previously added a 2.5 g/l solution of allyl thiourea at a rate of 0.5 ml per 100 ml (note ae).	(ae) It is important that the allyl thiourea solution be added before the test substance or industrial waste since interactions may otherwise occur which prevent the inhibition of nitrification.
	Repeat as required (C7.2.9, note 1). This gives R_{ATU+T_1}, R_{ATU+T_2}, etc.	

C7.6 Method CE — as Method CB, allowing for Nitrification

C7.6.1. Make up the control mixture of activated sludge and synthetic sewage (C7.3.1.) and mixtures containing the test substances or industrial wastes (C7.3.2.) and finally a second control, in time sequence but to all mixtures add a 2.5 g/l solution of allyl thiourea at a rate of 0.5 ml per 100 ml of mixture (note ae).

C7.6.2. Measure respiration rates 3 h after mixing (C7.3.4.).

C7.7 Method CF — as method CC allowing for Nitrification

C7.7.1. Prepare the series of test bottles (C7.4.1) but add 1.5 ml of a 2.5 g/l solution of allyl thiourea to each bottle before making the volume up to 300 ml (note ae).

C7.7.2. Measure the concentration of dissolved oxygen in each bottle at 30 min intervals for 3 h (note af).

(af) Nitrification may account for as much as 50% of the oxygen uptake of the sludge, therefore the control may not reach 2 mg DO/l in 3 h in the presence of ATU. The test duration may be extended if required.

C8. Expression of Results

Method of calculation

C8.1. For methods CA, CB, CD, CE, the respiration rate is calculated from the slope of the straight line portion of the respiration curve traced on the chart recorder and is expressed as mg oxygen/l min or mg oxygen/l h.

The specific respiration rate is taken as the mean of replicate values calculated from the following formula:—

$$R = \frac{\text{Respiration rate (mg/l h)}}{\text{concentration of suspended solids (g/l)}}$$

$$= \text{mg oxygen/g solids h.}$$

For methods CC & CF plot graphs of dissolved oxygen concentration against time at each test concentration; the respiration rates are given by the gradient of these lines.

C8.2. Calculation of EC_{50}

The percentage inhibition at each concentration is calculated as follows:—

$$\text{inhibition} = \frac{R_C - R_{T1}}{R_C} \times 100\%$$

where: R_C = the respiration rate of the control
R_{T1} = the respiration rate with test material at concentration 1.

For other test concentrations (T_2 to T_5) the appropriate rates are substituted.

If physico-chemical uptake of oxygen has occurred this must be taken into account as follows:—

$$\text{inhibition} = \frac{R_C - (R_T - R_{pc})}{R_C} \times 100\%,$$

where R_{pc} = the rate of physico-chemical uptake at the appropriate concentration.

Plot the percentage inhibition against logarithm of the concentration of test material, or in some cases, it may be preferable to plot percentage inhibition against linear concentration. Draw the best regression line between these points and estimate the EC_{50} by interpolation (see Figure 8). The EC_{50} estimated from the results of the preliminary test is expressed as one of four ranges of concentrations:—

greater than 100 mg/l
between 100 and 10 mg/l
between 10 and 1 mg/l
less than 1 mg/l.

The EC_{50} of the definitive test is more accurate and can be given directly.

C8.3. Toxicity to Nitrification and/or Carbonaceous respiration

The toxicity of the test material to nitrification can be calculated from the respiration rates with added allyl thiourea, and added test substance.

C8.3.1. *The respiration rate due to nitrification*

$$R_C - R_{ATU} = R_N$$

where R_C = the respiration rate of the control
R_{ATU} = the respiration rate with allyl thiourea added
R_N = the respiration rate due to nitrification
(R_{ATU} = the carbonaceous respiration rate).

C8.3.2. *Test material inhibitory to nitrification only*

If there is no further decrease on adding test material to the control activated sludge inhibited by allyl thiourea, the test material has no effect on the carbonaceous respiration rate:

$$\text{i.e. } R_{ATU} - R_{ATU+T_1} = 0$$

where R_{ATU+T_1} is the respiration rate with both allyl thiourea and test substance (at concentration 1) added.

Thus the reduction in respiration rate of the control sludge with added test substance (but no ATU) is due to inhibition of nitrification by the test substance:

$$R_C - R_{T_1} = R_{NT_1}$$

where R_{T1} is the respiration rate with test substance at concentration 1, and R_{NT1} is the respiration rate due to nitrification in the presence of the test substance at concentration 1.

The percentage inhibition of nitrification at concentration 1 is given by the formula:

$$\frac{R_{NT_1}}{R_N} \times 100$$

For other test concentrations (T_2 to T_5) the appropriate rates (R_{NT_2} to R_{NT_5} are substituted.

C8.3.3. *Test substance inhibitory to carbonaceous respiration*

If there is a further decrease in respiration rate of the activated sludge inhibited by ATU when the test substance is added, the test substance is inhibitory to carbonaceous oxidation processes at the concentration added.

The percentage inhibition of carbonaceous oxidation is given by:

$$\frac{R_{ATU} - R_{ATU+T_1}}{R_{ATU}} \times 100$$

C8.3.4. *Test substance inhibitory to carbonaceous respiration and nitrification*

If, on addition of test substance to the control sludge, the decrease in respiration rate is greater than that given by ATU added to the control, both carbonaceous respiration and nitrification are inhibited by that concentration of test substance. The amount of carbonaceous oxidation can be measured by adding allyl thiourea as above (C8.3.3.), and the diference ($R_{T_1} - R_{ATU+T_1}$) is the respiration rate due to nitrification.

C9. Sources of Error

C9.1. To avoid cross contamination, the electrode vessel must be carefully rinsed out between determinations (not between successive additions).

C9.2. The concentration of suspended solids affects the respiration rates obtained since the ratio of food: micro-organisms, or inhibitor: micro-organisms is a critical factor. Therefore the particular solids concentration used in the method (CA, CB, CD, CE 1500 mg/l, CC and CF 100 mg/l) should be adhered to as closely as possible.

C9.3. For determination of EC_{50} timing is another critical factor. If the respiration rate is taken directly after mixing the activated sludge, synthetic sewage and inhibitor, very little inhibition may be observed. The amount of inhibition may change with time as the synthetic sewage is metabolized; in methods CB, CC, CE, CF 3 h was chosen arbitrarily for comparative purposes, based on the retention time in the standard activated sludge simulation test.

C9.4. If the temperature has significantly changed, or the pH has fallen outside the range 6–8 in methods CB and CC then the test should be repeated.

C9.5. If significant physico-chemical uptake of oxygen occurs in methods CB or CC, controls should be included for each concentration of material tested, and the abiotic oxygen uptake subtracted to get the 'true' biotic uptake.

C10. Checking the Accuracy and Validity of Results

The accuracy of determinations in methods CA and CD can be checked by carrying out a number of replicates for each concentration of test substance (e.g. 6), and taking the mean respiration rate calculated from these; outliers are disregarded. The validity of the results in methods CB, CC, CE and CF can be checked using a standard substance e.g. 3,5-dichlorophenol. If the EC_{50} of 3,5-dichlorophenol falls within the range 5–20 mg/l the sludge can be assumed to have normal activity and the results for other test materials are considered to be valid.

If the EC_{50} of the standard substance does not fall within the normal range, repeat the test using activated sludge from another source.

C11. Example of a scheme for filling beakers in the preliminary test (method CB)

Beaker	1	2	3	4	5
Concentration of test substance (mg/l)	0 (control)	1	10	100	100 (physico-chemical control)
Concentrated synthetic sewage (ml)	16	16	16	16	16
Distilled water (ml)	234	233	219	84	334
*solution of test substance (ml)	0	0.5	5	50	50
†inoculum (ml)	250	250	250	250	0

* assuming a concentration of 1 g/l of test substance
† assuming a concentration of 3000 mg activated sludge/l

C12. Example of a scheme for filling the test bottles in the preliminary test (method CC)

Flask	1	2	3	4	5
Concentration of test substance (mg/l)	0 (control)	1	10	100	100 (physico-chemical control)
Concentrated synthetic sewage (ml)	9.6	9.6	9.6	9.6	9.6
*distilled water (ml)	100	100	100	100	100
†solution of test substance (ml)	0	0.3	3	30	30
‡inoculum (ml)	10	10	10	10	0

distilled water to make up to 300 ml (ie. to fill the bottles completely) in all cases

All solutions are saturated with air and brought to the required temperature before use.

* The activated sludge should not be allowed to come into contact with undiluted test substance

† Assuming a solution of 1 g/l of test substance

‡ Assuming a solids concentration of 3000 mg/l

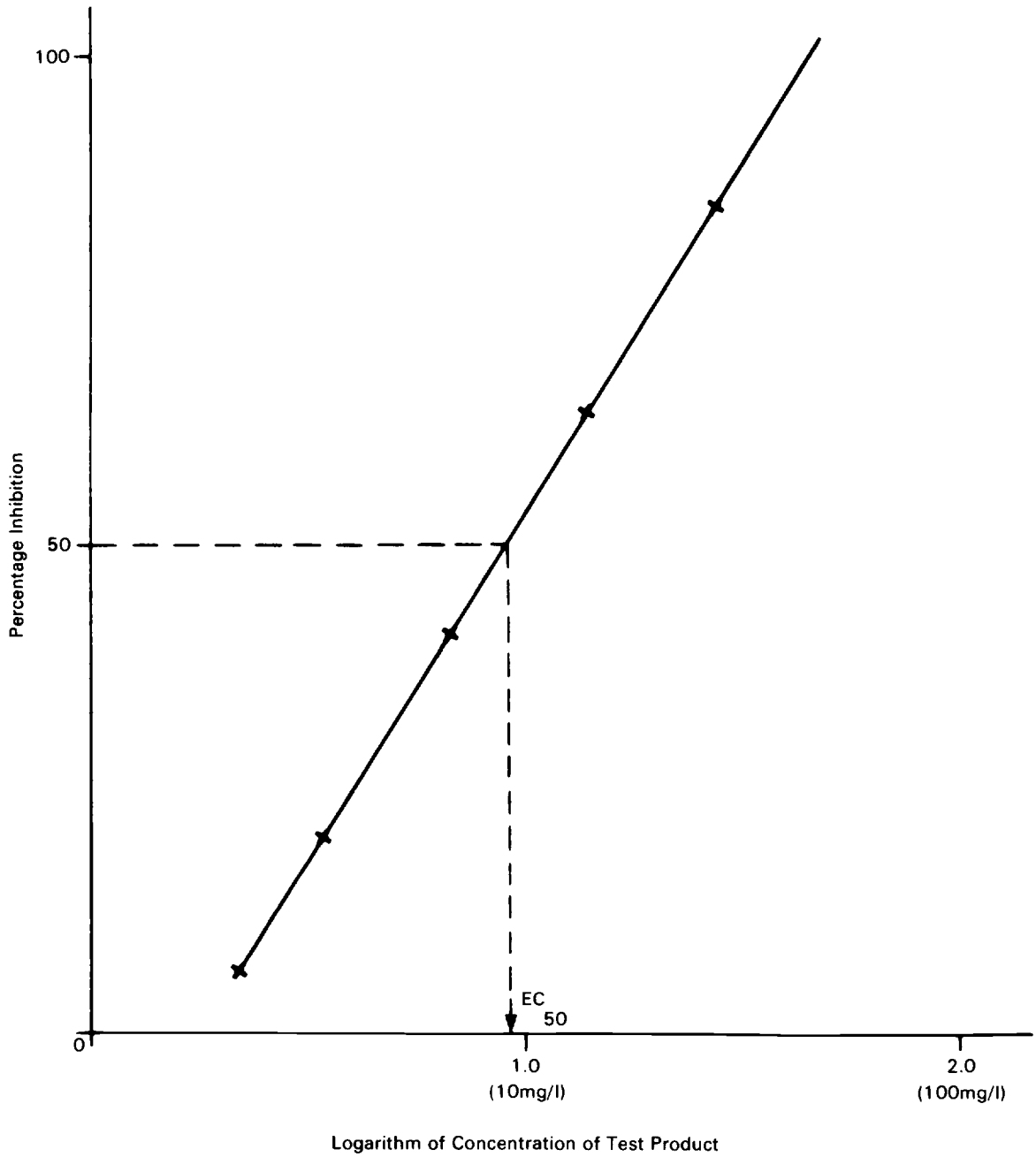


FIGURE 8 PERCENTAGE INHIBITION AGAINST LOGARITHM OF CONCENTRATION OF TEST PRODUCT (e.g. 3.5 dichlorophenol)

D. Assessment of Treatability and Toxicity by means of a Manometric Respirometer

Introduction

This method offers some advantages over others in that it requires little attention and also simulates quite closely the conditions found on a sewage treatment works. It can be used to assess the toxicity of a trade waste or specific chemical provided that its presence does not significantly alter the food/micro-organism ratio in the respirometric flask. It can also be used to provide information when assessing the treatability of a material: a material can be regarded as treatable if its concentration can be reduced to a level suitable for discharge to a river without impairing the sewage treatment processes used. The method will provide information on the rate of degradation, degree of removal and inhibition of the oxidation process. Also dissolved organic carbon (DOC)[25] or COD[24] can be determined on the mixtures at the beginning and end of the test.

D1. Performance Characteristics

D1.1.	Property Determined	Toxicity or treatability by means of oxygen uptake (respiration) rate.
D1.2.	Type of Samples	Domestic and industrial sewages, trade effluents, specific chemicals
D1.3.	Nature of test	Quantitative or semi-quantitative depending on application
D1.4.	Basis of Method	Comparison of the oxygen uptake rates of mixtures of settled sewage and activated sludge with similar mixtures containing industrial wastes or specific chemicals.
D1.5.	Sensitivity	For example: WRC respirometer — an oxygen uptake of 1 mg (see part A)
D1.6.	Precision	Varies — usually R.S.D. of O_2 uptake is ± 10% (see part A)
D1.7.	Interferences	Any substance inhibitory to bacterial growth not entering the system by design. Substances interfering in the rate of oxygen transfer.
D1.8.	Time required for test	Continuous uptake test usually running 1–5 days (operator time 6 h per run of up to 6 units)

D2. Principle

A comparison is made of the rate and amount of oxygen taken up in closed test vessels containing inoculated reference sewage with that taken up in vessels containing the test material either alone or in admixture with the reference sewage.

The contents of the vessels are stirred rapidly and the consumption of oxygen is determined either by measuring the quantity of oxygen required to maintain constant gas volume in the respirometer flask, or from the change in volume or pressure in the apparatus. Evolved carbon dioxide is absorbed in a solution of potassium hydroxide. Uptake of oxygen by nitrification may occur for nitrogen-containing organic compounds and this may be taken into account by use of a specific inhibitor (D5.4).

D3. Interferences

3.1. Toxic materials may cause inhibition and care should be taken to ensure that they do not enter the system except by design. Examples of materials which may be present adventitiously are chlorine from tap water and chromate from glass cleaning operations. No special precautions, other than rinsing with distilled water, are needed to ensure removal of these substances.

3.2. If silicone grease is used on the ground glass joints care should be taken to avoid its entering the main body of the flask as it may cause interference by reducing the rate of oxygen transfer.

D4. Hazards

D4.1. Hygiene

Sewages and derived activated sludges may contain potentially pathogenic organisms therefore appropriate precautions should be taken when handling them to avoid the risk of infection.

D4.2. Chemicals

Precautions should be taken if the test material is toxic or its properties are unknown.

D5. Reagents

D5.1. Water — Distilled or deionized water is normally used, although in some experiments it may be necessary to use the public supply because of quality considerations, e.g. hardness.

D5.2. Activated Sludge

The source of the activated sludge used will depend upon the aim of the experiment. Generally to determine the toxicity of a trade waste or a chemical, sludge from a plant treating predominantly domestic sewage is used. For certain treatability studies, sludge from the particular plant involved may be used. If an acclimatized sludge is required it can be obtained by running a laboratory unit with sewage and a given amount of the test material (or the test material alone).

The sample should preferably be taken from the end of the aeration unit so that little external substrate remains but the sludge is still in an aerobic condition.

If required the sludge may be concentrated by settling or slow centrifuging (e.g. to obtain 12000 mg MLSS/l).

For some applications it may be necessary to wash the sludge by settlement/centrifugation and resuspension in an isotonic medium.

D5.3. Reference sewage

For general purposes settled sewage of domestic origin (BOD 125–250 mg/l) is required, which should be freshly collected and kept aerobic before use. Alternatively, synthetic sewage may be used (e.g. OECD synthetic sewage[4]).

For treatability studies relating to a particular plant, settled sewage from that plant will be required.

D5.4. Allyl thiourea solution

Inhibition of nitrification in activated sludges which have not previously been exposed to the inhibitor can be achieved by adding allyl thiourea solution (5 ml of 2.5 g/litre solution to each litre of test sample to give a concentration of inhibitor of 12.5 mg/litre).

D5.5. 20% Potassium hydroxide solution W/V

D6. Apparatus

D6.1. Suitable respirometers are Hach[14] and WRC[5] (see Method A) the Sapromat[15] and that of the Japanese Ministry of International Trade and Industry (MITI)[16].

D6.2. Water bath or constant temperature room

D7. Procedure

Step	Procedure	Notes
	Preparation of Apparatus	
D7.1.	Assemble the reaction vessels (e.g. 6), according to the manufacturers' instructions (see method A) and place them in the constant-temperature water-bath, or in a constant-temperature room.	
	Preparation of Activated Sludge	
D7.2.	Collect the mixed liquor on the day of the test and prepare as described in section D5.2. Thicken the sludge if necessary and determine the concentration of suspended solids (note a). Place the activated sludge in a water bath and aerate until used.	(a) The inoculum should be a small proportion of the total volume and therefore high suspended solids in the seed are required (e.g. 6000 mg MLSS/l)
	Characterization of Test Materials	
D7.3.	Determine the BOD and COD of the test and reference materials by standard methods[10, 24].	

Step	Procedure	Notes
	Prior Assessment of Toxicity of Test Substance (if required)	
D7.4.	To obtain quickly a rough guide to the toxicity of the test material, the respiration rate of the sludge in an oxygen electrode respirometer may be measured and the test substance added at the required concentration during the measurement (note b). The degree of inhibition is indicated by the amount of lowering of the respiration rate.	(b) See method C
	Preparation of Reaction Vessels	
D7.5.	Calculate (note c) the required volumes of reference sewage, test material (if appropriate), ATU (if needed) and water and add to the vessels (note d), (see Tables 2 and 5 for examples of toxicity measurement and Tables 3 and 4 for examples of treatability measurement).	(c) The volumes calculated should provide a constant organic (COD) load in each flask. (d) Usually reaction vessels are prepared in duplicate for each concentration of test material, and a pair of controls with no test material are included.
	Measurement of Oxygen Uptake	
D7.6.	Inoculate the vessels with the required volume of activated sludge (note e) and start the measurement of oxygen uptake. Usually no further attention is required other than to take the necessary readings and make occasional checks to see that adequate stirring is maintained. Stop the experiment when desired, usually after 1–5 days.	(e) A convenient concentration of suspended solids in the final medium is 1500 mg/l. For studies on a particular activated sludge works the appropriate concentration should be used.

D8. Expression and Analytical Interpretation of Results

Calculate the oxygen uptake values from the readings obtained as described in method A.

D8.1. Toxicity

When measuring the toxicity of a material using an experimental protocol such as described in Table 2 the oxygen uptake curves will generally be of the form shown in Fig. 9 showing increasing toxicity with increasing concentration.

D8.1.1. *Calculation*

The toxicity in terms of percentage inhibition can be calculated at a given time as shown below:

$$I = \frac{O_2 \text{ uptake Control} - O_2 \text{ Uptake test}}{O_2 \text{ uptake control}} \times 100\%$$

On many occasions, however, different values of inhibition are obtained when calculated after different incubation periods. If a curve has a sudden 'jump', such as curve 5x in Fig. 9, this may indicate that some form of 'acclimatization' has taken place. The experiment can be repeated using an activated sludge which has been acclimatized to the test substance in a laboratory activated sludge unit.

Even when there is no sudden jump, different values of inhibition can still be found at different times. The value could be quoted at one specific time (e.g. 6 hours to relate to the retention time in an activated sludge unit) or the values at hourly intervals can be calculated and plotted against time. The most reasonable (steady) value can then be picked off this graph.

If it is required to characterize a substance for comparative purposes, then a range of concentrations can be used. The EC_{50} value can then be obtained (EC_{50} is the concentration required to give 50% inhibition of respiration of the sludge) by plotting percentage inhibition against logarithm of concentration of test substance (in some cases a linear-linear plot may be used). The EC_{50} value is obtained from the graph by interpolation.

D8.2. Treatability

When assessing the treatability of a substance, and assuming all the vessels have been set up containing the same organic load (Table 3) curves of various forms can be obtained as illustrated in Fig. 10.

A curve the shape of (A) which ultimately takes up the same amount of oxygen as the control indicates that the material in the test sample is degraded to the same extent but more rapidly than the reference sewage. A curve the shape of (B) would indicate that the test substance is degraded more slowly. A curve the shape of (C) would indicate either that the test substance is not degraded to the same extent or that there is inhibitory material present. It is extremely difficult to distinguish between these two effects. A curve the shape of (D) would indicate that inhibition was occurring.

D.8.2.1. *Calculations* To give an indirect measure of degradation, the oxygen uptake of the test can be compared with the control sewage.

$$D = \frac{O_2 \text{ uptake by test material}}{O_2 \text{ Uptake by Control sewage}} \times 100\%$$

Again values obtained will depend upon the times chosen. When considering the treatability of trade wastes for acceptance into a sewer, it is probably best to quote the value at a standard time (6 hours retention time in A/S unit).

Table 2. Typical Scheme for Preparation of Vessels when Measuring the Toxicity of a Test Material in WRC Respirometer

		VOLUME ADDED (ml)				
	Vessel	Reference sewage	Test material	Water	Buffer solutions	Inoculum
1, 2	Control test conc = O	470	0	10	20	50
3	Test material conc = ½x*	470	1	9	20	50
4	Test material conc = x	470	2	8	20	50
5	Test material conc = 2x	470	4	6	20	50
6	Test material conc = 5x	470	10	0	20	50

* x = the expected concentration of the test material in sewage.

Note: For many cases a scheme as shown above will be adequate as the presence of the material will not significantly alter the organic load in the flasks. If the loadings are significantly different then different respiration curves will be produced which makes direct comparison between test and control vessels difficult. Therefore if the test material is extremely strong or the proportion added is high (most likely when dealing with trade waste) the volumes of test material, reference sewage and inoculum must be calculated to give a constant organic load (in terms of COD) and hence a fixed food micro-organism (F:M) ratio. An example is given in table 3.

Table 3. Typical Scheme for Preparation of Vessels when Assessing the Treatability of a Strong Trade Waste in WRC Respirometer

	Vessel	VOLUME ADDED (ml)				
		Reference sewage	Test material	Water	Buffer solutions	Inoculum
1, 4	Control	461	0	0	20	69
2, 5	Test material	0	46	415	20	69
3, 6	endogenous respiration	0	0	461	20	69

Table 4. Preparation of Hach Bottles for Assessing the Relative Treatability of an Effluent

Bottle No.	Vol of test solution (ml)	Test solution components (per litre of distilled water)			
		Activated sludge[a] (mg)	OECD synthetic sewage (100 x)[b] (ml)	Test[c] effluent (ml)	Bicarbonate solution 5% w/v[d] (ml)
1, 2	157	30	15	0	3
3, 4	157	30	0	15	3
5, 6	157	30	0	0	3

Notes:

(a) Add the appropriate volume which contains 30 mg MLSS.

(b) 15 ml of a 100 x concentrate of OECD synthetic sewage per litre of test solution should produce a BOD of about 240 mg O_2/l over 5 days. Taking a 157 ml test solution volume per Hach bottle would permit the use of a 0–350 scale with a direct readout of the BOD.

(c) The volume of test effluent should be adjusted to ensure that the organic loading on the sludge is similar to that in the bottles to which the standard sewage has been added. As a guide the Total Organic Carbon (TOC) of the OECD synthetic sewage is approximately 180 mg/l. Test effluent should be added such that the TOC[25] of the test solution is approximately 180 mg/l.

(d) If it is suspected that the activated sludge is actively nitrifying then bicarbonate solution may be added to buffer the pH.

To determine the treatability of test effluent/synthetic sewage mixtures, bottles may be set up with varying proportions of each component. However the TOC of the test solution should not exceed 180 mg/l when a sample volume of 157 ml is chosen.

Table 5. Preparation of Hach Bottles for Measurement of the Toxicity of a Test Material to Activated Sludge

Bottle No	Vol of test solution (ml)	Test compound conc (mg/l)	Test solution components (per litre of distilled water)			
			Activated sludge[a] (mg)	OECD synthetic sewage (100 x)[b] (ml)	Test[c] compound (mg)	Bicarbonate solution 5% w/v[d] (ml)
1, 2	157	0	30	15	0	3
3, 4	157	3. 2	30	15	3. 2	3
5, 6	157	10	30	15	10	3
7, 8	157	32	30	15	32	3
9, 10	157	100	30	15	100	3
11, 12	157	320	30	15	320	3

Notes

(a) Add the appropriate volume which contains 30 mg MLSS

(b) 15 ml of a 100-fold concentrate of OECD synthetic sewage per litre of test solution should produce a BOD of about 240 mg O_2/l over 5 days. Taking a 157 ml test

solution volume per Hach bottle would permit the use of a 0–350 scale with a direct readout of the BOD.

(c) Add the appropriate volume of stock solution to give these amounts of test compound. A preliminary range-finding test may suggest that other concentrations are more appropriate.

(d) If it is suspected that the activated sludge is actively nitrifying then bicarbonate solution may be added to buffer the pH.

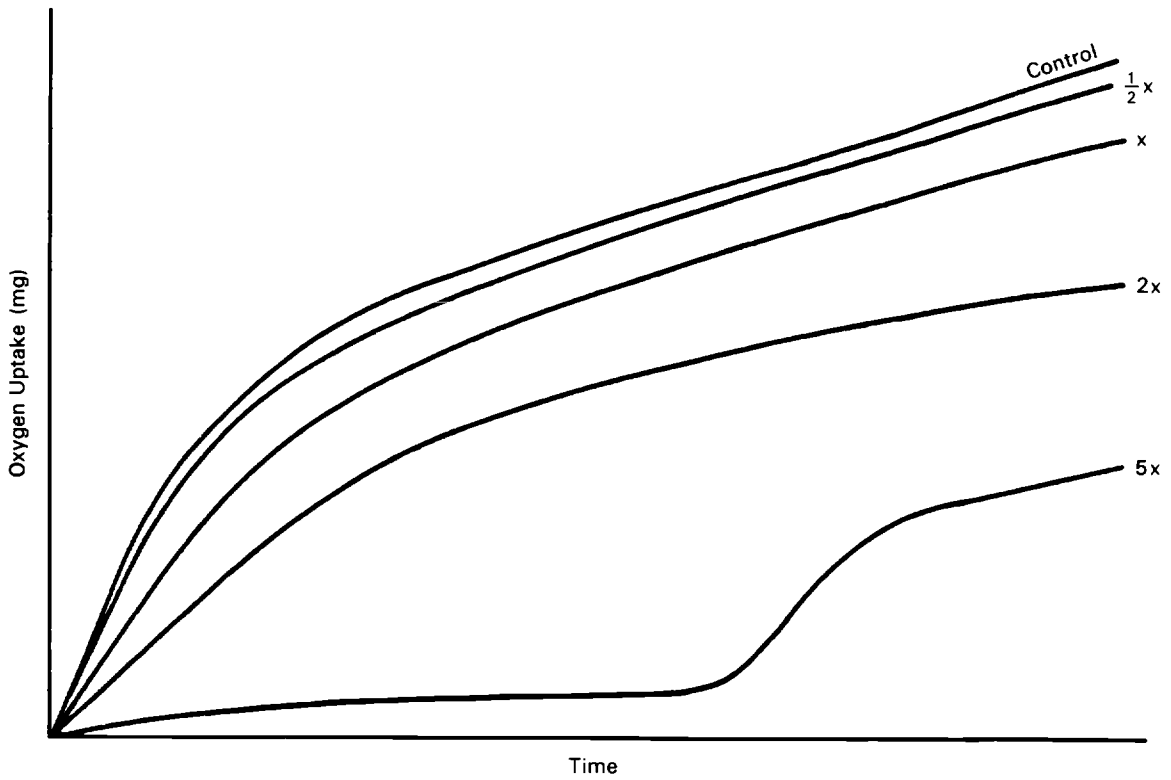


FIGURE 9 TYPICAL OXYGEN UPTAKE CURVES FROM A TOXICITY EXPERIMENT

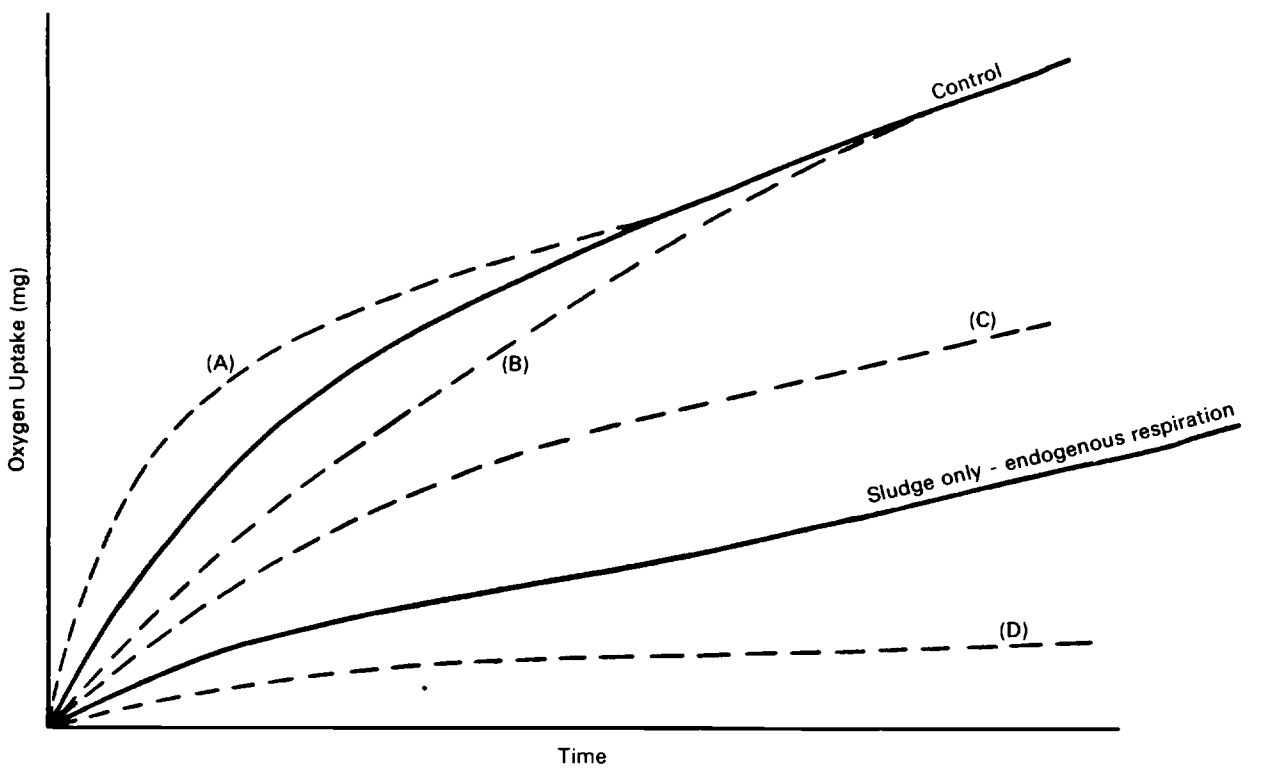


FIGURE 10 TYPICAL OXYGEN UPTAKE CURVE FROM A TREATABILITY STUDY

E. Continuous Simulation Test to Assess the Treatability of Chemicals and Industrial Waste Waters and their Toxicity to the Activated Sludge Process

Introduction

Simulation tests are applied to substances and industrial waste waters, which are expected to be discharged to a sewer, in order to ensure that, at the concentration used, they will not have any adverse effects on the sewage treatment processes. Alternatively, simulation tests may be used to find a suitable non-toxic concentration for discharge. Since they are relatively expensive tests, they are usually applied only to substances which are used in large quantities or which are of economic importance.

This test simulates activated-sludge sewage treatment and can be carried out using either the Husmann apparatus, which is the OECD[17] and EEC standard[2] for testing the biodegradability of synthetic surfactants, or the Porous Pot[18] apparatus developed at the WRC.

Both of these fail to simulate true conditions of sewage treatment, so that each apparatus is a compromise. For example, the Husmann unit has an air-lift pump which returns settled sludge at an abnormally high rate (greater than twelve times the sewage flow-rate), which does not allow anaerobic conditions to develop during settlement as happens in practice. The porous pot has no settlement phase at all.

Both treatability and toxicity are assessed by comparison of the performance of control units treating sewage alone with that of plants receiving a mixture of either sewage and test substance (or industrial wastewater) or industrial wastewater alone. The percentage removal of various parameters such as BOD, COD and ammoniacal-N is used to asses the treatability of the test material, while the percentage inhibition may be calculated by comparisons of the concentration of BOD, COD or NH_3 in the test and control effluents.

E1. Performance Characteristics

E1.1.	Properties determined	The treatability of an industrial waste water alone, or in admixture with sewage, by activated sludge sewage treatment, and its toxicity to activated sludge.
E1.2.	Type of sample	Single substances and industrial waste waters.
E1.3.	Basis of method	Determination of the effects of the test substance or industrial waste water on the performance of laboratory scale plants simulating the activated sludge process.
E1.4.	Range of application	0–100% removal of BOD or COD or DOC. 0–100% nitrification.
E1.5.	Standard deviation	No data.
E1.6.	Limit of detection	Limited by biological variation, and sensitivity of the analytical methods used.
E1.7.	Interferences	Any inhibitors of bacterial growth not entering the system by design; substances interfering in the analytical method used (see Section 3).
E1.8.	Time required for determination	Varies according to the treatability of the waste water tested. Generally a minimum of eight weeks. Operator time: 8 h/week for 4 units (excluding analysis)

E2. Principle

Comparison is made of the performance of laboratory-scale activated sludge units treating sewage alone with that of units receiving the industrial waste water alone or in a mixture of the waste and sewage. Performance is assessed by the removal of polluting matter, degree of nitrification, sludge growth and sludge settleability*; further indications of toxic effects of the industrial waste water may be given by microscopic examination of the sludges.

Ideally, the industrial waste water should be tested using the sewage to which it is, or is to be, discharged. If the waste appears to be untreatable when using a sewage already containing industrial wastes, the test should be repeated with synthetic or domestic sewage to eliminate any interactions between the industrial wastes.

E3. Interferences

Substances strongly adsorbing onto the walls of the plants will give false values. Some chemical substances in the air used for aeration may adversely affect the growth of the sludge micro-organisms.

E4. Hazards

E4.1. Hygiene

Sewage contains potentially pathogenic organisms, therefore appropriate protective clothing should be worn during maintenance of equipment and handling of samples.

E4.2. Mechanical and electrical

Guards should be fitted to the peristaltic pumps to avoid catching fingers in the rollers. Electric pumps and stirrers should be protected from splashes and leaks.

E4.3. Chemicals

The industrial waste water may have harmful properties and should be handled with care. Mercuric chloride, used in preservation of samples (except those for BOD analysis), and sodium hypochlorite, used for cleaning porous pots, should be handled with care.

* The use of large units is necessary when assessing settleability

E5. Reagents

E5.1. Settled sewage is collected from the overflow of the primary sedimentation tank. The sewage with which the industrial waste water is mixed for testing must be as similar as possible in composition and strength to that receiving the industrial discharge. The waste water may prove to be untreatable in this sewage as a result of the presence of other incompatible wastes. If this happens, its treatability in admixture with synthetic or domestic sewage should be determined.

E5.2. Synthetic sewage

A suitable synthetic sewage (OECD[17]) may be prepared as follows. For each 1 l of tap water add:—

160 mg peptone, 110 mg meat extract, (or 270 mg of mixed peptone-meat extract commercial preparation),

30 mg urea, 7 mg sodium chloride, 4 mg calcium chloride dihydrate, 2 mg magnesium sulphate heptahydrate, 28 mg dipotassium hydrogen phosphate (not in original recipe).

For convenience, this may be prepared as a 100-fold concentrated solution, which can be stored at 1°C for up to 1 week, and the synthetic sewage is prepared daily from this by appropriate dilution with tap water. The pH value should be in the range 7–7.5 and any adjustment to achieve this should be made using hydrochloric acid (1M) or sodium carbonate/bicarbonate (1M) (see section E10.2).

E5.3. Industrial waste water

Fresh, representative samples of the industrial waste water to be tested should be collected from the site and if necessary, stored at 1–4°C to avoid changes. This may be mixed with sewage in the appropriate proportion or dosed separately to the aeration vessels.

E5.4. Test substance

An appropriate amount of test chemical may be dissolved in the sewage to be used, or a stock solution of appropriate strength is prepared for dosing separately to the aeration vessels.

E5.5. Activated sludge

A supply of activated sludge is collected from the aeration tank or return-sludge line of an appropriate treatment plant and kept aerated until required. The time between collection and use should be kept to a minimum and should not exceed a few hours.

E5.6. Mercuric chloride

A 1% m/v solution of Analytical Reagent grade mercuric chloride for preservation of samples, if required, except for BOD determinations.

E5.7. Lubricant

Glycerol or olive oil may be used for lubricating the peristaltic pump rollers.

E6. Apparatus

Laboratory scale activated sludge plants are used; suitable systems are the EEC standard (Husmann) units and WRC porous-pots.

E6.1. EEC unit

The small activated sludge plants are constructed according to the OECD/EEC specifications (Figs. 11 and 12). They are made from acrylic polymer or glass and consist of a cylindrical aeration chamber (C) of 3-litre capacity with a conical base. The aeration vessel has an outlet at one side which passes into the conical base of a settlement chamber (D), which is also cylindrical and 3/5 of the diameter of the aeration chamber. Near the top of the settlement chamber is an effluent outlet fitted at a level to maintain 3-litre in the aeration chamber, while at the base is an air-lift pump (E) for returning settled sludge to the aeration vessel.

Filtered compressed air is supplied through a pressure regulator to the air-lift pump, and to the aeration vessel. Each air supply is controlled by a needle valve and the aeration chamber supply is metered by a direct reading flow meter (H). Two diffusers (G) are placed in the aeration vessel to produce fine bubbles and to achieve effective mixing (one diffuser may sometimes prove inadequate).

The 24-h supply of sewage is contained in a suitable polythene container (A) and a similar vessel (F) is used for collection of the effluent. Suitable tubing (e.g. silicone rubber) is used to supply the sewage and/or industrial waste to the aeration chamber by means of a peristaltic pump (B). A length of tubing notched at the distal end to prevent blocking reaches to the base of the influent vessel, and a curved piece of the same tubing at the outlet is clipped to the side of the aeration vessel. A removable curved tube may be fitted onto the outlet of the air-lift pump to deliver returned sludge below the rim and near to the centre of the aeration vessel so that loss of sludge by splashing is prevented.

E6.2. Alternative apparatus

WRC porous pot units

These units differ from the EEC units in one essential point; there is no settlement vessel, most of the solids in the mixed liquor being retained in the aeration vessel by the porous material of which it is made.

The porous pots are constructed from sheets of porous polythene (2 mm thick, maximum pore size 95 μm), which are made into cylinders 14 cm in diameter with a conical base at 45° (Figs. 13 and 14). The porous pot is contained in an impervious PVC vessel 15 cm in diameter with an outlet at a height of 17.2 cm on the cylindrical part, which determines the volume of 3 l in the porous pot. There is a PVC supporting ring round the top of the porous pot, so that there is an effluent space (of 0.5 cm) between the inner and outer vessels.

The porous pot units are set in sockets in the base of the thermostatically-controlled water bath. There is a metered air supply to the porous pot in which there are placed two diffusers.

Sewage is pumped from a polythene container through suitable tubing (e.g. silicone rubber) to the porous pot. A multichannel peristaltic pump may be used to serve several units. The effluent is collected in another polythene container of suitable size. Spare inner porous pots should be available to replace any which may block in use.

E6.3. Larger scale activated sludge plants

In some circumstances more precise information of the effects of industrial waste water on sludge properties is required. To determine the effects on stirred sludge volume (SSV) and capillary suction time (CST), larger-scale activated sludge plants have to be used from which enough sludge may be wasted to allow measurements, e.g. for SSV about 3.5 l of mixed liquor.

A suitable plant would consist of a complete-mixing aeration tank (e.g. of 400 l capacity) with a circular clarifier. Alternatively, a plug-flow system may also be used consisting of a number of similar completely mixed tanks in series, also with a circular clarifier.

Settled sludge is returned continuously from the clarifier to the aeration tank from which mixed liquor is wasted at a continuous rate to establish equilibrium conditions at a suitable mixed liquor suspended solids (MLSS) concentration.

In the plug-flow system settled sludge is returned to the first aeration vessel, and mixed liquor may be wasted from the last aeration tank of the series — activated sludge from the last tank should be used for SSV and CST determinations.

E7. Sample collection and preservation

Spot samples of sewage, sewage plus industrial waste water or industrial waste water feed are taken and preserved (e.g. with 40 mg/l mercuric chloride, by adding 2.0 ml of 1% m/v

mercuric chloride solution to 500 ml of sewage*), if analysis can not be carried out immediately. It may be necessary to allow for changes in the composition of the sewage and/or waste water over a 24 h period, e.g. by taking another sample at the end of the period, and using the average value of this and the initial sample.

24 h composite samples of effluent are collected and 500 ml samples taken after mixing are filtered through washed glass wool, preserved if necessary (e.g. with 20 mg/l mercuric choride by adding 1 ml of 1% m/v solution per 500 ml sample of effluent*).

E8. Procedure

Step	Procedure	Notes
E8.1	**Procedure for Industrial waste water in admixture with sewage**	
	Running in	
E8.1.1.	Collect 24 h supply of sewage freshly each day (note a) (or prepare the synthetic sewage), and aerate at a rate sufficient to maintain a minimum of 2 mg dissolved oxygen per litre.	(a) If available, a continuous supply of sewage should be used, since this avoids the necessity of regular collections of sewage and daily cleaning of storage vessels; also aeration of the sewage may be unnecessary.
E8.1.2.	Add the appropriate volume of activated sludge (note b) to the aeration vessel to start the test.	(b) Approximately 3000 mg/l is a suitable concentration.
E8.1.3.	Pump sewage to the aeration vessels of two pairs of plants (note c) at a suitable rate to give a 3 h retention (note d) in each. The air flow to the aeration vessel is set to give complete mixing and a concentration of dissolved oxygen of at least 2 mg/l (note e).	(c) Pump tubing should be lubricated (e.g. with glycerol) and it should be replaced at the first signs of wear (stretching or splitting). Influent tubing should be cleaned out regularly (e.g. twice weekly) to remove bacterial growth. (d) This is shorter than the average sewage retention for UK treatment works, and thus may underestimate removal of the industrial waste water components. To ascertain the treatability of the waste water at a particular works, the appropriate retention time should be substituted.
	Operation of Husmann units	
E8.1.4.	The air-lift pump should continuously return settled sludge (note f). Any sludge accumulating in the air-lift pump circuit or in the settlement vessel and around the rim of the aeration vessel should be returned to circulation as soon as possible e.g. by scraping or brushing — at least twice each day.	(e) For example: an air flow of 2.5 l/min at 21°C will result in a dissolved oxygen concentration of approximately 7 mg/l, but a high rate is necessary to create turbulence and thus to avoid sludge settling in the aeration vessel. (f) The airlift pump gives a high flow of returned sludge. Normally in sewage treatment the return flow of sludge is nearly equal to the inflow of sewage. However, in this design (Fig. 12) the return sludge flow cannot be reduced to less than 12:1.

* Samples for BOD analysis should not be preserved with mercuric chloride.

Step	Procedure	Notes
	Operation of porous pots	
E8.1.5.	The slude accumulating around the rim of the aeration vessel should be scraped down at least twice daily. The porous pot should be changed at the first sign of blocking of the pores, i.e. when the mixed liquor level rises above the effluent spout. A fresh porous pot is placed in the outer container and all the mixed liquor carefully transferred. Any sludge sticking to the sides of the blocked pot is also scraped off and transferred. The blocked pot is thoroughly cleaned before re-use (note g).	(g) A fine jet of water may be used to remove any remaining sludge from the pots before they are soaked for about 24 h in a 1:1 dilution of industrial grade sodium hypochlorite. The pots should be totally immersed. After 24 h, the pots are removed, rinsed thoroughly with water and then soaked for about 24 h in water. If there is any remaining odour of chlorine the soaking in water is continued until it is removed, or it may be removed with thiosulphate. Note: Sodium hypochlorite is hazardous and protective clothing (eye shields and gloves) must be worn when using the chemical.
E8.1.6.	Take sewage and effluent samples as required, e.g. twice weekly, for a period of 4–6 weeks. This is the running-in period (note h). Assess the performance of the plants by suitable parameters e.g. BOD, COD, DOC, NH_3 and Ox-N (note i). Determine mixed liquor suspended solids twice weekly (note i) and waste sludge to maintain approximately 2.5 g MLSS/l, or apply constant wasting, e.g. 10% of the mixed liquor each day. For larger-scale plants determine SSV and CST (note i) as required on the mixed liquor (note j).	(h) The running-in period ensures that test and control units are giving similar results and that they have reached a steady state. If results are not similar greater comparability may be attained by interchange of sludges i.e. by drawing off, mixing the two sludges and redistributing the resulting mixture equally between the two plants. (i) See Refs 10, 24, 25, 26, 27, 1 and 8. (j) The SSV and CST of the activated sludge should be the same for test and control units in the running-in stage.
	Steady State	
E8.1.7.	When the removal of BOD and COD have become steady and nitrification is complete in each plant, begin the addition of the industrial waste water or test chemical to one pair of units at the required proportion (note k). Continue the determinations of BOD, COD, DOC, NH_3, Ox-N, (MLSS, SSV and CST) twice weekly for a further 4–6 weeks (note l) or until a steady state is reached in the test plants.	(k) If the volume of the industrial discharge is not known, the ratio of waste water to sewage should be estimated and twice this concentration tested to allow a safety margin. Normally the proportion of industrial waste water to sewage would be less than 5%. If a continuous supply of sewage is used, the industrial waste water can be dosed separately at an appropriate rate to the aeration vessel. Alternatively, a mixing tank may be used in which an aliquot of sewage is mixed with an appropriate volume of waste water before dosing to the aeration vessel.
	Treatability	
E8.1.8	If the industrial waste water has adverse effects on the sludge and the performance of the plants does not show any signs of recovery after a few weeks, repeat the test using lower concentrations of industrial waste water until there are no adverse effects observed.	(l) The duration of the test depends on the treatability and toxicity of the waste water, eg. if nitrification is inhibited it may or may not be resumed after a period of acclimatization of the sludge to the test material.
	Toxicity	
E8.1.9	If it is required to know at what level the waste water causes adverse effects, the method can be repeated using a higher proportion of waste water to sewage until the concentration (if any) which is toxic is established. Determination of the effects of shock loads or intermittent discharging may be necessary, in which case a steady state is not applicable.	

E8.2 Procedure for Industrial waste water alone

The industrial waste water may contain sufficient nutrients to support sludge growth without requiring addition of sewage. Normally, a feed having organic carbon, nitrogen and phosphorus in the approximate ratio of 100:10:1 would support healthy sludge growth. If the waste water contains excess carbon, other nutrients may be added — e.g. if it contains a high proportion of carbohydrates, ammonium salts and phosphates can be added.

The procedure is similar to that described in procedure E 8.1 except that the industrial waste water is used as the feed (instead of sewage) from the start of the test. In this case control units are not required, as comparison of units with different feeds would not be valid.

Performance of the plants is assessed by determining the amount of carbonaceous oxidiation, nitrification and the properties of the activated sludge as for procedure E.8.1.

The acceptable amount of removal of the industrial waste water components depends on the purpose of the separate treatment — whether to reduce the BOD to a concentration which may be discharged to the sewer, or to biodegrade the waste water to an acceptable BOD for discharge to the environment. In both cases the required emission standards must be met.

It may be necessary to alter the operating conditions of the plant (e.g. increasing the retention time, or controlling the pH value) to obtain an effluent of the required quality.

Some components of the industrial waste water may be biodegraded, while others are not, so specific analysis may be required to determine the components which are resistant to biodegradation.

E9. Estimation of Treatability and Toxicity

E9.1. Calculation of Treatability

E9.1.1. Waste water in admixture with sewage

The percentage removals of BOD, COD, and NH_3 for the test and control plants can be calculated using the following formula:—

$$\text{Overall removal} = \frac{I - E}{I} \times 100\%$$

where I = BOD, COD, or NH_3 of the influent (for control) or sewage plus industrial waste water (for test), and E = BOD, COD or NH_3 of the effluent.

Assuming the loading of the test and control plants is the same (see section E10.3) the removal of BOD (or COD) present in the industrial waste water component can be calculated (see Section E9.4) to be:—

$$\left[\frac{(C_m - E_m) - (1 - x)(C_s - E_s)}{C_m - (1 - x)\ C_s} \right] \times 100\%$$

where x = fraction of industrial waste water in mixed sewage
C_m = BOD (or COD) of mixed sewage
C_s = BOD (or COD) of sewage alone
E_m = BOD (or COD) of test effluent
E_s = BOD (or COD) of control effluent.

The amount of oxidation of NH_3-N can be used as a measure of nitrification*. Percentage inhibition of nitrification by the industrial waste water may be calculated by the following formula:—

$$\left[\frac{(C_s - E_s) - (C_m - E_m)}{(C_s - E_s)} \right] \times 100$$

where C_s = ammonia-N concentration of the control influent (sewage alone)
E_s = NH_3-N concentration of the control effluent
C_m = NH_3-N concentration of the test influent
E_m = NH_3-N concentration in the test effluent.

* The calculation for ammonia removal is not applicable when OECD/EEC synthetic sewage is used, since it contains urea but no ammonium salts.

Alternatively, assuming no denitrification occurs, the production of nitrate and nitrite (oxidized nitrogen) may be used to calculate the percentage inhibition of nitrification:

$$\text{percentage inhibition} = \frac{(OxN_S - OxN_m)}{OxN_S} \times 100$$

where OxN_S = oxidized nitrogen concentration in control effluents
OxN_m = oxidized nitrogen concentration by test effluents.

If $OxN_m > OxN_S$ nitrification probably has been stimulated in the presence of the industrial waste water.

E9.1.2. Treatability of waste water alone

The percentage removals of BOD, COD and NH_3 can be calculated from the same formulae as those for the overall removals given in E9.1.1., where BOD_S, COD_S, NH_{3S} would be the BOD, COD, NH_3 respectively of the influent waste water and BOD_E, COD_E and NH_{3E} of the effluent.

E9.2. Calculation of toxicity

The toxicity can be expressed as a percentage reduction in the removal of BOD or COD in the test plant compared to that of the control plant:

$$\text{Percentage inhibition} = \frac{R_C - R_T}{R_C} \times 100$$

where R_C is the percentage removal of BOD, COD or NH_3 of the control unit, and R_T is the percentage removal of BOD, COD or NH_3 of the test unit.

E9.3. Interpretation of Results

E9.3.1. *Treatability*

An industrial waste water can be assumed to be adequately treatable if the polluting matter it contains is satisfactorily removed under the conditions of the test. What can be regarded as 'satisfactory' removal depends on the frequency of discharge, any subsequent treatment process, and ultimate destination of the effluent. Reduction in settleability or increased CST of the sludge may be an important factor in activated sludge treatment.

If there is good removal of the industrial waste water components only after a period of acclimatization, the waste water would be suitable for continuous discharge, but intermittent discharging would be unsuitable and may result in deacclimatization if the period between the discharges were too long.

If the industrial waste is not satisfactorily removed at twice the discharge concentration, the test concentration should be reduced until a treatable level is found.

If the industrial waste has adverse effects on the sludge at all the concentrations tested, it can be assumed to be untreatable by the activated sludge process and alternative means of treatment must be considered.

E9.3.2. *Toxicity*

The industrial waste water may be toxic to only one group of organisms in the sludge, or to the whole range of sludge micro-organisms.

If it is toxic to the nitrifying bacteria, a reduction in the concentration of nitrate in the effluent will occur, with a resulting increase in effluent ammonia concentration, the changes being a measure of the toxicity. It may also be toxic to carbonaceous oxidative processes, when a decrease in the removal of BOD and COD would be observed. The presence of the industrial waste water may also promote the growth of certain species of the sludge population, adversely affecting the settleability of the sludge or its capillary suction time.

One or any of these effects may occur, but the toxicity may be temporary and acclimatization of the sludge micro-organisms to the industrial waste water may follow. Therefore, if the inhibition is partial, it is important to continue the testing for a period of

weeks to allow some time for acclimatization. Some toxic materials (eg. metals), which build up slowly on sludge solids before reaching critical levels, may require several weeks testing before their effect on the process is established.

E9.4. Derivation of the Calculation of the Removal of BOD or COD present in an Industrial Waste Water

The removal of BOD or COD present in the industrial waste water component is calculated as follows (Section E9.1.1 refers):—

Let C_w = BOD (or COD) of waste water

E_w = equivalent effluent BOD and assume no interactions.

Then

$$C_m = (1 - x)\, C_s + xC_w$$

$$\therefore \quad C_w = \tfrac{1}{x}(C_m - (1 - x)\, C_s)$$

Similarly,

$$E_m = (1 - x)E_s + xE_w$$

$$\therefore \quad E_w = \tfrac{1}{x}(E_m - (1 - x)E_s)$$

$\therefore$ Removal of industrial waste water component

$$= \frac{C_w - E_w}{C_w} \times 100\%$$

$$= \frac{\tfrac{1}{x}(C_m - (1 - x)\, C_s) - \tfrac{1}{x}(E_m - (1 - x)\, E_s)}{\tfrac{1}{x}(C_m - (1 - x)\, C_s)} \times 100\%$$

$$= \frac{(C_m - E_m) - (1 - x)\,(C_s - E_s)}{C_m - (1 - x)\, C_s} \times 100\%$$

where x = fraction of industrial waste water in mixed sewage

C_m = BOD (or COD) of mixed sewage
C_s = BOD (or COD) of sewage alone
E_m = BOD (or COD) of test effluent
E_s = BOD (or COD) of control effluent

E10. Sources of error

E10.1 Comparability of test and control units before the addition of industrial waste is very important. The addition should not be started until the same steady removals have been achieved in both control and test units. Also, the performance of the controls must remain stable during the testing period. If any problems develop e.g. those described in the following paragraphs, the running in stage should be repeated until comparable results are obtained.

E10.1.1. Loss of sludge in the effluent (of Husmann plant)

If the sludge is allowed to accumulate in the settlement vessel, denitrification may occur causing bubbles of nitrogen gas to be formed which attach themselves to the sludge, resulting in floating masses of sludge, which are washed out in the effluent.

Filamentous growth will also result in poor settling and sludge in the effluent. These solids in the effluent would give rise to high COD values, and therefore effluent samples should be filtered or centrifuged before analysis.

E10.1.2. Non-return of settled sludge (in Husman plant)

Sometimes, particularly if the sludge is filamentous, 'bridging' occurs at the base of the settlement vessel, so that sludge accumulates there and is not returned to the aeration vessel, the returned liquid being low in suspended solids. This results in depletion of sludge in the aeration vessel and consequently low removal of COD and test substance. This can be temporarily remedied by dislodging the sludge with a brush.

E10.2 Synthetic sewage

The concentration of phosphate in the synthetic sewage is insufficient for adequate buffering of the mixed liquor if complete nitrification (an acid producing reaction) occurs and as a result the pH value may fall sufficiently to reduce the degree of removal of COD and perhaps of the substance under test. This may be remedied by adding suitable concentrations of bicarbonate to the synthetic sewage, using the fact that the oxidation of 1 mgN to nitrate produces an acidity equivalent to about 7 mg $CO_3^=$.

E10.3 Loading

Unless the BOD or COD of the sewage and the industrial waste water are identical, the organic loads to the plants will not be the same. Since the proportion of waste water added is low, usually $< 5\%$, the differences in loading are not likely to be high enough to alter the performance of the plants significantly. However, if the waste water is extremely strong or if the proportion added is high, due consideration must be given to the effect that the larger differences in loading might have. It might even be necessary to adjust the strengths of the influents by addition of water or of a solution of known biodegradable organic substances ('synthetic sewage').

E11. Checking accuracy

Duplicate plants are run simultaneously and mean values are taken to reduce the variability of results from similar units. The length of time taken to test a particular industrial waste water depends upon its behaviour in treatment. Therefore, if the waste is not removed readily in a short time with no adverse effects on plant performance, the test should be extended to allow time for acclimatisation of the sludge to the test material. If no signs of improvement occur after 6 weeks of addition, or if the sludge is killed on the first addition, a range of lower concentrations must be tried before assuming that the waste water is untreatable*.

The test described is normally carried out at room temperature i.e. 18–25°C. If the industrial waste water is expected to be discharged all year round, or in the winter season only, it may be necessary to assess its treatability at, say, 10°C.

* To obtain an approximate idea of the concentration of industrial waste water which can be treated without toxic effects preliminary respiration rate determinations can be carried out. (method C)

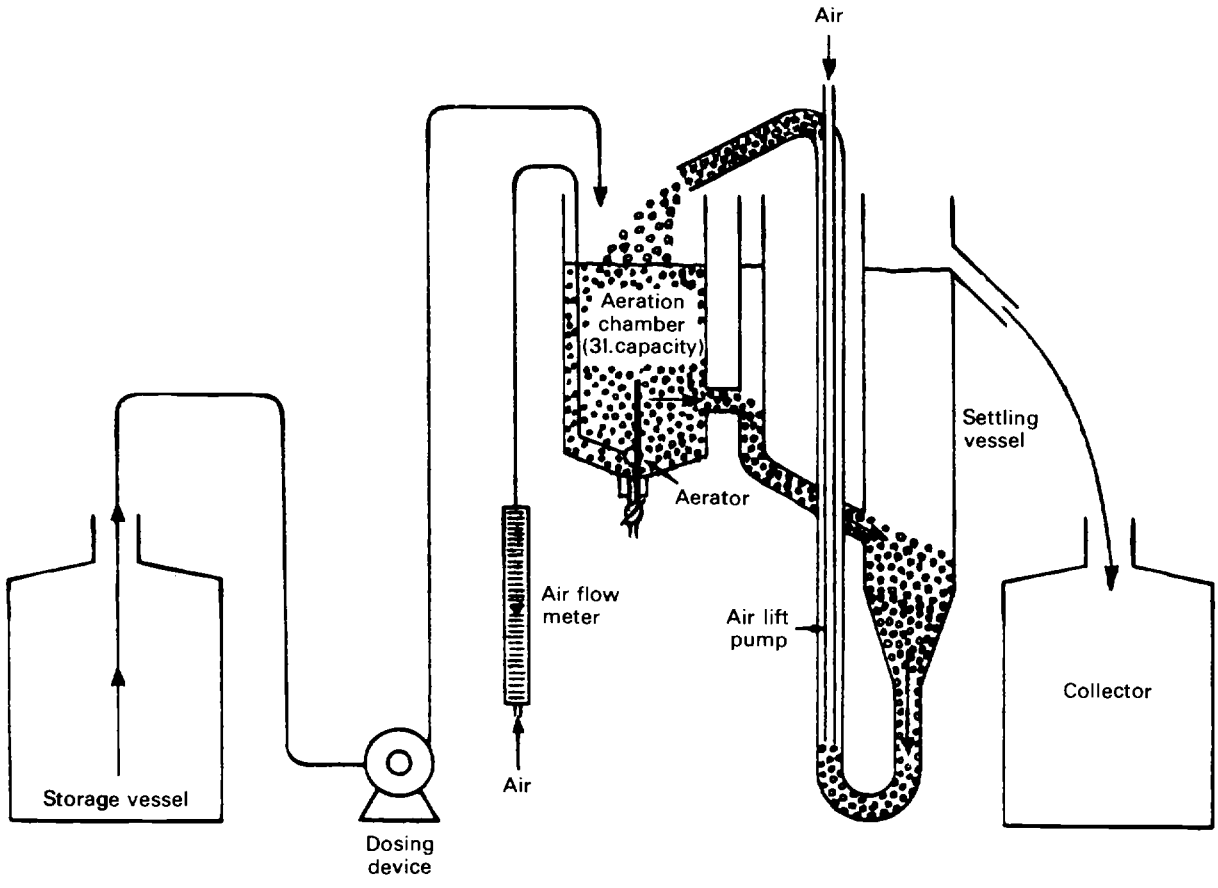


FIGURE 11 EQUIPMENT USED FOR ASSESSMENT OF TOXICITY/TREATABILITY (Husmann unit)

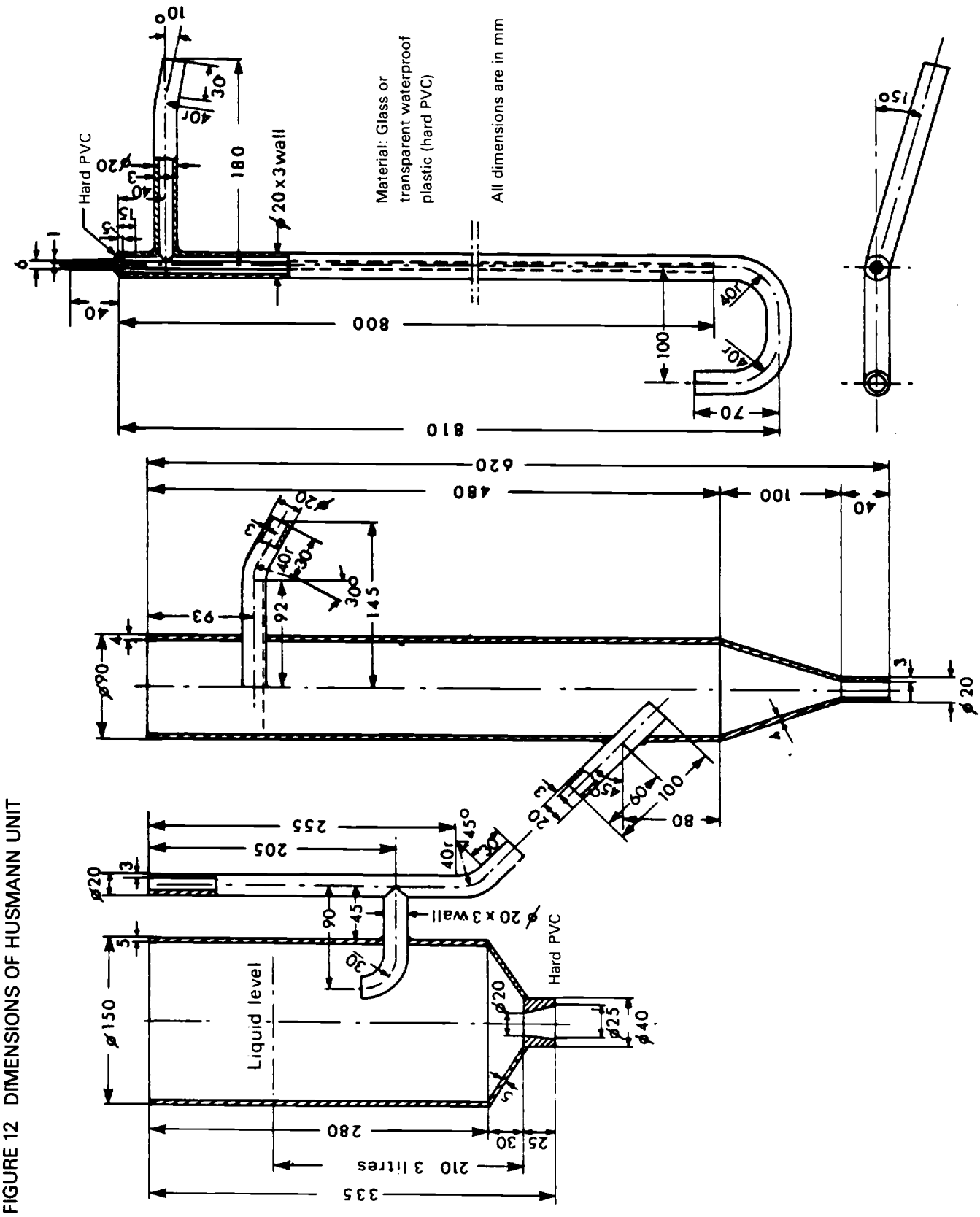


FIGURE 12 DIMENSIONS OF HUSMANN UNIT

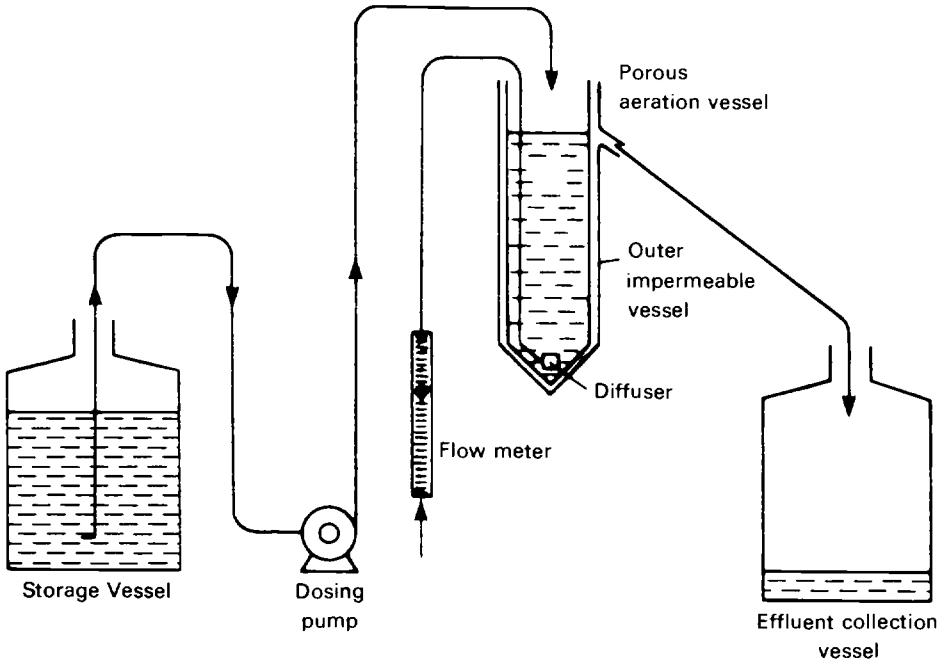


FIGURE 13 EQUIPMENT USED FOR ASSESSMENT OF TOXICITY/TREATABILITY (Porous pot)

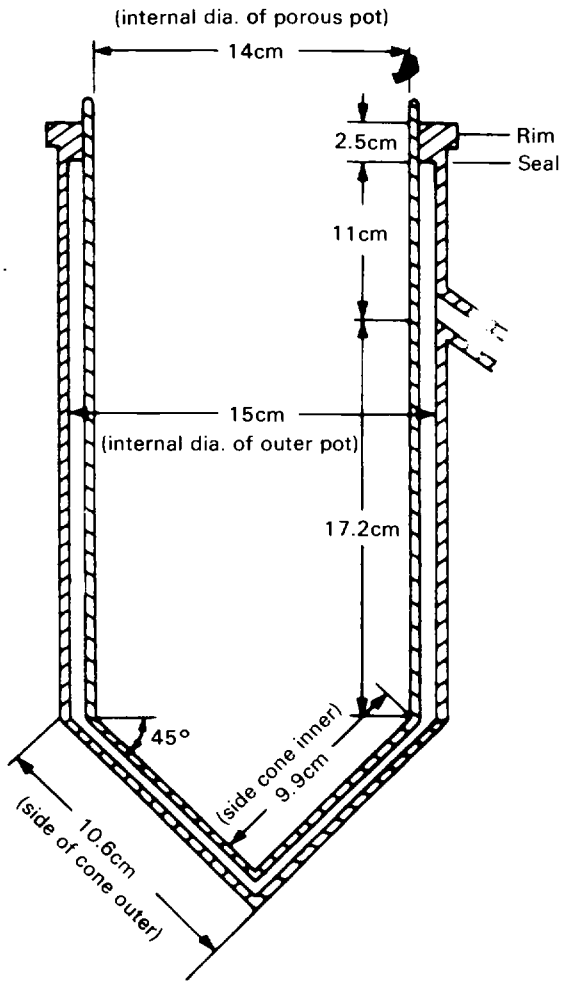


FIGURE 14 DETAILS OF 3-LITRE POROUS-POT AERATION VESSEL

F. Continuous Simulation Test to Assess the Treatability of Chemicals and Industrial Waste Waters and their Toxicity to the Biological Filter Process

Introduction

This simulation test is based on the continuous test originally described by Gloyna et al[19] and developed by Tomlinson and Snaddon[20]. It can be applied to chemicals or industrial waste waters which have given indications of treatability and/or toxicity (e.g. in tests such as methods C and D) and where additional information is required for application to the biological filter method of sewage treatment. If the industrial waste water is a mixture, and it proves toxic or untreatable, it may be necessary to separate the components for further study.

Since the test is relatively expensive and time-consuming it is usually only resorted to in the case of industrial waste waters of importance either in terms of their potential effect on a wide range of treatment plants or of particular local significance.

Procedure FA of this method describes the assessment of treatability and/or toxicity of chemicals and waste waters in admixture with sewage. However, if the industrial waste water contains sufficient nutrients for bacterial growth it may be tested alone (Procedure FB).

The conditions of the test may be varied to simulate more accurately the performance of a particular treatment plant (See Section F12).

Where the chemical or industrial waste is tested in admixture with sewage, the treatability and toxicity are assessed by comparison of the performance of test and control units. This is only valid if the loading of the test and control units is the same (See Section F10.2).

F1. Performance Characteristics

F1.1.	Properties determined	The treatability of an industrial waste water or chemical alone, or in admixture with sewage in the biological filter process, and the toxicity of the test material to that process.
F1.2.	Type of sample	Industrial waste waters and chemicals which are soluble and non-volatile.
F1.3.	Basis of method	Determination of the effects of an industrial waste water or chemical on the performance of laboratory scale plants, simulating the biological filter process.
F1.4.	Range of application	0–100% removal of BOD or COD or DOC. 0–100% nitrification
F1.5.	Standard deviation	Within test 3.5% Between tests 5.0%
F1.6.	Limit of detection	Limited by biological variation, and the sensitivity of the analytical methods used.
F1.7.	Interferences	Any substance present in the air or the test media, which inhibits the growth of micro-organisms. Any substance which interferes in the analytical method being used (see Section 3).
F1.8.	Time required for determination	A maximum of nine weeks. Operator time: (excluding analysis) for a 6 tube installation, approximately 12 hours/week.

F2. Principle

F2.1. Comparison is made of the performance of laboratory scale units simulating the percolating filter process treating sewage alone with that of units receiving an industrial waste water or the test chemical, either alone or in admixture with sewage.

F2.2. The test simulates the performance of a biological filter by the application of sewage and/or the material to be tested to the internal surface of a slowly rotating inclined tube. A layer of micro-organisms similar to that present on the surface of filter media is built up on the internal surface of the tube. The effluent from the tube is collected and either allowed to settle or filtered before analysis.

Performance is assessed by the removal of polluting matter, or degree of nitrification.

F2.3. Ideally, the industrial waste water should be tested using the sewage to which it is, or is to be, discharged. If the waste appears to be untreatable when using a sewage already containing industrial wastes, the test should be repeated with synthetic or domestic sewage to eliminate any interactions between the industrial wastes.

F3. Interferences

Any chemical substance in the sewage or in the air which may adversely affect the growth of sludge micro-organisms. Examples are: organic solvents, toxic metals, strong acids and alkalies, bactericides. Any substance which interferes in the analytical methods used.

F4. Hazards

F4.1. Hygiene

Sewage contains potentially pathogenic organisms, therefore appropriate precautions should be taken during maintenance of equipment, and handling of samples.

F4.2. Mechanical and Electrical

Guards should be fitted to the moving rollers of peristaltic pumps, electric motors, gears, shafts and wheels driving the totating tubes.

All electrical equipment should be protected from splashes and leaks.

F4.3. Chemicals

The industrial waste water or test chemical may have harmful properties, and should be handled with care.

Mercuric chloride used in the preservation of samples (except those for BOD analysis) also should be handled with care.

F5. Reagents

F5.1. Settled Sewage

When sewage is to be used for the test it should be collected each day from the overflow channel of the primary sedimentation tank or, if possible, the feed to the biological filters. The waste water may prove to be untreatable in this sewage as a result of the presence of other incompatible wastes. If this happens, its treatability in admixture with synthetic or settled domestic sewage should be determined.

F5.2. Synthetic Sewage

The synthetic sewage is composed as follows for each litre of tapwater:— 160 mg peptone, 110 mg meat extract, (or 270 mg of mixed peptone-meat extract commercial preparation), 30 mg urea, 7 mg sodium chloride, 4 mg calcium chloride dihydrate, 2 mg magnesium sulphate heptahydrate, 28 mg dipotassium hydrogen phosphate.

For convenience, this may be prepared as a 100-fold concentrated solution in distilled water, which can be stored at 1°C for up to 1 week, and the synthetic sewage made daily from this by appropriate dilution with tap water.

The synthetic sewage after dilution contains approximately 106 mg/l organic carbon, 46 mg/l nitrogen, 5 mg/l phosphorus.

The pH value of the applied sewage should be in the range 7.0—7.5 and any adjustment necessary to achieve this should be done using dilute hydrochloric acid (1M) or dilute sodium carbonate/bicarbonate (1M) (See Section F10.3).

F5.3. Test substance

An appropriate amount of test chemical may be dissolved in the sewage, or alternatively a stock solution of appropriate strength may be prepared and dosed separately to the tube.

F5.4. Industrial waste water

Fresh representative samples of the industrial waste water to be tested should be collected from the site, and if necessary stored at 1–4°C to avoid changes, and added as in F5.3.

F5.5. Mercuric Chloride

1.0% m/v solution of mercuric chloride for preservation of samples when necessary.

F5.6. Lubricant

Glycerol or olive oil may be used for lubricating the peristaltic pump rollers: both are suitable for use with silicone rubber tubing.

F6. Apparatus (Figs. 15 and 16)

This consists of a bank of acrylic tubes 30.5 cm long x 5 cm internal diameter, supported on rubber-rimmed wheels contained within a supporting metal frame. The tubes (Fig. 15) have an outside lip approximately 0.5 cm deep to retain them on the wheels, the internal surface is roughened by abrasion with coarse wire wool, and there is a 0.5 cm deep internal lip at the feed end to retain the liquid. The tubes are inclined at an angle of

approximately 1° to achieve the required contact time when the test medium is applied to a clean tube. The rubber-tyred wheels are rotated using a slow variable-speed motor. The temperature is controlled by installation in a constant temperature room. A 24 hour supply of settled sewage or material to be tested is contained in a 20-litre storage vessel (A) (Fig 16).

The vessels have outlets near the bottom and are connected by suitable tubing, e.g. silicone rubber, via a peristalic pump (B), to a glass or acrylic delivery tube which enters 2–4 cm into the higher end of the inclined tube (C). Effluent is allowed to drip from the lower end of the inclined tube to be collected in another storage vessel (D), and is settled or filtered before analysis.

F7. Sample collection and preservation

F7.1 A sample of feed solution or solutions (if test material is dosed separately) is taken after approximately six hours settlement in the feed vessel and preserved, if necessary with 40 mg/l mercuric chloride*, by adding 2.0 ml of 1% w/v mercuric chloride to 500 ml of sample.

A further sample may be taken 16 hours later if it is considered that the composition may vary in that time. The mean on the two analyses is then used in calculations: this takes into account any changes in the feed during the day.

F7.2. The effluent from the tube is collected over a period of approximately 16 hours starting at the time of the initial sampling of the feed solution. The bulk effluent is thoroughly mixed before a sample is removed for settlement and/or filtration prior to analysis. If samples are to be preserved but BOD determinations are not required, the effluent may be preserved by collecting directly into mercuric chloride solution (2.0 ml of 1% w/v for each 500 ml).

F8. Procedure

Step	Procedure	Notes
	Procedure for Industrial Waste Water or Test Chemical in Admixture with Sewage	
F8.1	**Collection or Preparation of Sewage**	
F8.1.1.	Settled sewage is collected, preferably each day, from the overflow channel of the primary sedimentation tank or if possible from the feed to the biological filters (see F5.1), and kept aerated before use to maintain a minimum of 2 mg dissolved oxygen per litre.	
F8.1.2.	Alternatively, synthetic sewage is freshly prepared each day from a concentrated stock solution by appropriate dilution (note a). The sewage is added to clean influent vessels (note b).	(a) 100 x stock stored at 1°C for no longer that 1 week. (b) Influent and effluent containers and feed tubes should be thoroughly washed out to remove bacterial (influent) or (algal effluent) growths.
F8.2.	**Operation of Rotating Tubes**	
F8.2.1.	Two identical pairs of tubes should be used.	
F8.2.2.	The peristaltic pump is controlled to deliver 250 ± 25 ml/h (note c) of settled sewage or synthetic sewage into the inclined tube which is rotated at a speed of 18 ± 2 revolutions per minute.	(c) This rate of application may have to be varied if attempts are made to simulate flow at a particular sewage treatment works. (Section F12).
F8.2.3.	The angle of inclination of the tubes is adjusted to produce a mean residence time of 125 ± 12.5 sec for the mixed feed in a clean tube (note d).	(d) The residence time is determined by spot dosing of readily detectable materials, e.g. dyestuff or chloride and detection of the peak concentration in the effluent.
F8.2.4.	The air temperature is maintained at 18–25°C, but for special investigation a lower temperature may be required.	

* Samples for BOD analysis should not be treated with mercuric chloride.

Step	Procedure	Notes
F8.2.5.	No inoculum is normally needed to start the growth of micro-organisms, but if inoculation proves necessary (e.g. with synthetic sewage), 1 ml settled sewage per litre may be added to the feed for 3 days.	
F8.3.	Sampling	
F8.3.1.	A sample of feed is taken as described in section F7.1, the volume being dependent on the analysis required (note e).	(e) A minimum volume of 250 ml is collected in a 500 ml measuring cylinder.
F8.3.2.	A sample of effluent is taken as described in section F7.2, the volume again depending on the analysis to be undertaken (note e). This sample is either settled (note f) or filtered (note g) before analysis.	(f) The sample is allowed to settle for 60 minutes before removal of sufficient quantity for analysis from below the surface to avoid any floating solids. (g) The sample is filtered e.g. through a Whatman No. 1 filter paper and the first 100 ml of filtrate rejected before collection of the material for analysis.
F8.4.	**Running-in**	
	Influent and effluent samples are taken during an initial 'running-in' (note h) period of the test, to check the performance of the tubes. Appropriate analyses may include COD, BOD, DOC, NH_3 and Oxidized N[24, 10, 25, 26, 27].	(h) 'Running-in' period is the time taken for the surface film to reach a "steady state". It is usually about 2 weeks, but should not exceed 6 weeks.
	When the removal of the determined parameters has reached a steady and similar level in each tube the mean removals can then be calculated. The COD removal in the control tubes should remain consistent (note i) during the subsequent period when analytical data are collected.	(i) For synthetic sewage the normal percentage removal of COD is 80 ± 4% based on the mean of 5 daily samples. The extent of nitrification will depend on the conditions of the test.
F8.5.	**Introduction of Test Material**	
	When removal of COD etc. has become steady, the industrial waste water or test chemical is added to the sewage of one pair of rotating tubes in the required proportion. (Note j)	(j) The industrial waste water or test chemical may be mixed with the sewage in the feed vessel in the required proportions, or dosed separately with another pump (See F5.3). Usually twice the concentration expected to be discharged is tested (See F10.2).
F8.6.	**Test Period**	
	Continue the analytical determinations for a further four to six weeks (note k) or until a steady state is reached in the test tubes (note l). Daily analysis (5 times weekly) over a period of 3 weeks are used for the calculation of toxicity and treatability. If it is clear after six weeks that the performance of the test tubes is significantly reduced, it may be necessary to repeat the entire experiment at a lower concentration.	(k) The duration of the test depends upon the treatability and toxicity of the waste water; it may be some time before a steady state is reached. (l) During the "steady state" period the surface film may slough from the tube surface. If this occurs, the period for collecting of analytical data should cover at least 12 full cycles.

Step	Procedure	Notes
F8.7.	**Toxicity** If it is required to know at what level the waste water causes adverse effects, the method can be repeated using a higher proportion of waste water to sewage or concentration of test chemical in sewage until the concentration (if any) which is toxic is established. Determination of the effects of shock loads or intermittent discharges may be necessary in which case the 'steady state' is not required.	

F8.8. Procedure for Industrial Waste Water Alone

F8.8. The industrial waste water may contain sufficient nutrients to be biodegraded on a biological filter without the addition of sewage. Normally a feed containing organic carbon, nitrogen and phosphorus in the approximate ratio 100:10:1 would support healthy growth.

The procedure is similar to that described in procedure F8 except that the industrial waste water is used as the feed from the start of the test. Control tubes are not necessary in this case although the use of a synthetic sewage standard may be beneficial to ensure that testing conditions are suitable, or for comparability if different waste waters are being tested at different times.

Performance of the plants is assessed by determination of the degree of carbonaceous oxidation and/or nitrification as for procedure F8. The acceptable amount of removal of the waste water components depends on the purpose of the separate treatment; whether to reduce the pollutants to a concentration which may be discharged to a sewer or to a level suitable for discharge to the environment.

Some components of the waste water may be biodegraded while others are not, so specific analysis may be required to determine the components which are resistant to biodegradation.

F9. Estimation of Treatability and Toxicity

F9.1. Calculaton of Treatability

F9.1.1. Chemical or waste water in Admixture with Sewage

The percentage removals of BOD, COD etc for the test and control plants can be calculated using the following formulae:—

$$\text{Overall removal} = \frac{I - E}{I} \times 100\%$$

where I = BOD, COD etc of the influent (for control) or sewage plus test material and E = BOD, COD etc of the effluents. Assuming the loading of the test and control plants is the same (see section F10.2), the removal of BOD (or COD) present in the industrial waste water component can be calculated from the following formula (see Section F13 for derivation).

$$\frac{(C_m - E_m) - (1 - x)(C_s - E_s)}{C_m - (1 - x) C_s} \times 100\%$$

where x = fraction of industrial waste water in mixed sewage,

C_m = BOD (or COD) of mixed sewage,
C_s = BOD (or COD) of sewage alone,
E_m = BOD (or COD) of test effluent,
E_s = BOD (or COD) of control effluent.

F9.1.2. *Treatability of Waste Water Alone*

The percentage removals of BOD, or COD, can be calculated using the same formulae as those in 9.1.1. where BOD_S or COD_S would be the BOD and COD respectively of the influent waste water and BOD_E and COD_E the BOD and COD of the effluent.

The percentage removal of ammonia can be calculated in the same way as the BOD or COD (F9.1.1.)

F9.2. Calculation of Toxicity

The toxicity can be expressed as a percentage reduction in the removal of BOD, or COD or NH_3 in the test plant compared to that of the control plant:

$$\text{Percentage inhibition} = \frac{R_c - R_T}{R_c} \times 100,$$

where R_c is the percentage removal of BOD (COD or NH_3) of the control unit and R_T is the percentage removal of BOD (COD or NH_3) of the test unit.

Alternatively, assuming no denitrification occurs, the production of nitrate and ntirite (oxidized nitrogen) may be used to calculate the percentage inhibition of nitrification:

$$\text{percentage inhibition} = \frac{(OxN_s - OxN_m)}{OxN_s} \times 100$$

where OxN_s = oxidized nitrogen concentration in control effluents.
OxN_m = oxidized nitrogen concentration in test effluents.

If $OxN_m > OxN_s$ nitrification probably has been stimulated in the presence of the industrial waste water.

F9.3. Interpretation of Results

F9.3.1. Treatability

An industrial waste water can be assumed to be adequately treatable at a sewage works if the polluting matter it contains is satisfactorily removed under the conditions of the test. What can be regarded as 'safisfactory' removal depends on the frequency of discharge, subsequent treatment process, and ultimate destination of the effluent.

If there is good removal of the industrial waste water components only after a period of acclimatization, the waste water would be suitable for continuous discharge, but intermittent discharging would be unsuitable and may result in deacclimatization if the period between the discharges were too long.

If the industrial waste is not satisfactorily removed at twice the discharge concentration, the test concentration should be reduced until a treatable level is found.

F9.3.2. Toxicity

If the average performance of the tubes receiving the waste water is not significantly lower than those of the controls, the material may be said to be non-toxic at the concentration tested.

If, however, the average performance of the tubes for one or more of the parameters tested is lower than the control, the material may be exhibiting a toxic effect.

The waste water may be toxic to only one group of organisms in the microbial film on the rotating tube, or to the whole range of micro-organisms present. If it is toxic to the nitrifying organisms, a reduction in the nitrate concentration in the effluent will occur, with a corresponding increase in effluent ammonia concentration, the changes being a measure of toxicity. It may also be toxic to carbonaceous oxidative processes, when a decrease in the BOD and COD removal would be observed.

One or any of these effects may occur, but the toxicity may be temporary, and acclimatization of the micro-organisms to the waste water may follow. Therefore, if the inhibition is partial, it is important to continue the testing for a period of weeks to allow

time for acclimatization. Some toxic substances (e.g. metals) may absorb onto the film and build-up to toxic levels, and therefore require several weeks testing before their effect on the process is established.

F10. Sources of Error

F10.1 Comparability of test and control units before the addition of chemical or industrial waste is very important. The addition should not be started until the same steady removals have been achieved in both control and test units. Also the performance of the controls must remain stable during the testing period. If any problems develop (e.g. sloughing, see F8.6 note 1), the running-in stage should be repeated until comparable results are obtained.

F10.2. Unless the BOD or COD of the sewage and the industrial waste water are identical, the organic loads to the plants will not be the same. Since the proportion of waste water added is low, usually $<5\%$, the differences in loading are not likely to be high enough to alter the performance of the plants significantly. However, if the waste water is extremely strong or if the proportion added is high, due consideration must be given to the effect of the larger differences in loading. It might even be necessary to adjust the strengths of the influents by addition of water or of a solution of known biodegradable organic substances ('synthetic sewage').

F10.3 Usually it is preferable that one batch of peptone and beef extract should be used throughout the test to minimise any changes in constituents which may arise by different amounts of purification or by different protein sources. The synthetic sewage may have insufficient buffering capacity if complete nitrification (an acid-producing reaction) is achieved, and the resultant fall in pH may reduce the degree of removal of COD and perhaps the material under test. This may be remedied by addition of suitable concentrations of bicarbonate/carbonate to the synthetic sewage. The conversion of 1 mg ammonia to nitrate produces acidity equivalent to approximately 7 mg carbonate. This procedure is unlikely to be necessary using natural sewage where the buffering capacity is likely to be much greater.

F10.4 Since the simulation test is usually carried out at room temperature in the laboratory (at 20°C), it may be necessary to check that the test material is equally treatable at lower temperatures found in practice (e.g. in winter). This may be done by installing the tubes in a cold room (e.g. at 10°C).

F11. Checking accuracy

Duplicate plants are run simultaneously and mean values are taken to reduce the variability of results from similar units. The length of time taken to test a particular industrial waste water or chemical depends upon its behaviour in treatment. Therefore, if it is not removed readily in a short time with no adverse effects on plant performance, the test should be extended to allow time for acclimatization of the sludge to the test material. If no signs of improvement occur after 6 weeks of addition, a range of lower concentrations must be tried before assuming that it is untreatable*.

F12. Operational use of the Test

If the test is being used to assess the effect of a material on a particular treatment plant it is first necessary to simulate the performance of that plant without the material.

For a test attempting to simulate the performance at a particular works, it will be necessary to carry out a series of tests at different flow rates (F8.2.2.) and plot the performance (e.g. COD removal) against flow, to obtain the closest agreement between the performance of the tubes and the biological filters.

A flow which more accurately simulates the works performance can thus be found and the subsequent tests for treatability/toxicity carried out at this flow rate.

* To obtain an approximate idea of the concentration of industrial waste water or chemical which can be treated without toxic effects preliminary respiration rate determinations can be carried out. (method C).

F13. Derivation of the Calculation of the treatability of industrial waste water in admixture with sewage (Section 9.1.1 refers).

Let C_w = BOD (or COD) of waste water
E_w = equivalent effluent BOD and assume no interactions

Then

$$C_m = (1 - x)\, C_s + C_w$$

$$\therefore \quad C_w = \tfrac{1}{x}(C_m - (1 - x)\, C_s)$$

Similarly

$$E_m = (1 - x)\, E_s + xE_w$$

$$E_w = \tfrac{1}{x}(E_m - (1 - x)E_s)$$

Removal of industrial waste water component

$$= \frac{C_w - E_w \times 100\%}{C_w}$$

$$= \frac{\tfrac{1}{x}(C_m - (1 - x)\, C_s) - \tfrac{1}{x}(E_m - (1 - x)E_s) \times 100\%}{\tfrac{1}{x}(C_m - (1 - x)\, C_s)}$$

$$= \frac{(C_m - E_m) - (1 - x)\,(C_s - E_s) \times 100\%}{C_m - (1 - x)\, C_s}$$

where x = fraction of industrial waste water in mixed sewage
C_m = BOD (or COD) of mixed sewage
C_s = BOD (or COD) of sewage alone
E_m = BOD (or COD) of test effluent
E_s = BOD (or COD) of control effluent

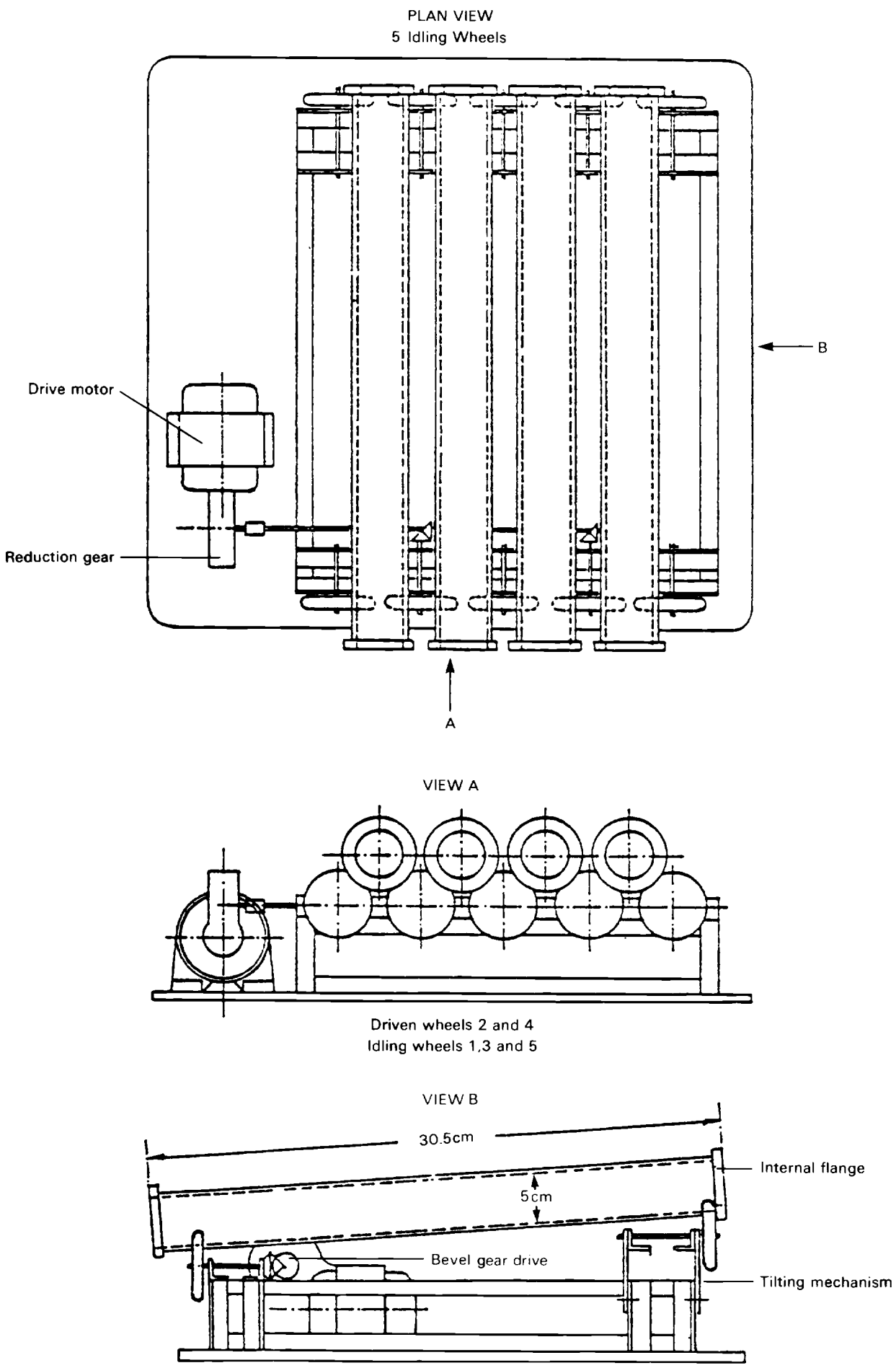


FIGURE 15 LAYOUT OF ROTATING TUBES

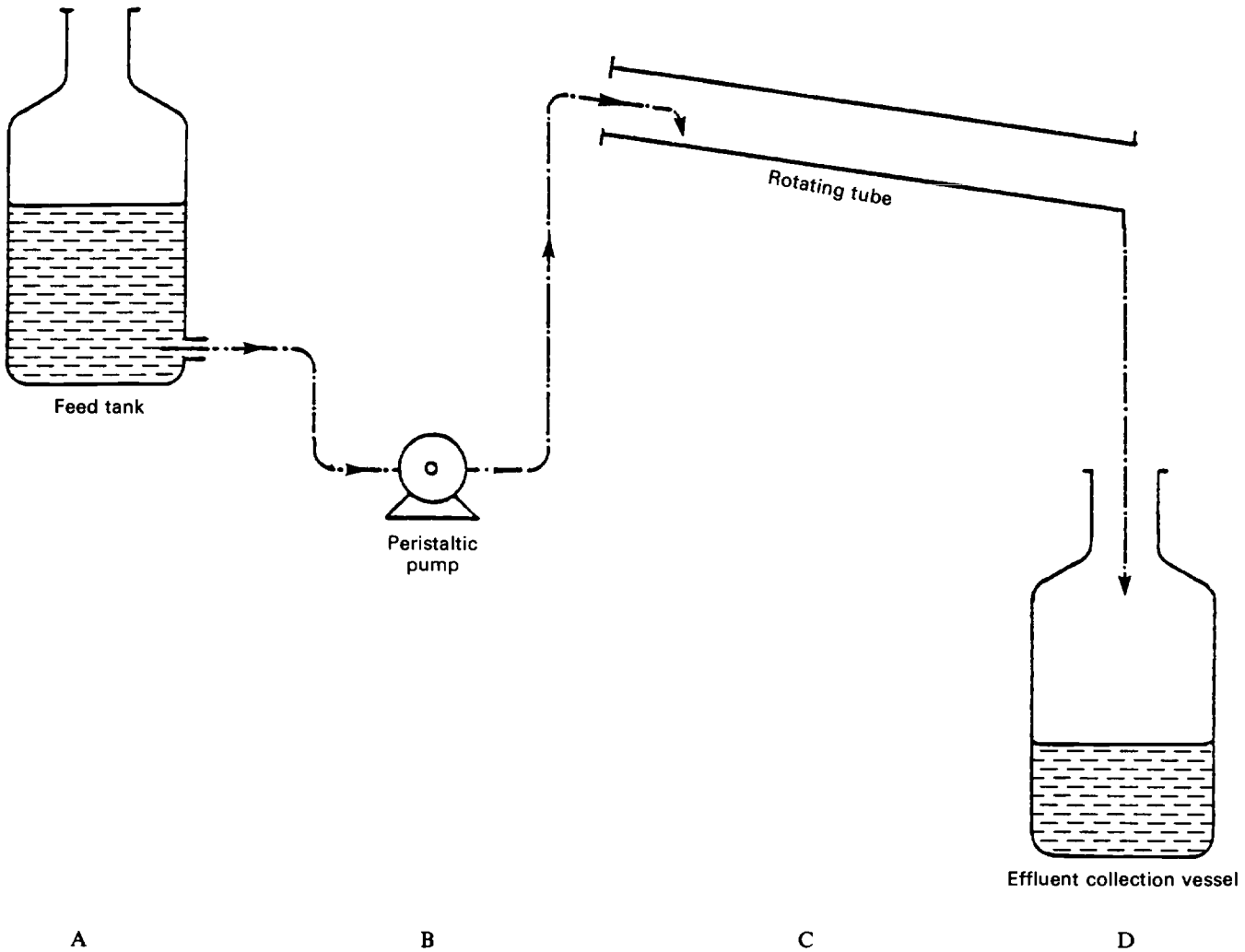


FIGURE 16 FLOW DIAGRAM

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