Examining Biological Filters, Toxicity to Aerobic Bacteria, Effect of SRT and Temperature 1985–6.

The Examination of Percolating Biological Waste Water Filters 1985. The Determination of the Toxicity of Substances to Aerobic Bacteria by Measurement of Growth Inhibition, and The Assessment of the Effect of Sludge Retention Time and Temperature on the Treatability of Chemicals in the Activated Sludge Process 1986.

Methods for the Examination of Waters and Associated Materials

This booklet contains three methods used in monitoring different aspects of aerobic sewage treatment processes.

Method B supplements but does not supercede 'Methods for Assessing the Treatability of Chemicals and Industrial Waste Waters and their Toxicity to Sewage Treatment Processes 1982. HMSO, in this series.

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About this Series

This booklet is part of a series intended to provide both recommended methods for the determination of water quality, and in addition, short reviews of the more important analytical techniques of interest to the water and sewage industries.

In the past, the Department of the Environment and its predecessors, in collaboration with various learned societies, have issued volumes of methods for the analysis of water and sewage culminating in 'Analysis of Raw, Potable and Waste Waters'. These volumes inevitably took some years to prepare, so that they were often partially out of date before they appeared in print. The present series will be published as series of booklets on single or related topics; thus allowing for the replacement or addition of methods as quickly as possible without need of waiting for the next edition. The rate of publication will also be related to the urgency of requirement for that particular method, tentative methods and notes being issued when necessary.

The aim is to provide as complete and up to date a collection of methods and reviews as is practicable, which will, as far as possible, take into account the analytical facilities available in different parts of the Kingdom, and the quality criteria of interest to those responsible for the various aspects of the water cycle. Because both needs and equipment vary widely, where necessary, a selection of methods may be recommended for a single determinand. It will be the responsibility of the users—the senior technical staff to decide which of these methods to use for the determination in hand. Whilst the attention of the users 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 Analysis (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 nonmetallic 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

1 July 1986

Warning to Users

The analytical procedures given in this booklet should only be carried out by competent trained persons, with adequate supervision when necessary.

Local Safety Regulations must be observed.

Laboratory procedures should be carried out only in properly equipped laboratories.

Field Operations should be conducted with due regard to possible local hazards, and portable safety equipment should be carried.

Care should be taken against creating hazards for one's self, one's colleagues, those outside the laboratory or work place, or subsequently for maintenance or waste disposal workers. Where the Committee have considered that a special unusual hazard exists, attention has been drawn to this in the text so that additional care might be taken beyond that which should be exercised at all times when carrying out analytical procedures. Reagents of adequate purity must be used, along with properly maintained apparatus and equipment of correct specifications. Specifications for reagents, apparatus and equipment are given in manufacturers catalogues and various published standards. If contamination is suspected, reagent purity should be checked before use.

Lone working, whether in the laboratory or field, should be discouraged.

The best safeguard is a thorough consideration of hazards and the consequent safety precautions and remedies well in advance. Without intending to give a complete 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 waste, containment in the event of breakage, access to taps, escape routes and the accessibility of the correct and properly maintained first-aid, fire-fighting, and rescue equipment. Hazardous reagents and solutions should always be stored in plain sight and below face level. Attention should also be given to potential vapour and fire risks. If in doubt, it is safer to assume that the hazard may exist and take reasonable precautions, rather than to assume that no hazard exists until proved otherwise.

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

It cannot be too strongly emphasised that prompt first aid decontamination, or administration of the correct antidote can save life; but that incorrect treatment can make matters worse. It is suggested that both supervisors and operators be familiar with emergency procedures before starting even a slightly hazardous operation, and that doctors consulted after any accident involving chemical contamination, ingestion, or inhalation, be made familiar with the chemical nature of the injury, as some chemical injuries required 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. If an ambulance is called or a hospital notified of an incoming patient give information on the type of injury, especially if poisoning is suspected, as the patient may be taken directly to a specialized hospital.

A The Examination of Percolatoring Biological Waste Water Filters

A1 Performance and Characterisation of the Methods

A1.1	Biota sampled	Microbial film Protozoa Macrofauna Emerging insects
A1.2	Habitats sampled	Conventional stone or clinker filled percolating filters High rate filters Rotating contact-disc units
A1.3	Type of sampler	Garden spade for surface samples Sieve for filter effluent Shafts for depth sampling *Neutron probe for film determination Fly traps for emerging insects
A1.4	Basis of operation	Samples from surface of conventional or high rate filters Depth samples from shafts throughout the depth of the filter Examination of effluent by sieving Examination of film on rotating contact-disc units Trapping insects from known surface area or from general filter area *Radioactive neutron source emitting fast neutrons which are reflected by the hydrogen atoms within film water
A1.5	Forms of data	Qualitative or quantitative assessment of film, protozoa, macrofauna or emerging insects
A1.6	Limitations of methods	Surface samples restricted to the topmost 300 mm of filter, although the film accumulates throughout depth of filter Depth samples need installation of sample shafts and time for stabilisation prior to sample collection Sieving methods unsuitable for protozoa High rate filters with non-random medium need special sampling methods *Neutron probe inaccurate near surface and bottom of the filter due to air/medium boundary
A1.7	Efficiency	Qualitative surface samples are subjective and may be influenced by the operator

*This equipment contains a radioactive source and must be used in accordance with regulations as given in A Appendix 2.

A2 Introduction

The purpose of this booklet is to outline and describe the methods which may be used to examine biological filters (percolators) found on water reclamation works. The examination of the biota and condition of biological filters is an important adjunct to the operation of a works for the more efficient purification of the applied sewage and for minimising complaints concerning the operation of the works. Biological examination is intended to supplement rather than replace traditional chemical methods of assessing works performance, although its use may permit fewer chemical samples to be analysed. The numbers and species of organisms which occur in such filters are related to the strength and nature of the sewage, the type and operation of the filters and are also subject to seasonal variations.

The rationale for the examination may include the following considerations.

A2.1 To determine the characteristic fauna and flora of each biological filter for reference.

A2.2 To investigate the biological effects of changes in operational treatment eg flow rate, recirculation, frequency of dosing.

A2.3 To quantify the magnitude and variation of nuisances and problems related to the filters and the effects thereof eg fly emergence, incipient 'ponding', uneven distribution.

A2.4 To investigate the biological effects of new or unauthorised discharges to the works eg milk, blood, fuel oil, toxic metals.

The examination of biological filters can often illustrate or explain deficiencies in the operation of the filters. Film growth can be uneven over the filter and this may be caused by imbalance of the whole rotor assembly or by inefficient distribution of sewage caused by poor design of the dosing arms. An investigation of the effect of unauthorised discharges into a works will, to some extent, depend upon knowing the normal biological condition of the filters. This emphasises the benefits of a regular monitoring programme of the filters to establish a reference point against which to compare any subsequent examination made after the discharge. The effects of changes in operational treatment are better assessed if proposed changes are notified first so that inspections of the filters can be made before any changes occur.

A3 Hazards

Sewage, sewage sludge and sewage effluents may contain harmful organisms. Wear suitable protection to avoid skin contact and ingestion. In the event of contamination clean up at once. To avoid accidental infection, wash at the conclusion of the examination. Appropriate first aid equipment and disinfection should be available.

All regulations for maintenance of mechanical equipment must be observed. Power to all moving parts such as rotor arms must be switched off and the switches so marked and the parts so braked that they cannot be restarted accidently. Disconnection is usually preferable to simple switching.

Hot oil is used in A Appendix 3. Use a spillage tray adequate for the amount of oil used, wear proper gloves and protective clothing and wear a face visor. Materials added to the oil should be dry. Ensure that the container cannot be knocked over.

A4 Method of Examination

A4.1 Locations for examination

Sampling a biological filter may consist of an examination of the biota either living within the filter or emerging from the filter. Sampling the fauna within a filter can be undertaken simply by means of kicking the surface stones by foot or digging with a spade or by more elaborate methods such as having vertical metal or plastic shafts sunk into the depth of the filter. The simpler methods invariably suffer from the disadvantages that only the top-most portion of the filter is examined due to the difficulty of digging to any great depth into the filter without the use of retaining shuttering. The insertion of vertical sampling shafts is best done when the filter is being constructed, before the medium is placed into position. Even if work is not planned

at the time, it enables sampling canisters to be placed into the filter in the future. However, consideration should also be given to the fact that iron shafts which do not contain sample containers are open to the air and this, together with the continuously wet conditions, is likely to lead to corrosion of the shaft, thereby making future insertion of sampling containers a more difficult task.

For most operational purposes, qualitative sampling of the filter surface is sufficient. However, if the distribution of film and fauna throughout the depth of the filter is needed, thus necessitating sampling shafts, then quantitative sampling would be performed, although each sampling canister could be examined qualitatively as described below. If only quantitative assessment of film is required throughout the depth of the filter, then consideration should be given to the use of a neutron probe moisture meter, also described below.

A4.2 Preliminary Observations

In assessing works performance, adequate coverage of the filter needs to be given. The distribution of sewage onto the filter may be uneven causing irregular film growth, possibly leading to 'ponding' of the filter, and this may be observed by differences in the distribution of the film. On a works with more than one filter, unless there is differentiation into primary and secondary filters, it is usually desirable that all filters receive a similar loading. Biological and chemical samples taken from each filter on a works can be used to determine the efficiency of sewage distribution.

A4.3 Qualitative Sampling

The simplest method of sampling, suitable for a quick qualitative assessment, is by the use of a spade and digging into the surface of the medium. This method is usable down to approximately 200 mm before it becomes necessary to remove the surface layers. If deeper inspection is required, an area with a radius approximately equal to the required depth should be cleared at the filter surface to minimise collapse of the surrounding medium. Hand sampling can be used to investigate the surface layers but becomes difficult if deeper inspection is required. A rough qualitative assessment of the macrofauna is possible by noting the approximate frequency of occurrence of the various species. The following symbols have been used to indicate the relative abundance of the macrofauna present, together with an approximate number of individuals per sample (volume equivalent to 1 litre filter medium).

r = rare	1 – 9
o = occasional	10 – 99
c = common	100 – 999
vc = very common	1000 +

Where variations occur, such as those caused by uneven film growth, the report may be written to include two classifications eg r/o, o/c, o/vc. A rapid semi-quantitative assessment of the film can be made at the same time and reported within one of the following categories:

small — thin covering of film over the medium which still appears as separate pieces.

moderate — thicker covering, some coalescing of film between adjacent pieces of medium.

heavy — larger quantity of film, almost or completely filling all medium void spaces; can become anaerobic if very heavy when it may appear slimy and grey-coloured with 'septic' odour.

Recirculation may impose the need to sample below the top-most 100–150 mm which is normally examined since the film commonly extends deeper into the filter medium when recirculation is used.

A specimen report form is included as A Appendix 1.

A4.4 Quantitative Sampling

Quantitative sampling involves sampling a known volume of filter medium. Surface samples can be collected immediately but for those from within the depth of the filter, a period of stabilisation is necessary to ensure that the sample is representative of the surrounding filter medium.

A4.4.1 Surface Sampling

The easiest method of quantitatively sampling the surface portions of a biological filter is to fill a container of known volume with medium at the time of sampling. This method is considered satisfactory for the top-most 150 mm of the medium, and provided the surface 100 mm is first moved to one side, can be used with care down to approximately 300 mm. Filter fauna may show large spatial variability and a number of replicates may need to be taken to reduce sample variability. Alternative methods include the use of plastic mesh bags or wire mesh baskets sunk into the surface layers and left to stabilise before being removed for laboratory examination. The collection of a known volume of medium by water displacement can be used but requires suitable apparatus and a supply of water.

A4.4.2 Depth Sampling

Sampling throughout the depth of a biological filter is much more difficult than surface sampling. A suggested method is to use perforated tubes placed vertically within the filter medium and containing samples of medium of known volume (Fig 1). These samples can be contained in perforated metal or plastic baskets on lifting rods or by plastic mesh bags with draw strings attached. Mesh bags may, however, bind in the sample shaft and be difficult to remove later. The sample shafts are best positioned in the filter during construction and may be of either metal or plastic, the latter not being subject to corrosion. Although possibly more expensive initially, the use of corrosion proof material and manufacturing to close tolerance will increase the reliability of removing and replacing the containers as required. Shafts limited to the upper section of the medium may suffice for some work and are more easily inserted into existing filters. If rigid containers are used for the samples, a suggested convenient size for filter medium up to approximately 50 mm diameter is 146 mm external diameter to fit into a 150 mm internal diameter shaft, each with a height of either 150 or 180 mm. These will then represent a volume of either 2.5 or 2.0 l of medium and divide a filter of average depth 1.8 m into 12 or 10 samples respectively. To reduce the 'edge-effect' of using small containers or to contain larger filter medium, a larger container size may be necessary, with a suggested size of 250 mm diameter and 300 mm deep. Containers of this size become more difficult to handle and may need to be lowered into and removed from the sample shaft individually. The sample containers should ideally be perforated to about 50% void area, for example, 19 mm holes on a centre-to-centre spacing of 25 mm. Plastic piping can be used for the sample containers but is difficult to perforate to the required extent without weakening the material. If plastic is used, particular attention should be given to the bottom of the container which should be screwed as well as glued or plastic welded. Plastic mesh bags can be used, these being filled with medium and lowered into the shaft by cords which can be used for subsequent removal of the bag from the shaft.

Since all sample containers are likely to be filled with medium from near the surface of the filter, it is important that the material is left in the shafts long enough for complete stabilisation with the surrounding filter medium to have taken place. A minimum of two months is suggested, longer if possible, and preferably six months. This will ensure that the medium from the surface placed at the bottom of a shaft is then representative of the bottom of the filter, not of its original position.

The insertion of sample shafts into an existing filter creates a major upheaval of the medium and destroys any existing biological strata. Because of the instability of the filter medium, digging to clear an area of 1 m^2 at the bottom of a 1.8 m deep filter usually involves starting with a hole some 4.5–5 m across at the surface. Six to twelve months must be allowed for the normal biological structure to become re-established and before any sampling is undertaken. The filled sample containers should be inserted

into the shafts as soon as these are in position so that the medium samples become conditioned to their correct depth.

A4.4.3 High-rate Filters

High-rate filters are usually of greater depth than conventional biological filters with stone medium. Those with random-pack filling may be sampled in a similar manner to conventional filters by using shafts and sample baskets of welded steel mesh through the depth of the medium. Because of the much lighter weight of the plastic medium per unit volume, the sample units can be larger than those used in biological filters containing stone medium. A suggested size for the baskets is 500 mm diameter by 500 mm deep with a volume of approximately 100 litres, although it should be remembered that the total weight of the basket will increase as the film accumulates.

High-rate biological filters employing welded corrugated plastic sheets or plastic tubing as the medium are much more difficult to sample. Only the top-most portion can be sampled easily unless special provision has been made to sample medium within the depth of the filter. One method which has been used successfully is the provision of 'drawers' in the side of the filter (Fig 2). These are constructed at the same time as the filter and contain a block of filter medium. When required, the 'drawer' is opened allowing access to the filter medium normally within the central portion of the filter.

High-rate biological filters which are filled with very large medium, for example, rock portions of 200–300 mm largest dimension, are almost impossible to sample except at the surface. The only alternative is to examine the effluent to determine the species living within the filter. A net or sieve positioned so as to filter a proportion of the effluent may be useful in providing a qualitative estimation of the species occurring within the filter. In one case, Psychoda pupal exuviae were observed on the surface of a humus tank and which were considered to have originated from the preceding high-rate filter.

A4.4.4 Rotating Contact-disc Units

Sewage treatment plants employing rotating contact-disc units also need special provision to be made in order to enable samples to be collected. Unless the outer cover of the plant is large enough to allow access to the contact-discs, and walk-ways are installed inside the unit, access is restricted to the use of doors fitted to the outer cover such that samples can be obtained from the contact-discs. Samples of film from one contact-disc unit which was examined showed that the microbial population resembled that of an activated sludge plant rather than that of a conventional stone filled biological filter where a grazing fauna is normally found. This absence is considered to be due to the periods of immersion caused by the rotation of the contact-disc.

A4.4.5 Film measurement by neutron probe equipment

The relative amount of film and its distribution throughout the depth of the biological filter can be measured 'in-situ' by the use of neutron probe equipment. Aluminium tubes, approximately 50 mm diameter and 2 m long, are inserted vertically into the filter medium and into which a radioactive source of neutrons can be lowered. Emitted fast neutrons collide with the hydrogen atoms within the water molecules and produce a cloud of slow neutrons. These are detected and counted by the equipment to give a number which is proportional to the water content of the surrounding film. Assuming that the proportions of water to dry film remains uniform, then the count is proportional to the amount of dry film. Readings taken near the surface and bottom of a filter are subject to inaccuracies caused by the non-uniformity of the surrounding medium. These can be reduced near the surface by the use of a medium-filled container which can be moved into position when measurements are being made. Anaerobic conditions within a filter tend to produce an increase in the water content of the film. Standardisation is necessary each time the probe unit is used but is usually restricted to measurements made at 0% and 100% saturation of void space in a sample of filter medium. This should obviously be the same as the experimental filter; sewage can be

used for the 100% saturation standard. The insertion of the aluminium tubes into the filter medium can be assisted by the use of a special tool. This is made from iron rod, turned to be a sliding fit into the tubing, the top end with a flange to retain the tubing, the bottom end shaped to a rounded point. The length is such that the pointed end will just protrude from the bottom of the tube. After driving the rod, with the tubing, into the filter, the rod is withdrawn and the top of the tube trimmed to clear the distributor. The top of the tubing should be sealed with a rubber bung when not being used for measurements.

A limitation of the method is the high cost of the equipment, in excess of £3000. The equipment contains a radioactive source and special regulations apply to its use, transport and storage, details of which are given in A Appendix 2.

A5 Sample Examination

After the samples have been collected from the biological filter, the film must be removed before quantitative measurements can be made. A number of methods have been used, their suitability depending upon the type of medium and the fauna to be examined.

A5.1.1 'Dunking' Method

The simplest method of film removal is to place the sample of medium into a container of suitable size and to add a quantity of water. The contents can then be stirred to wash the film from the stones, followed by sieving to separate the stones from the washings. This process can be made easier if apparatus as shown in Fig 3 is available, and could, provided the inner section is of suitable size, be used for washing samples still in shaft containers, thus avoiding the problem of transport of medium since only the washings, once transferred to suitable containers, have to be transported back to the laboratory. When sampling some medium, such as clinker, the very rough surface may trap some organisms, such as enchytraeid worms, and the numbers may be under-estimated.

A5.1.2 'Sieving' Method

The sample of medium is placed onto a sieve of suitable mesh size so as to retain the medium but allow the washings to pass (Fig 3). Water is used to wash the film from the stones and the washings are then sieved to retain the film. The method is suitable for the examination of the larger fauna species but is unsuitable for protozoa or smaller species which would be lost from the second sieve. Attempts to use a finer mesh second sieve usually results in blockages and loss of material. The sievings can be transferred to a laboratory for examination where they can be re-suspended in water before sub-sampling.

A5.1.3 'Scrubbing' Method

Provided that the number and size of the samples are small, scrubbing with a brush can be used to remove the film from the medium. The method is more suited to smooth medium, such as cracked stones or plastic medium, where either a toothbrush or a small scrubbing brush can be used. It is preferable for the medium to be scrubbed under water to avoid splashing and loss of material. Some damage to delicate animals may occur if 'speared' by the brush bristles and the use of warm or hot water alone may suffice to remove them from the medium.

A5.1.4 'Stone-washer' Method

A method which has been used at one laboratory for a number of years employs a 'stone-washer'. This has a perforated metal cylinder, hexagonal in cross-section, approximate length 300 mm, diameter 180 mm and volume 6 l, and which is rotated by an electric motor. The cylinder is partially immersed in water in a trough fitted with an outlet tap at the bottom. A sample of filter medium of approximately 2.5 l is placed into the cylinder, 2 l of water added to the trough and the motor switched on for 0.5–1 minute. The water and film washings are drained off into a bucket and the process repeated with another 2 l of water. This is added to the first 2 l and the stones removed from the cylinder. Similar apparatus could be constructed from plastic material and

rotated by hand if only a few samples are to be washed (Fig 4). Problems can arise when using a 'stone-washer' with small medium, such as crushed slag road chippings. For this medium, the use of either the 'dunking' or the 'sieving' method with a medium retaining sieve of 6–10 mm mesh size is recommended, although only the 'dunking' method is suitable if protozoa are to be examined. Observations made on the fauna after removal from the medium by the 'stone-washer' show little physical damage to most specimens, only the larger species such as lumbricid worms sometimes being damaged.

A5.1.5 Plastic Medium

The removal of the film from plastic medium is much more difficult and may necessitate washing individual pieces of medium by hand. A scalpel can be used to scrape film from the inside of corrugated sheet or tubular medium. Only with the random pack plastic medium is it relatively easy to remove the film and the 'dunking' method is suggested. Since there is often a lack of larger grazing fauna on plastic medium, the 'sieving' method is not recommended and, in addition, the protozoa will be partially lost for examination.

A5.2 Sub-sampling

In most cases, the sample of film removed from the medium will need to be sub-sampled before a detailed examination is made. Where the film has been washed off into water, this can be diluted to a given volume, otherwise the film can be re-suspended in water before dilution. The use of an octagonal container when sub-sampling helps prevent inaccuracies caused by vortex formation when stirring. A known volume, for example, 100 ml, is used for the determination of dry and organic contents of the film and sub-samples of either 100 or 500 ml can be taken for faunal estimation. These should be kept in a cold-room or refrigerator or preserved until examination can be made. Formalin added to give a final concentration of about 4% can be used for preservation, subject to laboratory safety precautions. An alternative is the use of either methylated ethyl alcohol or isopropyl alcohol. Samples may need sieving to remove excess water before the alcohol is added. The number of sub-samples to be examined will depend upon the nature of the work and upon the frequency of the species being examined. Experiments have shown that very good agreement between the numbers of specimens present in three sub-samples could be obtained provided there were approximately one hundred specimens in each sub-sample. For those species which occur only rarely, additional sub-samples may need to be examined in order that the estimate of the number in the original sample is valid.

A6 Examination of Sub-Samples

A6.1 Film

The 100 ml sub-sample for dry weight determination is transferred to a preweighed silica crucible and evaporated to near dryness on a hotplate. After drying overnight in an oven at 105°C, cooling and reweighing, the crucible is heated in an electric furnace at 600°C for 20 minutes and the ash weight obtained. Subsequent calculations allow the dry and volatile solids, representing the total film and organic content, to be expressed as g/l of filter medium.

A6.2 Macrofauna

The subsamples for the examination of the macrofauna may need sieving on a fine mesh sieve, say 250 μm, to remove fine silt and make the counting easier. The washed sample is then transferred to a plastic dish of suitable size, for example, 150×100 mm for the 100 ml sub-samples or 300×220 mm for the 500 ml sub-samples. In the larger dish, two areas, each 10% of the total area, are marked off and are used to count the more frequent species, such as enchytraeid worms. Counting each area separately enables a check to be made on the uniformity of distribution within the dish, as both areas should contain the same number of specimens. When the two numbers are added together, the number equivalent to a 100 ml sub-sample is obtained. If the two numbers are markedly different, it is unlikely that they represent 10% of the total number and the sample should either be redistributed and recounted or the total number in the dish counted.

For the larger species, or for those which are rare in the film samples, for example, the beetles of the genus *Cercyon*, the remainder of the washings can be sieved to recover the wanted species and the number present added to the number counted in the sub-samples. Comparisons made between the number calculated from the sub-samples and the total number by counting will give an indication of the necessity to count the total number present.

To enable comparisons to be made more easily, the number of each organism/litre of filter medium should be calculated for each sample.

A6.3 Protozoa

Protozoa from biological filter samples can be examined from a sub-sample of 5 or 10 ml taken from the volume of diluted washings. This sub-sample should be kept in a cold-room or refrigerator until required to prevent or reduce changes in the protozoan populations. Care must be taken to ensure that any subsequent sub-samples for examination are representative of the origianl sample, and this can be aided by the use of an air-line to mix the sample while small aliquots are withdrawn. Aeration of the sample for approximately ten minutes before sub-sampling will assist in the subsequent identification of many *Vorticella* and *Opercularia* species. Various methods have been used for the protozoa examination and which depend upon the extent of identification required. A simple method is to place a drop of film suspension, diluted if necessary, onto a microscope slide and cover with a cover-slip. This method enables high power ($\times 400$ or $\times 1000$) to be used to assist in the identification but a disadvantage is that the pressure from the cover-slip can distort some species. To avoid this problem, a Sedgewick-Rafter cell can be used but only on low power ($\times 100$). A modified version consists of two pieces of 1 mm plastic sheet, each 75×25 mm. One piece has a central hole of 12.5 mm diameter and is glued onto the second piece which has the corresponding area marked by lines scratched at 1 mm intervals on the upper surface. The volume enclosed by such a cell is approximately 0.125 ml. Other alternatives are haemacytometers but these are of very small volume (0.1×0.0025 mm^2), or Lund cells.

A7 Frequency of Examination

The frequency of examination should be determined by its purpose. If it is desired to record the characteristic fauna of each filter, inspection should be undertaken at three-monthly intervals, or at least once every six months. This same frequency may also suffice to determine the effects of operational changes on works although it is probably preferable to inspect filters at monthly intervals in order to monitor any changes more efficiently. If monitoring for fly emergence, the frequency may need to be increaseed to either once or twice weekly.

A8 Insect Emergence

The enumeration of insect emergence from a filter is normally undertaken on a quantitative basis, although the efficiency at capturing all the insects emerging from a known filter area varies with the method employed. Hawkes (1983) discusses the methods in some detail and provides examples of the results obtained, together with an extensive review of the relevant literature. The methods employed may be classified into four groups.

A8.1 Tray Traps

The trap consists of a metal tray 300 mm square by 65 mm deep which is inverted over the surface of the filter medium in order to provide a refuge for flies similar to the natural environment. Insects congregate within the tray and, after a given period, are killed and counted. Hawkes discovered that the number captured was influenced by light and temperature and concluded that it was not a valid method for the assessment of flies either within or emerging from a filter. However, it is usable for comparisons made on different filters at the same time.

A8.2 Emergence Traps

Early designs of emergence traps used methods previously adapted for fieldwork on aphid migration. Later, Solbé *et al* designed a trap which maintained conditions within

the trap as near as possible to the natural conditions. The lower section consists of a metal flange, 300 mm square and 75 mm high, supporting the top section which allows air, light and sewage to reach the enclosed filter area (Fig 5). Increasing the length of each side to 316 mm will enclose an area of 0.1 m^2. Glass or plastic sheets, partially coated with a sticky material on one side, are placed sticky side downwards to cover the top of the trap for a given period. The captured insects can then be identified and counted, the traps being left uncovered between successive trappings. Details of the sticky material are given in A Appendix 3.

Styles (1979) used a smaller trap, 280 mm long, 140 mm wide and 70 mm deep, in the form of a bottomless box with nylon netting ends (Fig 6). Small plastic sandwich boxes can be adapted by removing the bottom and inserting mesh covered apertures into the sides. The lid, covered with a sticky material on the underside, is placed on top of the trap when trapping. The small size of the complete trap allows the unit to be located on the filter surface between distributor jets, thereby avoiding the necessity for sewage distribution and reducing manufacturing costs. The traps should be removed from the filter surface when not in use to avoid adverse effect on the filter fauna.

A8.3 Dispersal Screens

To determine the distribution of flies leaving the filter area, and thus possibly causing nuisance, screens can be erected around the periphery of the filter area (Fig 6). Their purpose is to provide a suitable resting place for the flies which can then be enumerated and related to prevailing wind direction. It may be necessary to examine both sides of the screen, some species preferring different conditions to others. Hawkes found that the number of flies recorded from nearby houses was related to the numbers on the screens. This method is more suitable for *Sylvicola* and *Psychoda* as opposed to chironomids which tend to form swarms around nearby trees or buildings.

A8.4 Aerial Traps

These are traps which will assess the aerial density of insects in the vicinity of the filters. Hawkes (1983) describes three types which he used at Minworth WRW in field investigations of the nuisance caused by *Sylvicola* (*Anisopus*) *fenestralis* (Fig 7). A 'Canister Trap' has been used for general site surveillance but relied on the natural shelter-seeking habit of Sylvicola. An alternative design, the 'Cross' trap, consisted of vertical plastic sheets, coated with a sticky material, and shielded from rain by a protective hood. This was attached to an upright support and the whole assembly fastened to a distributor arm. Examination can be made to determine the daily and seasonal variations in the aerial density of flies. This passive method was later replaced by the Johnson suction trap, an apparatus containing an electric fan which sucked air into the apparatus. Flies which were also drawn in were collected between discs released by a timer at hourly intervals, thus enabling the aerial density to be related to environmental factors such as wind or sunshine.

A9 References for Identification

The current literature which deals specifically with the macrofauna associated with biological filters is limited, although identification guides are usually available for particular groups. References for protozoa are more common, although mostly concerned with species found in activated sludge plants.

Curds C R 1969. *An illustrated key to the British freshwater Ciliated Protozoa commonly found in activated sludge.* Water Pollution Research Technical Paper No 12. HMSO.

Curds C R and Hawkes H A 1975. *Ecological Aspects of Used-water Treatment. Vol. 1 The Organisms and their Ecology.* Edited by Curds C R and Hawkes H A. Academic Press

Hawkes H A 1983. *The Applied Significance of Ecological Studies of Aerobic Processes In Ecological Aspects of Used-water Treatment. Vol. 3 The Processes and their Ecology.* Edited by Curds C R and Hawkes H A. Academic Press

Martin D 1968. *Microfauna of Biological Filters.* University of Newcastle-upon-Tyne Bulletin 39. Oriel Press.

Styles P D 1979. *Control of chironomid flies breeding in sewage filters*. Ph.D. Thesis, University of Aston in Birmingham.

Tomlinson T G 1946. *Animal life in percolating filters*. Water Pollution Research Technical Paper No 9. HMSO. (Unfortunately now out of print)

Appendix 1

Typical Report Sheet

Works: GROVEWOOD
Date: 19-3-86

Inspected by: T. SMITH
Report Ref: 38

Last inspected: 15-12-85

Filter No.	Film		Fauna										Observations
	Quantity	Quality	Enchytraeids	Lumbricids	Sylvicola	Psychoda	Chironomids	Tomocerus	Collembola	Spiders	Others		
1	SMALL	GOOD	C	R	R	I	O	I	R	I	CERCYON	R	SOME SMALL PATCHES OF MOSS
2	MODERATE	GOOD	O/C	R	I	C	I	I	I	I			SLIGHT "PONDING" IN PLACES NEAR CENTRE OF FILTER

Remarks: FILTER 2 APPEARS TO BE RECEIVING GREATER FLOW THAN FILTER 1, ALSO DISTRIBUTOR ARMS NEED SUPPORT WIRES ADJUSTED TO ENSURE EVEN DISTRIBUTION OVER FILTER SURFACE

Signed: J. Smith

Date: 2/-3-86

A Appendix 2

Neutron Probe Moisture Meter

Although the neutron probe moisture meter was originally developed for the determination of the moisture content of soil, it can also be used for the determination of the water content of film in biological filters, and which is related to the quantity of film on the medium. A suitable apparatus, the 'Wallingford Soil Moisture Probe, Model 225', can be obtained from:

D A Pitman Ltd
Jessamy Road
Weybridge
Surrey KT13 8LE

Telephone 0932 46327/8

The cost of the complete equipment, including rechargeable batteries and charger, is in excess of £3000.

This equipment contains a radioactive source and consequently certain procedures need to be followed by prospective users before a radioactive source may be obtained. Applicatioon for registration under the 'Radioactive Substances Act 1960' must be made on form RSA2 and which may be obtained from:

HMIP
Radiochemical Inspectorate
Department of the Environment
Romney House
43 Marsham Street
London
SW1P 3PY

Switchboard 01 212 3434

Although the DOE will notify the local appropriate County and District Councils, it is probably preferable to contact the local Chief Fire Officer of the area in order that inspection of the premises may be performed.

In addition, the local office of the Health and Safety Executive and appropriate local Safety Officer should be informed that a radioactive source is being kept on the premises.

In general, persons involved in the use of radioactive sources are required to obtain and wear 'film badges' to monitor exposure to radioactivity. The radioactive source in neutron probe moisture meters is a 50 millicurie Americium/Beryllium source, and, because of this low level, exemption from wearing 'film badges' has been granted by the HSE. This is included in Certificate of Exemption No 14 (General) issued by:

Health and Safety Executive
Nuclear Installation Inspectorate
Thames House
Millbank
London
SW1P 5QJ

Copies of this exemption may be obtained from the National Radiological Protection Board:

Southern Centre
Chilton
Didcot
Oxford
OX11 0RQ

Telephone 0235 831600
Mr W F Bland*

Northern Centre
Hospital Lane
Cookridge
Leeds
LS16 6RW

Telephone 0532 679041
Mr Sutherland*

Scottish Centre
155 Hardgate Road
Glasgow
G51 4LF

Telephone 041 440 2201
Mr G Jardine*

Reference is made in the exemption to the appropriate Statutory Instruments, in this case SI 1985/1333: The Ionising Radiations Regulations 1985. Copies of this may be obtained from HMSO, price £5.40.

Although pocket type dosimeters are available for the detection and measurement of exposure to radiation, these are designed for use with beta, gamma and X-rays and are not suitable for use with neutrons. An instrument suitable with neutrons is likely to be as large as the neutron probe equipment itself and of similar cost.

See also Four Essay Reviews on applications of Radiation Measurement in the Water Industry 1984—Regulations Governing the Uses of Radioactive Materials. HMSO, in this series.

* At time of going to press, will change with time.

A Appendix 3

Preparation of Sticky Grease for Fly Traps

If a large number of fly traps are likely to be used in fly monitoring work, it may be more economical to prepare the 'sticky' material as opposed to purchasing ready made material. For persons not wishing to prepare such material, or who only wish to undertake small amounts of monitoring, commercial 'tree-banding' products are available from garden centres. However, the only one commonly available is very dark coloured and can make the subsequent identification of captured insects slightly more difficult than if a light coloured material is used. The following method is suggested for those persons wishing to manufacture their own, the recipe being a simplified version of a previously commercially manufactured material.

WARNING: THE METHOD INVOLVES HEATING CASTOR OIL TO OVER 150°C

Care is needed to avoid splashing the hot oil when adding the solid ingredients and the preparation is best performed in a fume cupboard while wearing suitable protective clothing.

Ingredients

Castor Oil	5 l
Gum Copal	1 kg
Colophony Resin	1 kg
Carnuba Wax	250 g
Cerasine Wax	100 g

Other items needed are a 'tar-bucket' or iron pail with a pouring spout, a gas ring or other heat source and metal containers to contain the finished product.

Method

The solid materials should be broken into small portions, either using a pestle and mortar for the gum copal and colophony resin or with a sharp knife or scalpel for the waxes.

Pour approximately 3.5 l of the castor oil into the bucket and heat strongly. Add the gum copal, a portion at a time, and heat strongly until melted into a homogeneous mass. Add the colophony resin and heat until melted, then the carnuba wax and cerasine wax likewise. Finally, after removing the heat source, add the remainder of the castor oil and stir into the melted mass. After allowing to cool to 80–100°C, the material is poured into the metal containers and allowed to cool. A small quantity of solids may remain in the bucket, this being mainly inorganic debris originally adhering to the gum copal, and it may be advantageous to filter the hot material into the metal containers through wire mesh of approximately 1 mm aperture.

To prepare a sticky plate for a fly trap, a small quantity of the grease is placed on the plate and spread thinly over the capturing area with the aid of a serrated-ended tool of the type normally used for tile or plastic laminate adhesive application. The grease does not harden on the sticky plate although it may lose some of its adhesive properties after several weeks. Between periods of use, the plates are stored in slotted wooden boxes and the surface moisture allowed to evaporate in a heated room maintained at approximately 30°C. Before the plates are reused, the grease is redistributed using the original spreader. If the plates need cleaning, the old grease is removed using a flat-ended scraper and the plates immersed in hot alkaline cleaning solution. A complete solvent for the grease has not been found but insects required for detailed examination have been treated with hot acetone and/or alcohol which removes the majority of the grease.

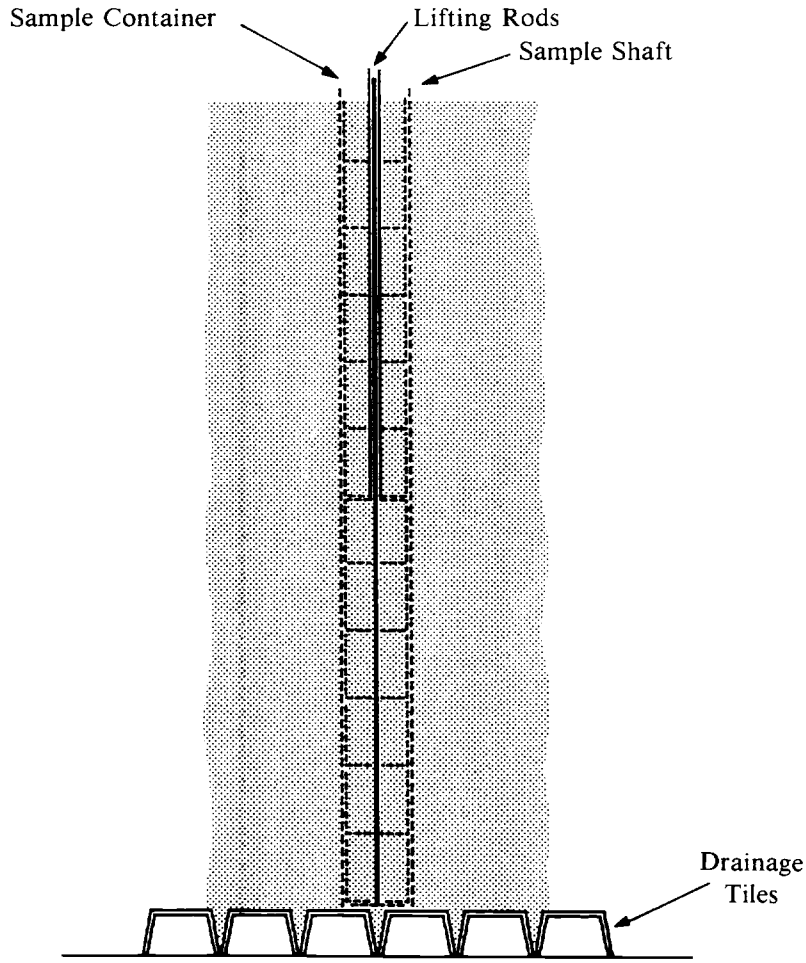
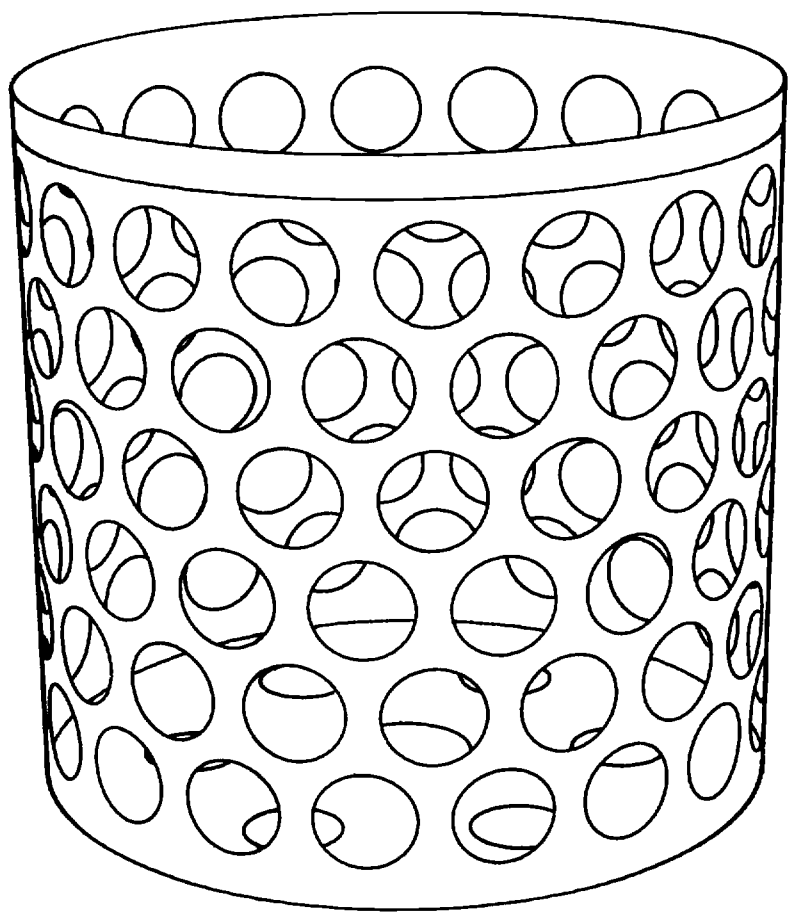


Fig 1A Sample Shaft in Filter with Sample Containers fitted onto Lifting Rods.



See Text
for
Dimensions

Fig 1B Perforated Sample Container

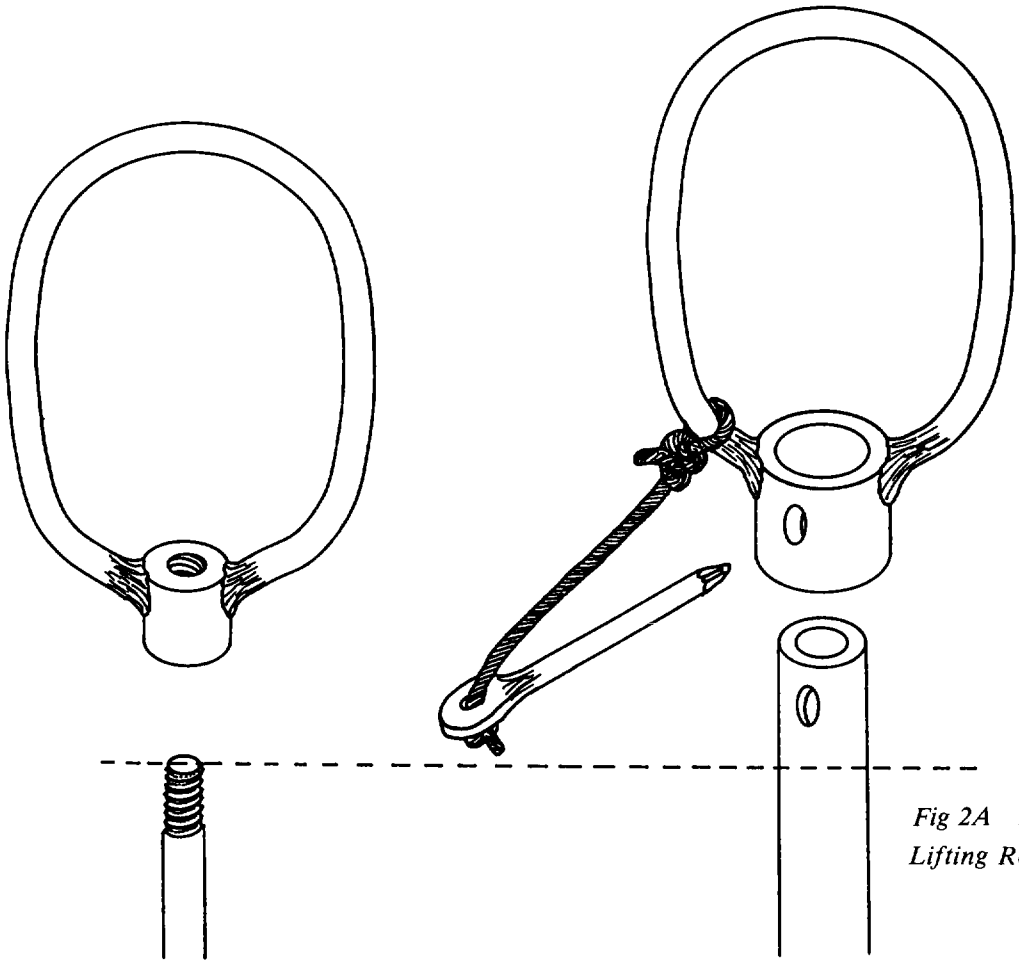


Fig 2A Handles for Lifting Rods

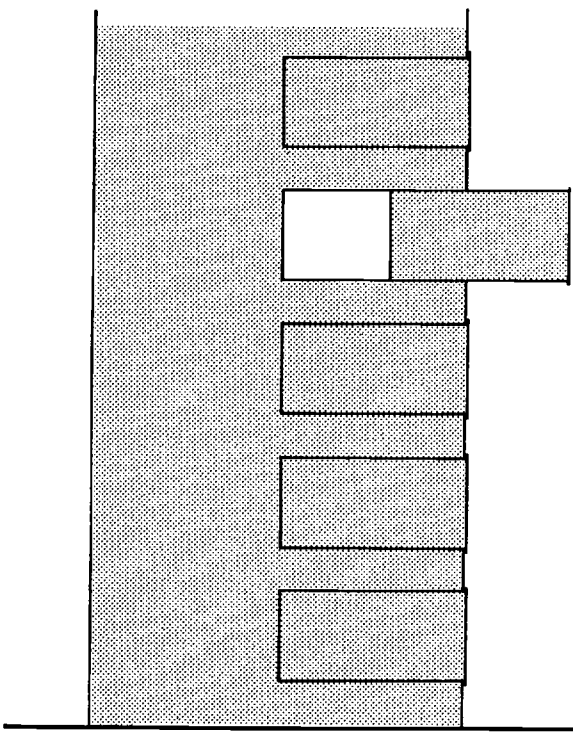


Fig 2B High Rate Filter Showing Sample Drawers

Dimensions to suit Filter but it is suggested that a suitable size might be 300 mm × 300 mm for the drawer fronts and the drawer extending into the Filter for 1 m.

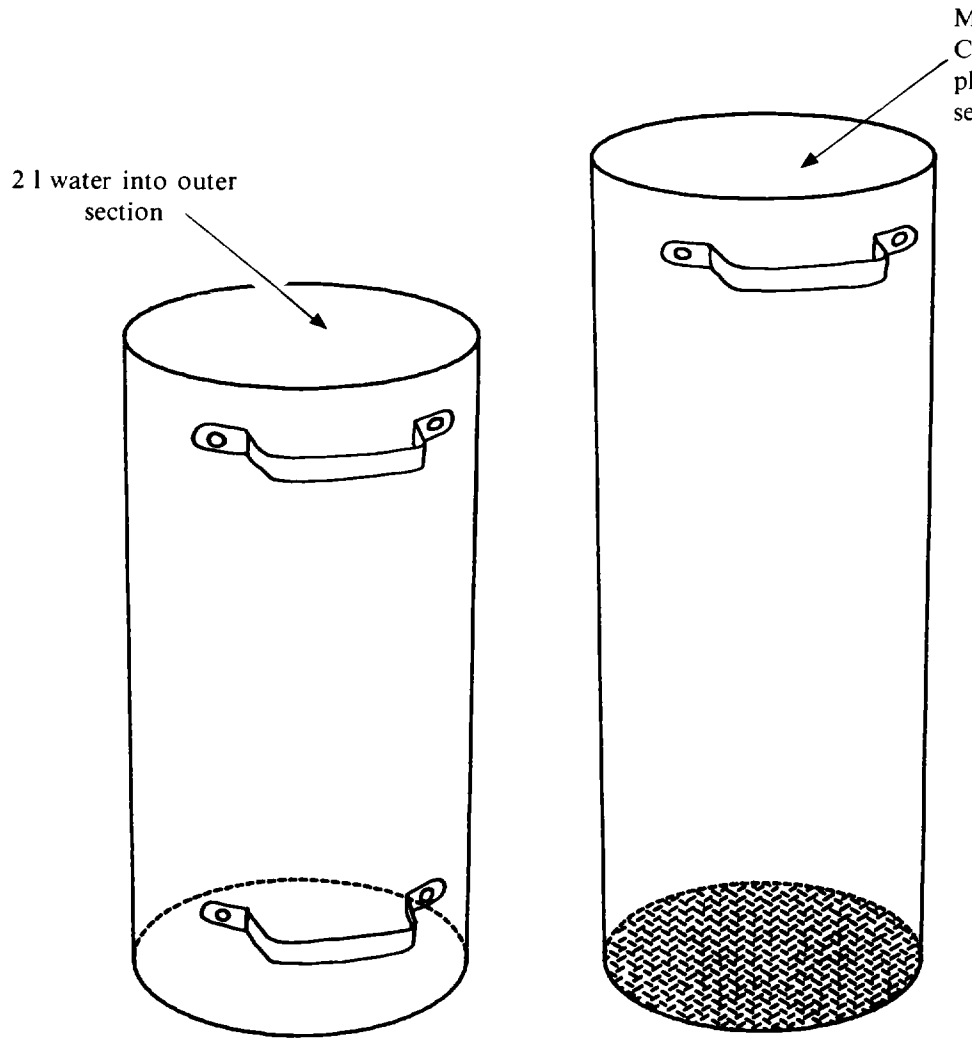


Fig 3A Washing apparatus for 'Dunking' method

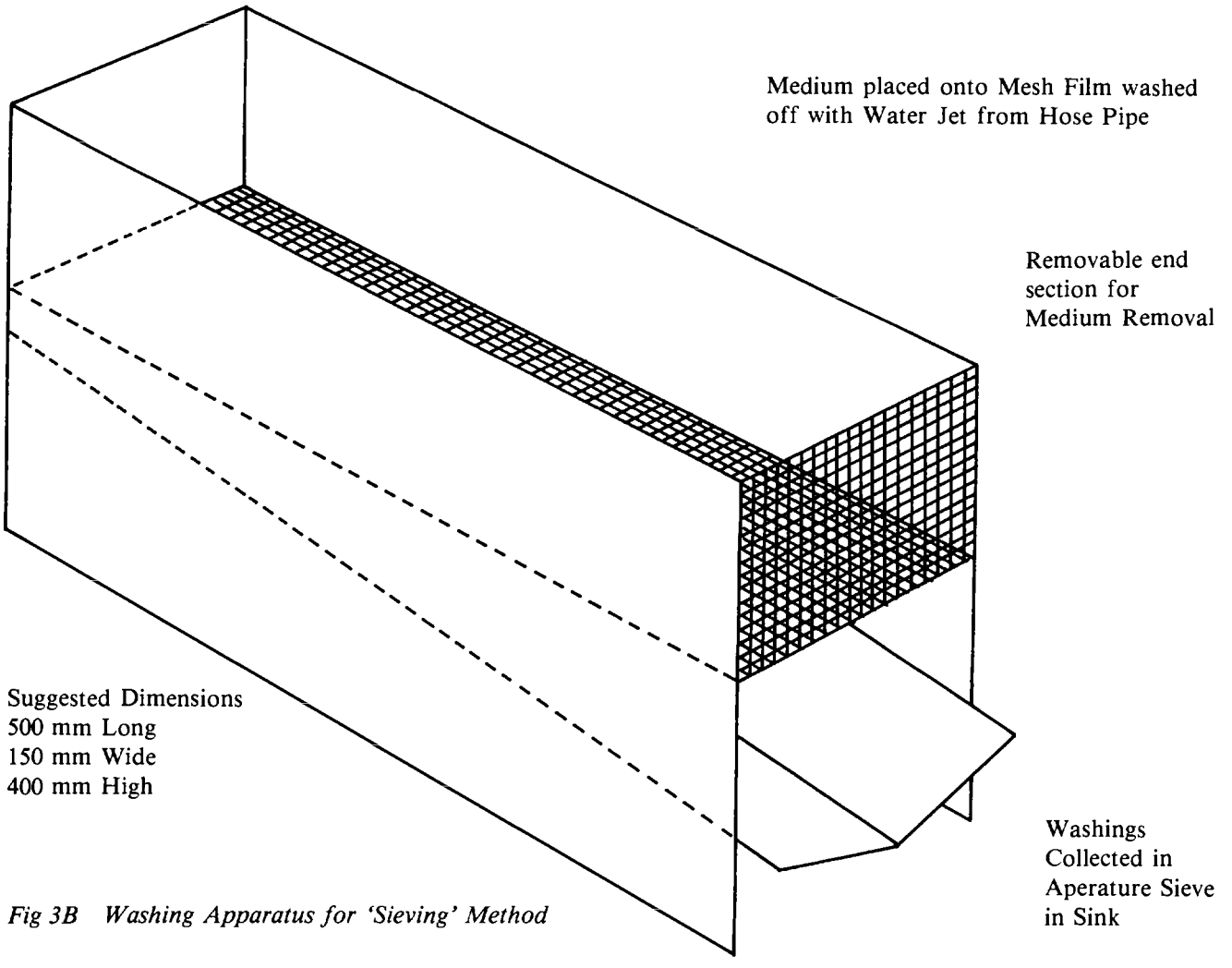
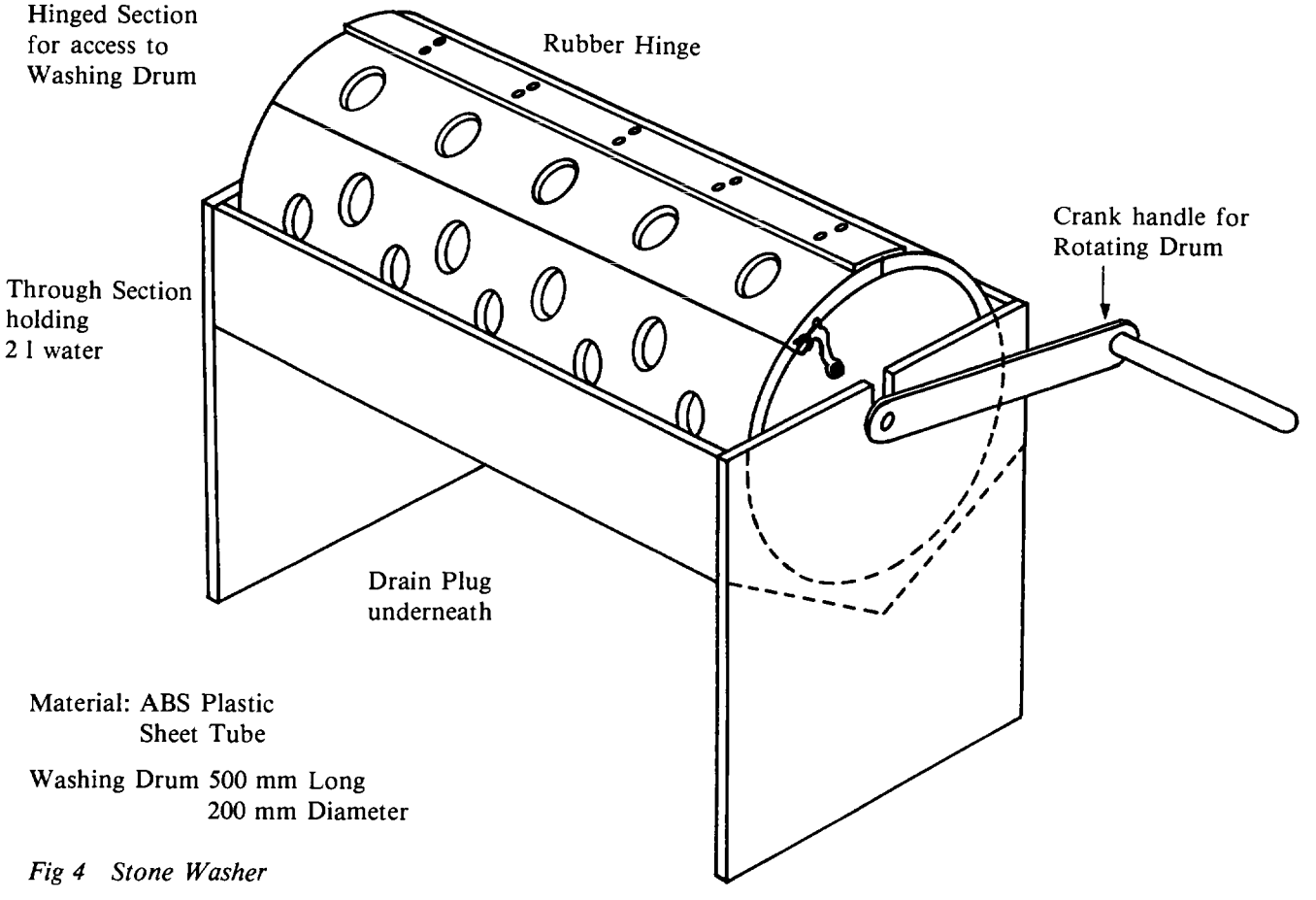


Fig 3B Washing Apparatus for 'Sieving' Method



Material: ABS Plastic Sheet Tube

Washing Drum 500 mm Long
200 mm Diameter

Fig 4 Stone Washer

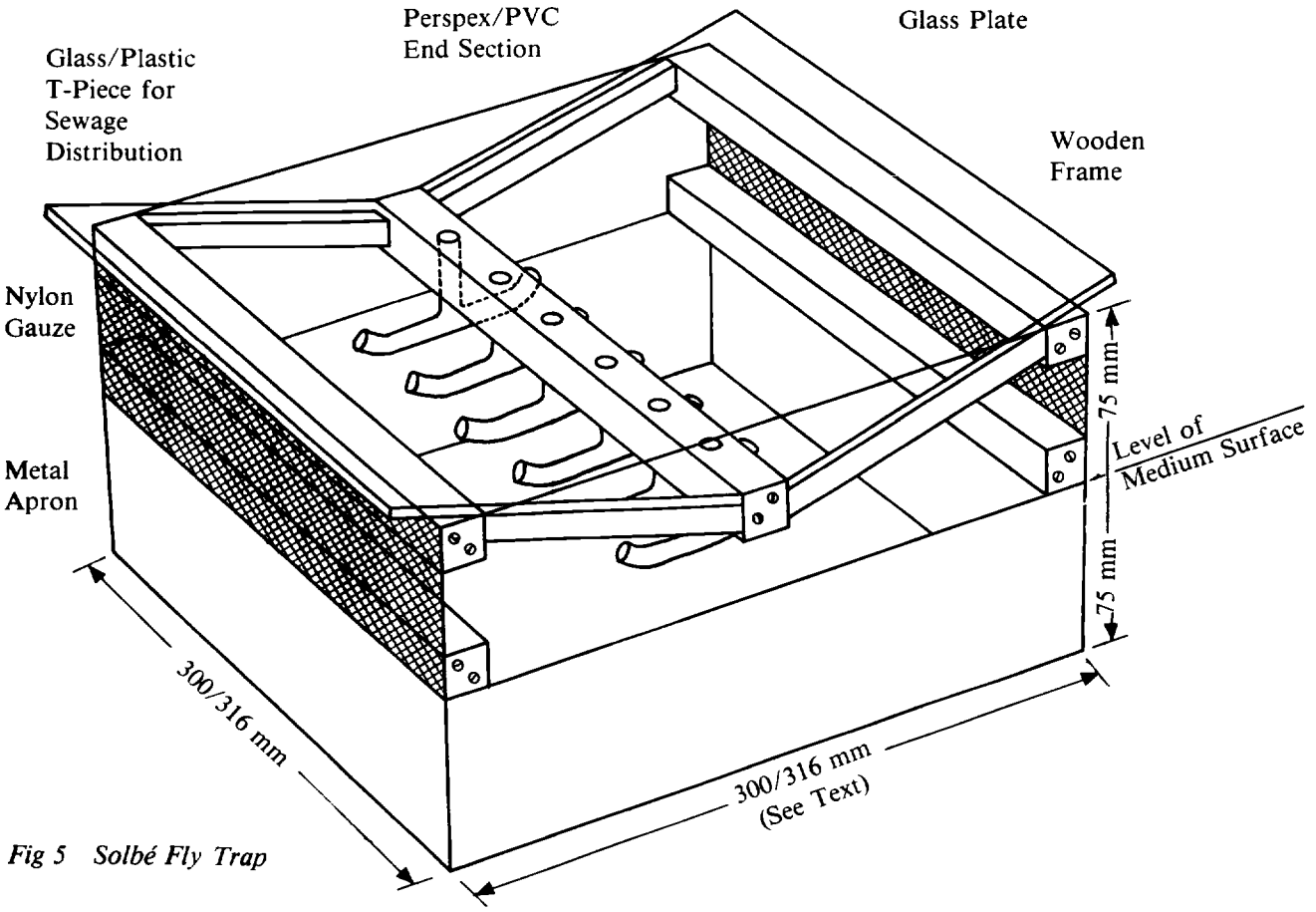


Fig 5 Solbé Fly Trap

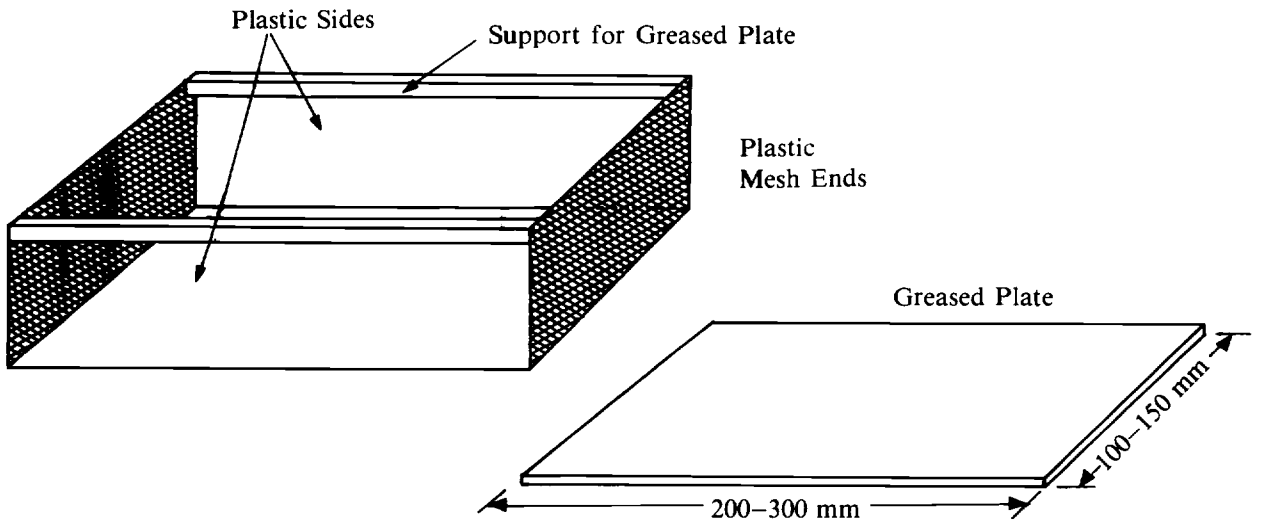


Fig 6A Plastic Fly Trap

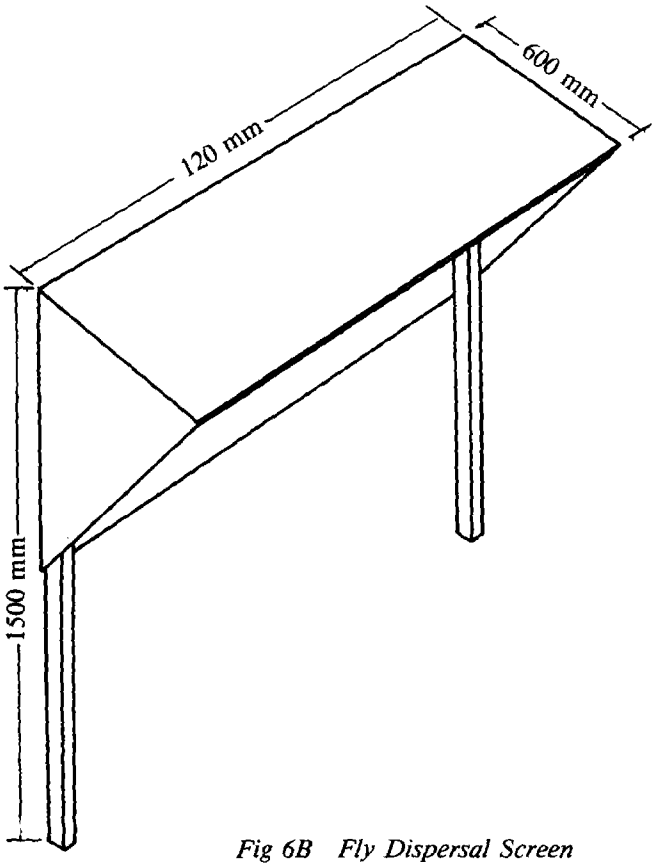


Fig 6B Fly Dispersal Screen

'Suction' Trap

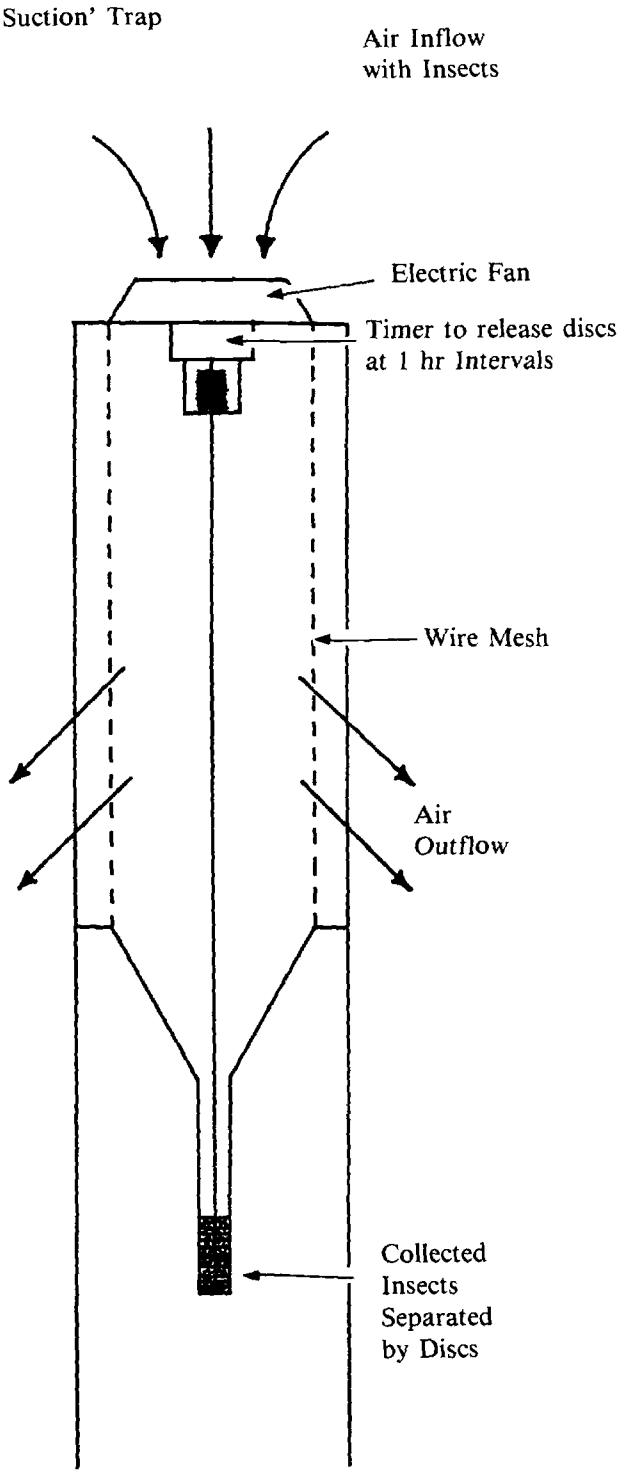
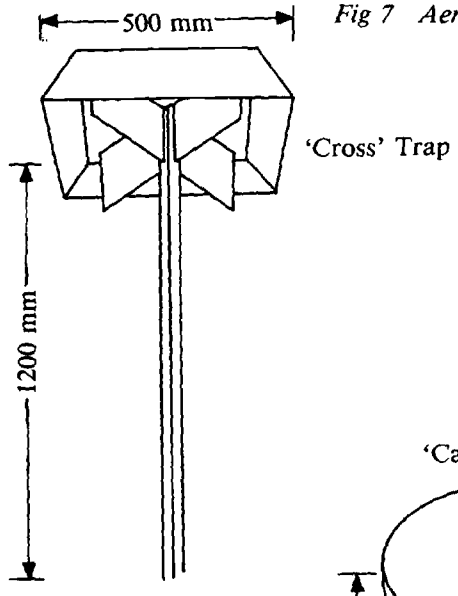
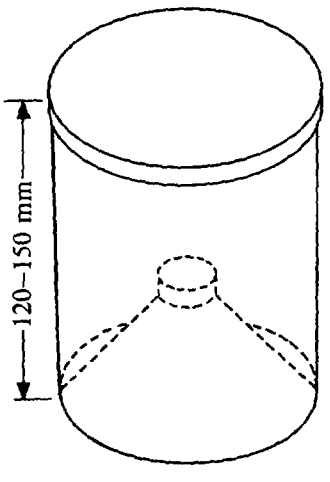


Fig 7 Aerial Traps



'Canister' Trap



B Determination of the Toxicity of Substances to Aerobic Bacteria by Measurement of Growth Inhibition

BO Introduction

The use of results of tests such as the inhibition of respiration of activated sludge (SCA 1982) to predict the effects of a chemical in the environment, eg on the activated sludge process, can lead to wrong conclusions. The principal reason for this is the greater effect some chemicals have on growth than on metabolic activity, such as oxygen uptake, of preformed cells. Since it is not possible to predict which chemicals fall into this category, it is necessary to carry out a test to ascertain the effect of chemicals on growth. (There are other reasons why predictions of environmental behaviour cannot be made accurately from respiration tests—adsorption onto solids, acclimatisation leading to tolerance and/or biodegradation. For such chemicals growth-inhibition tests are not satisfactory and only simulation tests will decide on their true behaviour.)

The Standing Committee of Analysts has devised a test to assess inhibition of growth, based on that described by Alsop *et al* (1980) but the choice of inoculum presented a problem. For better reproducibility a pure culture should be chosen but since no single species can be considered representative of such a varied population as exists in the aquatic environment, sewage was chosen as the inoculum source, with a consequent sacrifice of reproducibility.

The original method, in which the turbidity of a medium inoculated with settled sewage was measured, gave inconsistencies and was not reproducible because of the variable nature of sewage from day to day and source to source. The present method greatly reduces this variability by using an inoculum containing a more constant number of cells which are in the logarithmic phase of growth and which contains less inert colloidal matter. This inoculum was achieved by incubating overnight portions of medium containing various amounts of settled sewage and selecting that culture which gave a pre-determined optical density at the end of 16 h incubation.

This method describes a procedure for determining bacterial growth inhibition due to the presence of toxic substances. Since the method utilises low concentrations of microorganisms, it can be applied as a toxicity screening test prior to ready biodegradability assessment. The method augments those toxicity methods described in a previous publication (Standing Committee of Analysts 1982).

B1 Performance Characteristics of the Method

B1.1	Parameter determined	Toxicity of soluble substances to aerobic microorganisms, eg EC50, that concentration giving 50% inhibition of control growth.
B1.2	Type of sample	Pure substances, mixtures or industrial waste waters.
B1.3	Basis of methods	Comparison of bacterial growth rate in the presence and absence of varying amounts of test substance or waste-water.
B1.4	Range of application	0–100% inhibition.
B1.5	Standard deviation	See Section B8.2.
B1.6	Sources of error	Test substance colour, turbidity or tendency to precipitate in the test medium.
B1.7	Time required for analysis	A total of 22 hours. Actual operator time: 6 hours over this period.

B2 Principle

Shake flasks (see B6.1) containing buffer, nutrients and growth substrate are innoculated with an overnight culture of microorganisms, taken from domestic sewage, and incubated at 22±2°C for up to 6 hours. The growth rate of this culture is determined by measurement of turbidity increase at a wavelength of 530 nm. Test substances are evaluated at a range of concentrations and their effect on the rate of bacterial growth determined. The toxicity of test substances is plotted as a function of concentration and the EC50 value obtained (concentration causing a 50% reduction in growth rate).

B3 Interferences

Test substances or waste waters that are coloured or hazy, or precipitate in the test medium may give rise to background optical densities at 530 nm. These can be corrected for by including controls. Flocculent growth of micro-organisms could interfere but does not usually occur; it has largely been eliminated by the use of a pre-grown culture as inoculum.

B4 Hazards

B4.1 Hygiene

Sewage may contain potentially pathogenic organisms, therefore suitable handling precautions should be taken to avoid infection.

B4.2 Chemicals

Test and reference substances may be toxic or their properties unknown, and therefore suitable handling precuations should be taken to avoid unnecessary contact.

B5 Reagents

B5.1 Deionised or distilled water

B5.2 Phosphate buffer

Dissolve 8.5 potassium dihydrogen phosphate, 21.75 g dipotassium hydrogen phosphate, 33.4 g disodium hydrogen phosphate dihydrate in about 500 ml distilled water and make up to 1 litre.

B5.3 Nutrient broth/sodium acetate solution

Dissolve 8±0.2 g Bacto nutrient broth and 6±0.2 g sodium acetate in 1 litre distilled water. Solutions B5.2 and B5.3 are stored in the dark, preferably in a refrigerator, and discarded at the first sign of sediment, turbidity or biological growth.

B5.4 pH adjustment reagents

Sodium hydroxide (1 M) and sulphuric acid (0.5 m).

B5.5 Test substance stock solutions

Prepare a 1 g/l solution of the test substance in distilled water. The pH of this solution should be checked and adjusted to pH 7±1 if necessary before making up to volume.

B5.6 Reference substance stock solution

Prepare a 1 g/l solution of 3,5 dichlorophenol in distilled water.

B5.7 Waste water

A representative sample of the waste water is freshly collected from the source. The sample may be clarified, if necessary, by centrifuging or filtration to eliminate interference in the determination.

B5.8 'Seed' microorganisms

Supernatant from the primary settlement tank of a sewage works treating predominantly domestic sewage. This should be obtained freshly but may be stored at a temperature of 0–4°C for up to 5 days if necessary, but this is not recommended. Before use filter 200 ml through glass wool or glass fibre paper A to remove coarse material, discarding the first 180 ml and collecting the final 20 ml for use in the test.

B6 Apparatus

B6.1 250 ml conical flasks with polyurethane foam bungs.

B6.2 Incubator/shaker capable of maintaining a temperature of 22±2°C and a shaking speed of 150 rpm. Alternatively, a shaker in a constant temperature room (22±2°C) may be used.

B6.3 UV-visible spectrophotometer and matched cell (1 cm or 4 cm light path).

B7 Test Procedure

Step	Procedure	Notes
	Preculture	
B7.1	16 h prior to commencement of the test set up shake flasks (B6.1) containing 25 ml water (B5.1), 4 ml buffer (B5.2) and 10 ml nutrient broth/sodium acetate solution (B5.3).	
B7.2	Inoculate three flasks with a range of volumes of sewage micro-organisms (B5.8) eg 0.01, 0.1 and 1 ml and place in the incubator shaker (B6.2) for 16±1 h (note a).	(a) It is convenient to carry out this preculture stage overnight.
	Setting up the Test	
B7.3	After the preculture period take a sample from each flask and measure the optical density at a wavelength of 530 nm. Select the flask in which the culture is in the logarithmic phase of growth (note b).	(b) The optical density should be 1.0±0.2 for 4 cm cells (or 0.3 for 1 cm cells) at 16 h to ensure that the culture is in the log phase of growth. In any series of measurements only one size of cell should be used.
B7.4	Label the test flasks and add the appropriate volumes of water, buffer, nutrients and test substance or waste water (note c).	(c) Suggested experimental designs are shown in Tables 1 and 2. Note that unseeded control flasks containing the test substance or waste water are set up.

B7 Test Procedure—continued

Step	Procedure	Notes
B7.5	Place all flasks in the incubator/shaker and allow the contents to reach thermal equilibrium (note d).	(d) This step is advisable to minimise temperature shock on transfer.
B7.6	Inoculate appropriate flasks with 1 ml of precultured inoculum (B7.3) (note e).	(e) The volume of inoculum should be adjusted if necessary to ensure that the control flasks are still in log phase at the end of the 6 h measurement period.
B7.7	At hourly, or other convenient intervals, remove 4 ml samples from each flask and measure the optical density as indicated in B7.3. During the sampling procedure, which should be as short as possible, leave the flasks in the incubator (note f).	(f) Aim to reduce sampling time to a minimum in order to maintain bacterial growth in the log phase.
B7.8	Take the final reading at 6 h (note g) and treat the results as indicated below (Section B8).	(g) The absorbance of the controls is usually 0.3±0.05 in 1 cm cells.

B8 Calculation and Interpretation of Results

B8.1 Correct for any turbidity, colour or precipitation obtained in unseeded control flasks by subtracting the appropriate optical density readings from the corresponding values obtained in seeded flasks.

B8.2 Plot $\log_{10}$ corrected optical density against time for each test substance concentration and the mean of seeded controls. From these plots the specific growth rate of the cultures can be calculated as 2.303 × slope of the line ($= u\ h^{-1}$)*

B8.3 Three types of curve may be obtained (Figure 1). The plot may be a straight line up to 6 h (A) after a short initial lag, but in some cases there may be a departure from linearity before 6 h is reached (B); the inoculated controls always, and most test substances usually, fall into these two categories. A third type (C), observed in the presence of some toxic chemicals shows an initial lag followed by an increase in the rate of growth indicating a rapid acclimatisation or development by the test organisms of tolerance towards the test chemical.

B8.4 Calculate % inhibition as

$$\frac{\mu_c - \mu_t}{\mu_c} \times 100\%$$

where μ_c = specific growth rate of the inoculated control.

μ_t = specific growth rate of culture containing the test substance.

Alternatively, the optical density after 6 h (A), or at the latest time for which the line is straight (B), may be taken as a measure of the biomass present at that time. Calculate the % inhibition for each concentration of test substance as

$$\frac{\text{control optical density—test optical density}}{\text{control optical density}} \times 100$$

It is important to note that these two methods do not give the same values; the % inhibition of biomass increases with time, while that for rate is constant. (The relationship between these two values is indicated in the Appendix).

B8.5 If the curve is of type C and if an inflexion point is recognised, these facts should be reported.

Calculate the % inhibition from the growth rates both before and after the point of inflexion, if the data available justify this.

* At one laboratory using sewages from three sources, linearity was obtained with seeded controls up to absorbances of 0.3±0.05 (1 cm cell) at times corresponding to u values of 0.6–0.8 h^{-1}.

B8.6 Lastly, plot % inhibition against the logarithm of the concentration of test substance and calculate or interpolate from the graph the EC50 as that concentration which inhibits the growth of the control by 50%.

If suitable data are available, the 95% confidence limit of the EC50, the slope of the curve and suitable values to mark the beginning of inhibition (for example, EC10 or EC20) and the end of the inhibition (for example EC80 or EC90) can be calculated or interpolated.

In view of the variability often observed in the results it may in many cases be sufficient that the results be expressed in orders of magnitude for example

EC50 1 mg/l
1– 10 mg/l
10–100 mg/l
>100 mg/l,

but the actual value should be recorded.

B8.7 The results from this test can be used to select a concentration of substance for use in the ready biodegradability tests. A suitable concentration would be that at which 10% inhibition of growth occurs.

The results also give a more accurate indication of the likely effect of a test substance on sewage treatment processes than does the test for inhibition of the respiration of activated sludge (SCA, 1982). Even so, because of possible adsorption effects and possible reactions with other chemicals present in sewage as well as biodegradation after a period of acclimatisation, a final judgement of the effects of chemicals on sewage treatment may not be made until a simulation test is carried out.

B8.8 By determining the degrees of inhibition caused by a number of dilutions of an industrial waste water, an assessment can be made of the effect the waste water may have when treated with sewage at the dilution which is likely to occur in practice.

B9 Validity of the Results

The sensitivity of the sewage microorganisms should be checked by means of a reference substance.

In a ring test, the EC50 of 3,5-dichlorophenol was found to lie in the range of 1 to 10 mg/l.

If the EC50 of the reference substance does not lie in the expected range, the test should be repeated with sewage from another source.

B10 Reference

Standing Committee of Analysts (1982) Methods for assessing the treatability of chemicals and industrial waste-waters and their toxicity to sewage treatment processes. ISBN 011 751959 6, HMSO, London.

Table 1 Experimental design for determining the degree of microbial inhibition on a number of test substances

Flask No	Contents (1)	Water (ml)	Phosphate buffer (ml)	Nutrient broth/ sodium acetate solution (ml)	Inoculum (ml)	Test substance (2) (ml)	Concentration (mg/l)
1	Control	25	4	10	1	—	—
2, 3	Test substance A	21	4	10	1	4	100
4, 5	Test substance B	21	4	10	1	4	100
6, 7	Test substance C	21	4	10	1	4	100
8, 9	3,5 dichloro-	21	4	10	1	4	100
	phenol (3)	24.6	4	10	1	0.4	10
10, 11	Control	25	4	10	1	—	—
12	Unseeded control	26	4	10	—	—	—
13	A (unseeded)	32	4	—	—	4	100
14	B (unseeded)	32	4	—	—	4	100
15	C (unseeded)	32	4	—	—	4	100

Notes (1) Total volume in each flask = 40 ml
(2) Test substance stock solution = 1 g/l
(3) Reference substance tested

Table 2 Experimental design for determining the IC50 of a single test substance

Flask No	Contents (1)	Water (ml)	Phosphate buffer (ml)	Nutrient broth/ sodium acetate solution (ml)	Inoculum (ml)	Test substance * (ml)
1	Control	25	4	10	1	—
2, 3	1 mg/l test substance	24.6	4	10	1	0.4(2)
4, 5	3.2 mg/l	23.7	4	10	1	1.3(2)
6, 7	10 mg/l	21	4	10	1	4.0(2)
8, 9	32 mg/l	23.7	4	10	1	1.3(3)
10, 11	100 mg/l	21	4	10	1	4.0(3)
12	Control	25	4	10	1	—
13	Unseeded control	26	4	10	—	—
14	3,5 dichloro-phenol 2.5 mg/l	25	4	10	1	0.1
15	10 mg/l	24.6	4	10	1	0.4
16	100 mg/l	21	4	10	1	4.0

Notes (1) Total volume in each flask = 40 ml
(2) Test substance stock solution = 100 mg/l
(3) Test substance stock solution = 1 g/l
* Alternatively, various volumes of waste water may be used and the volume of water added so that the final volume is 40 ml in all flasks.

B Appendix

Let μ = specific growth rate of control culture having an absorbance A_t at time t,

μ^1 = specific growth rate of inhibited culture having an absorbance A_t at time t,

and A_o = absorbance of both cultures at time 0

then $\ln A_t = \ln A_o + \mu t$ (1)

and $\ln A_t^1 = \ln A_o + \mu^1 t$ (2)

(This assumes no lag in, and logarithmic growth over, the whole period.)

Subtracting (2) from (1):

$$\ln A_t - A_t^1 = \mu t - \mu^1 t$$

$$\text{Therefore } \mu^1 = \mu + \frac{1}{t} \ln \frac{A_t^1}{A_t}$$

Now % inhibition of growth rate, I_g,

$$= \left(1 - \frac{\mu^1}{\mu}\right) 100 = \left(1 - \frac{\left\{ \mu + \frac{1}{t} \ln \frac{A^1}{A} \right\}}{\mu} \right) 100$$

$$\text{therefore } I_g = - \left(\frac{1}{\mu t} \ln \frac{A_t^1}{A_t} \right) 100$$

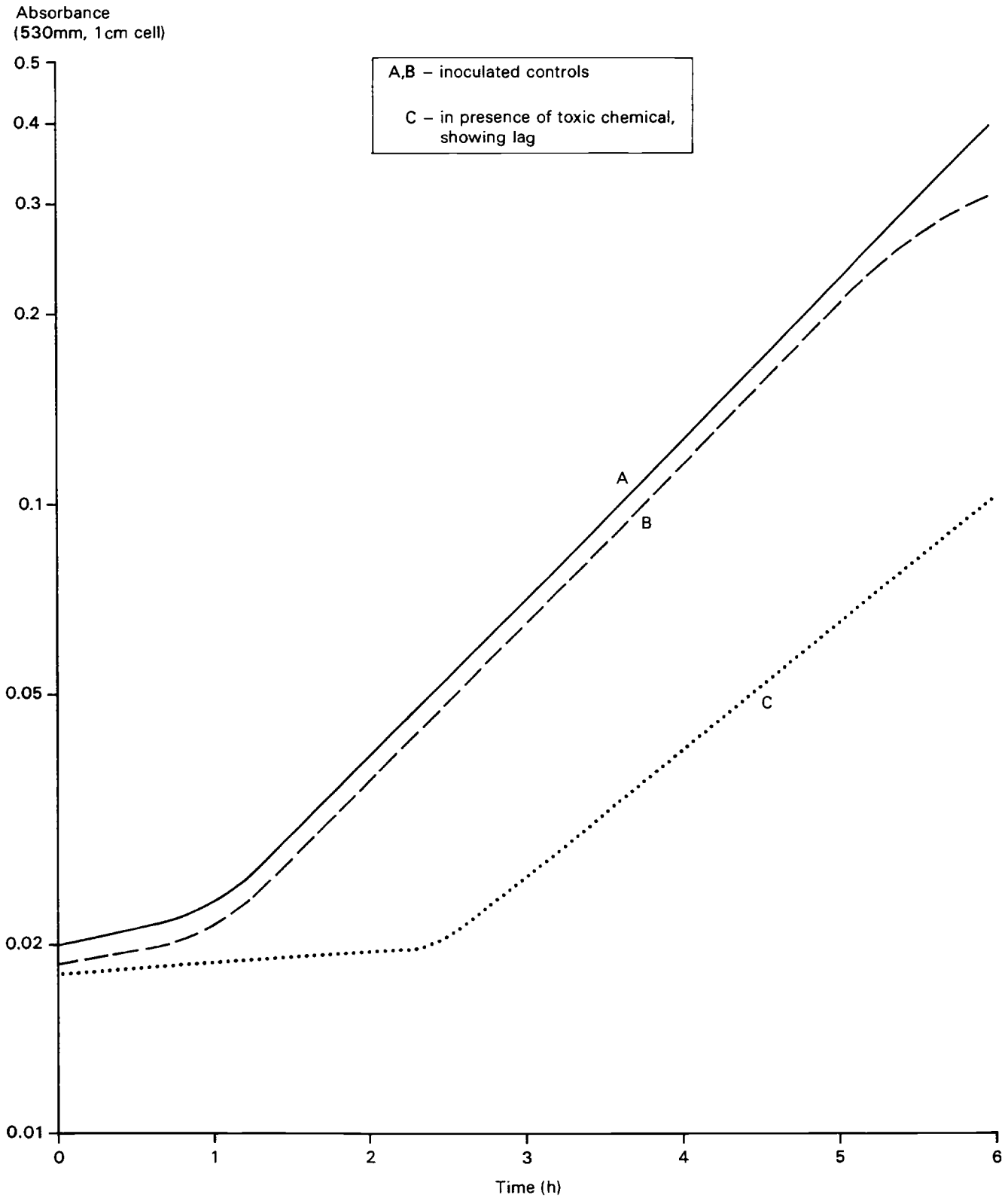
The relationship between I_g and inhibition of biomass, I_B, can thus be calculated since

$$I_B = \frac{(A_t - A_o) - (A_t^1 - A_o)}{(A_t - A_o)} \times 100$$

$$\therefore \quad I_B = \frac{A_t - A_t^1}{A_t - A_o} = 100$$

If $A_o = 0.02$, it may be calculated that
for t = 4h and $A_t = 0.3$, $\mu = 0.677\ h^{-1}$;
for t = 6h, $\mu = 0.451\ h^{-1}$.

Figure 1 Observed types of growth curves



C

Assessment of the Effect of Sludge Retention Time and Temperature on the Treatability of Chemicals in the Activated Sludge Process

CO Introduction

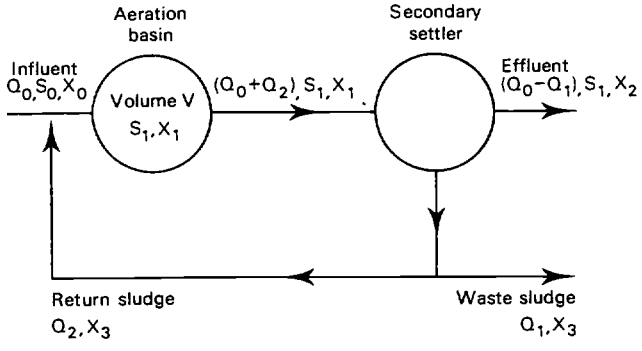
In the overall hazard evaluation of chemicals, which are discharged to the environment after sewage treatment, reliable tests for predicting the fate of materials in the process are essential. It is also necessary to establish if the material has any effect on the sewage treatment process.

One of the major difficulties in the development of such a test is the considerable variation in plant performance resulting from differences in plant design, process operation and temperature. The test described here is aimed at overcoming some of these difficulties and improving the predictive power of laboratory activated sludge simulation tests. Further, application of relatively simple sanitary analyses (eg BOD, COD, DOC, NH_3-N) allows adverse effects of the test material on the process to be detected.

Methods currently in use for assessing the treatability of materials in the activated sludge sewage treatment process (1) fail to take into account the effect of sludge retention time (SRT) and temperature on the concentration of test compound in the plant effluent. The importance of these parameters is apparent from consideration of the mathematical model of the process given below.

A flow diagram of a completely mixed activated sludge process is given in Figure 1.

Figure 1 Flow diagram of activated sludge process



where Q_0, Q_1 and Q_2 are the influent, waste sludge and return sludge flows (L/d)

S_0, S_1 are the substrate concentrations in the influent and effluent in (mg/L)

X_0, X_1, X_2 and X_3 are the concentrations of microorganisms in the influent, aeration basin, effluent and return sludge (mg/L)

The net change in the concentration of micro-organisms, X, as a result of synthesis and decay can be expressed by:

$$\frac{dX}{dt} = uX - KdX$$

where u is the specific growth rate and Kd is the specific decay rate, and the effect of substrate concentration, S, on the specific growth rate is given by the Monod function:—

$$u = \frac{\hat{u}S}{Ks + S}$$

where $\hat{u}$ is the maximum specific growth rate and Ks is the saturation constant, ie the concentration at which

$$u = \frac{\hat{u}}{2}$$

A mass balance of the active solids across the system gives:—

$$\frac{VdX_1}{dt} = u X_1 V - KdX_1 V + Q_0 X_0 - (Q_0 - Q_1) X_2 - Q_1 X_3 \ldots \text{(eqn. 1)}$$

For most of the materials for which detailed biodegradability studies are necessary, efficient treatment occurs only after a period of acclimatisation, ie when a significant population of competent micro-organisms becomes established in the activated sludge. Hence it is not unreasonable to assume that the levels of competent organisms in the influent sewage are low, ie $X_0 = 0$.

At steady state $\frac{dX_1}{dt} = 0$ and, since $u = \frac{\hat{u}S_1}{Ks + S_1}$, equation 1 reduces to:

$$\frac{\hat{u}S_1}{Ks + S_1} - Kd = \frac{(Q_0 - Q_1) X_2 + Q_1 X_3}{VX_1}$$

Since $\frac{VX_1}{(Q_0 - Q_1) X_2 + Q_1 X_3}$ is the mean sludge retention time (SRT), then:—

$$\frac{1}{\theta_s} = \frac{\hat{u}S_1}{Ks + S_1} - Kd$$

Where θ_s is the mean sludge retention time.

On rearrangement, the following expression for the level of substrate in the plant effluent at steady state is obtained:

$$S_1 = \frac{Ks\,(1 + Kd\theta s)}{\theta s\,(\hat{u} - Kd) - 1}$$

Examination of this equation leads to the following conclusions:

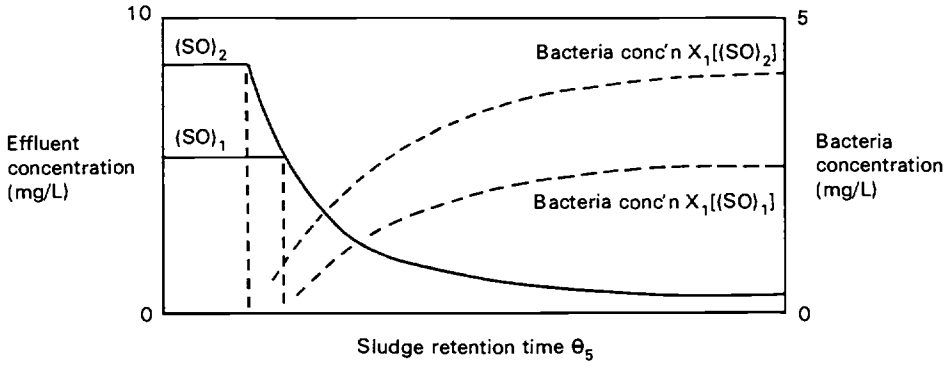
(i) The effluent concentration is independent of the influent concentration, S_0, since this parameter does not appear in the expression.

(ia) The % biodegradataion or removal will vary with the influent concentration since % biodegradation $= (S_0 - S_1)/S_0 \times 100$.

(ii) The only plant control parameter affecting the level of substance in the effluent is the sludge retention time, and the variation of effluent substrate concentration with SRT will be as shown in Figure 2.

$\hat{u}$ is usually written as u max

Figure 2 Effect of SRT on effluent concentration



(iii) For any given influent concentration there will be a critical sludge retention time θsc, such that $\frac{1}{\theta sc} = \frac{\hat{u}S_o}{K_s + S_o} - Kd$ below which the competent microorganisms will be washed out of the plant and the rate of biodegradation will fall to zero.

(iv) Since all other parameters in the equation are associated with the growth kinetics of the microorganisms degrading the substrate, then the temperature is likely to affect the effluent substrate level and the critical sludge age, ie if over the selected temperature range the biological coefficients vary appreciably with temperature a shift in position of the curve shown in Figure 2 would be observed and the SRT required to obtain efficient treatment would increase with decreasing temperature.

The above considerations suggest that to assess the treatability of materials in the activated sludge process it is necessary to control both the sludge retention time and the temperature and to determine how these factors affect the biodegradability of the material under test.

The laboratory scale activated sludge plant described below does not involve the use of a settler the sludge being retained by a porous liner. Consequently sludge is wasted directly from the aeration basin and hence:

$$SRT = \frac{VX_1}{(Q_0 - Q_1)X_2 + Q_1 X_1}$$

Further the suspended solids level in the plant effluent is low such that

$$(Q_0 - Q_1)X_2 + Q_1 X_1 \approx Q_1 X_1$$

$$\text{and } SRT = \frac{VX_1}{Q_1 X_1} = \frac{V}{Q_1}$$

It is therefore possible to control the SRT at any preselected value by the control of the waste sludge flow rate.

A further important implication of the model is that the % biodegradation will vary with influent concentration and consequently it is not valid to calculate effluent concentration from the relationship:

effluent conc'n = influent conc'n × (1 − % Biodeg'n/100)

The main purpose of the test is therefore to allow the effluent concentration and hence the levels of the test compound in the receiving waters to be predicted. In the procedure described the main emphasis is on the measurement of effluent concentration although the percentage biodegradation/removal can of course be calculated in the usual way.

C1 Performance Characteristics of the method

C1.1	Property determined	The concentration of test compound in the plant effluent and/or the concentration of DOC* in excess of that present in a corresponding control plant effluent under a variety of plant operating conditions.
C1.2	Type of sample	Compounds which are soluble at the concentration used in the test and non-volatile.
C1.3	Basis of method	Determination of the level of test compound in the plant effluents and the effect of SRT and temperature on these levels.
C1.4	Standard Deviation (within batch)	not known.
C1.5	Limit of detection	Limited by the analytical method for test substance or for DOC.
C1.6	Interferences	Inhibitors of bacterial growth. Any substance interfering in the analytical method used (See Section C3).
C1.7	Time required for analysis	Up to 6 months. Operator time 14 h/week per 10 units (excludes analysis) for 5 values of SRT at three different temperatures.

C2 Principle

The plants are used to treat domestic sewage and a solution of the test compound is dosed directly into the plant. The sewage and test compound solution flow rates are chosen to give the desired sewage retention time and influent test compound concentration. The plant temperature is controlled and adjusted to obtain data at different temperatures within the desired range, usually between 5 and 20°C. The air supply is arranged to ensure complete mixing of the system. The plants are designed to facilitate the continuous wastage of mixed liquor which allows precise control of the sludge retention time (SRT). Plants are operated to give a range of SRT's usually between 2 and 10 days. At each temperature and for each SRT samples of the plant effluents are analysed to obtain a number of values for each set of conditions. Control units receiving no test substance are operated in parallel for comparative purposes.

C3 Interferences

Any chemical substance in solution or in the air that may adversely affect the growth of sludge microorganisms. Examples are: organic solvents, toxic metals, strong alkalis, biocides.

Substances strongly adsorbing onto the walls of the aeration vessels may give false removal values. Substances interfering in any specific chemical methods used may give false results.

C4 Hazards

C4.1 Hygiene

Sewage and activated sludge may contain pathogenic microorganisms and it is therefore necessary to take appropriate precautions when carrying out plant maintenance and handling samples.

C4.2 Mechanical and Electrical

Guards should be fitted on peristaltic pumps to prevent catching fingers in the moving rollers, and electric stirrers and pumps should be guarded from splashes and leaks.

* dissolved organic carbon

C4.3 Chemicals

If the test substance is toxic or its properties are unknown, it should be handled with care.

Mercuric chloride, used to preserve samples, and sodium hypochlorite, used to clean apparatus, should also be handled with care.

C5 Reagents

C5.1 Sewage

The sewage used should be primary settled sewage of predominantly domestic origin. Sewage containing more than a small proportion of industrial waste should be avoided, since bacterial inhibitors may be present and spurious results may be obtained.

Alternatively a synthetic wastewater[1] may be used and this has a number of advantages such as availability and constant strength. However, the nature of the sludge is very different from that produced by domestic sewage and the porous liners block more rapidly and hence must be changed and cleaned more frequently. Further, for some materials, biodegradation has been observed to be less extensive in synthetic wastewater than in domestic sewage. Consequently tests using synthetic wastes may seriously under-estimate biodegradation observed in practice. Fully operational plants are established more easily if at start-up the pot is filled with activated sludge. This should be obtained from a suitable sewage treatment works treating a predominantly domestic sewage containing little or no industrial effluent.

C5.2 Stock Solutions of Test Substances

To avoid biodegradation of the test substance before it is dosed to the apparatus the test substance solution and sewage are dosed separately. The strength of the dosing solution is calculated from the flow rates of the two pumps delivering this solution and the settled sewage to the apparatus as follows.

If the sewage flow rate delivered to the apparatus is designated SF ml/min the dosing solution flow rate designated DF ml/min; the desired concentration of test substance in the settled sewage designed C mg/L.

Then the concentration of the dosing solution $= \frac{(DF+SF)\times C}{DF}$ mg/L

For the size of apparatus described here DF is set at 0.5 ml/min. Unless the test substance is particularly insoluble the dosing solution is prepared daily by diluting a suitable stock solution ten or twenty fold.

If the solubility of the test compound precludes the preparation of concentrated stock solutions then the dosing solution is prepared directly.

C5.3 Formalin (10 ml, 40% formaldehyde solution/L) or mercuric chloride (40 mg/L) solution for preservation of samples if necessary.

C5.4 Lubricant

A lubricant, eg glycerol, is required for the peristaltic pump rollers.

C5.5 Sodium Hypochlorite Solution

This is used to clean the porous liners.

C6 Apparatus

C6.1 Porous pot aeration vessel see Figure 3.

The plants should be located in a constant temperature room which can be controlled at temperatures in the range 5°–20°C±1°C.

The porous pot aeration vessel liners are constructed from porous polythene of 3.2 mm sheet thickness and a pore size of approximately 90 μm. The sheet is made into cylinders 14.9 cm in diameter and 32 cm in length. The join is butt-welded and the cylinders may be obtained prefabricated from the manufacturers such as Porvair Ltd.

The porous liner is fitted into an impervious polythene outer vessel, which consists of two parts; a circular base in which holes are bored to accommodate two air lines and a sludge wastage line, and an upper cylinder which screws onto the base. This cylinder has an outlet at a height of 25.5 cm above the base.

Note that two air lines are supplied to the base of the pot, one line is fitted with a diffuser stone and the other is set at right angles to the stone in the pot and open ended. The air line produces the necessary turbulence to ensure the pot contents are completely mixed.

C6.2 Oil-Free Compressor supplying compressed air to the aeration vessel.

C6.3 Suitable pumps are required to dose test substance solution and settled sewage at the desired rates (0–1.0 ml $min^{(1)}$ for the test substance solution and 0–25 ml min^{-1} for the settled sewage). Also another pump is required to pump waste sludges from the apparatus. Since this flow has to be very low it is obtained by using a pump set at a higher flow rate and operated intermittently by the use of a timer eg 10 sec. every 1 minute; pump delivery rate 3 ml min^{-1}; wastage rate 0.5 ml min^{-1}.

C6.4 Timer for operating sludge wastage pump.

C6.5 1 litre glass bottles for test substance dosing solutions.

C6.6 Suitably sized measuring cylinders for collecting daily sludge wastage.

C7 Procedure

Step	Procedure	Notes
C7.1	Fill the aerator vessel to the overflow with mixed liquor obtained from an activated sludge plant treating predominantly domestic sewage.	
C7.2	Start the aeration and set the air flow to about 2.5 L/min (note a).	(a) The air flow should be sufficient to maintain the solids in suspension and the concentration of dissolved oxygen about 2 mg/L.† The air flow is split 50–50 through an air stone and a right-angled open ended tube.
C7.3	Place 1 litre of test substance dosing solution in the dosing jar.	
C7.4	Start the sewage and test compound dosing pumps. (note b).	(b) To prolong the life of the peristaltic pump tubes lubricate lightly with glycerol.
C7.5	Set the sewage dosing pump and test substance solution dosing pump to the required rates.	
	Daily Operation	
C7.6	Any sludge adhering to the liner above the mixed liquor level should be returned to the aeration vessel by brushing at least once a day (note c).	(c) This should be done prior to sampling for the mixed liquor suspended solids determination.
C7.7	Measure the temperature, dissolved oxygen and pH of the mixed liquor (see notes d, e).	(d) If the temperature difference is greater than 1°C from the required value the temperature control unit should be readjusted. (e) If the dissolved oxygen† is less than 2 mg/L check air supply and diffuser stone.

Step	Procedure	Notes	
C7.8	Note the time, measure and record the volume of wasted sludge (note f) and discard.	(f)	Calculate the expected volume wasted from this time and the corresponding time the previous day. If the measured volume is greater than 10% away from the calculated value adjust the waste pump rate accordingly and either waste or return sludge to the aeration vessel to compensate for this error.
C7.9	Measure the settled sewage dose rate (note g). Adjust flow if greater than 0.5 ml/min from the required value.	(g)	This may be done using either a 25ml receiving cylinder or similar sized pipette and a stopwatch.
C7.10	Note the time and measure and record the volume of test substance dosing solution remaining (see note h). Discard the remaining solution and replace with 1 litre±10 ml of fresh solution.	(h)	Calculate the expected value and adjust the relevant pump as outlined in note (g) if necessary. The adjusted pump rate should be checked using a 1 ml graduated pipette and stopwatch.
C7.11	The porous liner should be changed at the first sign of blocking of the pores, ie when the mixed liquor level rises above the level of the effluent outlet. The sludge is syphoned from the pot into a clean bucket and the blocked liner is removed. After wiping out the impervious cylinder, a clean liner is slotted into the base and the sludge returned to the pot. Any sludge adhering to the sides of the blocked liner is also scraped off and transferred. The blocked liner is then cleaned before re-use (note i).	(i)	A fine jet of water may be used to remove any remaining sludge from the pots before they are soaked for at least 24 hours in a 1:1 solution of industrial grade sodium hypochlorite. The pots should be totally immersed during soaking. The pots are removed from the hypochlorite, rinsed thoroughly with tap water and soaked for at least 24 hours more in tapwater. If after this time there is any remaining chlorine on the liners; (this may be tested for with KI/starch papers) it may be removed by longer soaking or by immersion in dilute (1%) sodium thiosulphate solution.
	Checking Plant Performance		
C7.12	Determine the mixed liquor suspended solids† level three times a week.		
C7.13	Determine the organic carbon† concentration in the effluent at least twice a week (notes j & k).	(j)	The dissolved organic carbon concentration in the effluent should be determined on a filtered or centrifuged sample.†
C7.14	Ammoniacal nitrogen and MBAS* removal may also be measured.†	(k)	The total organic carbon (TOC) in the influent should be determined in an homogenised sample if required.†
C7.15	All samples should be analysed on the day of collection unless this proves impossible. Should this be the case the methods of preservation will vary depending upon the analysis to be perfored (note 1).	(l)	For carbon analysis the samples should be frozen in glass beakers (taking care not to crack the beakers) or, in the case of effluents, filtered, acidified and stored at 4°C after adjusting the pH to 4 with hydrochloric or phosphoric acid. For surfactant analysis a 1% v.v. formalin or 1% w/v mercuric chloride solution may be used as preservative.

* methylene blue reacting substance = anionic surfactants.
† Methods for these analyses are included in other booklets in this series (2).

C7.16 Bioelimination

This method measures bioelimination, and so it may be necessary to distinguish between true biodegradation and physical adsorption of test substance onto the sludge. Adsorption, if it occurs, is usually most marked at the start of the test and generally an equilibrium is reached, so it may be identified by careful examination of the course of bio-elimination. Biodegradation is usually shown as a steady upward trend of removal of test substance to a constant plateau at the 'steady state'. However, a special investigation is necessary to determine the extent of adsorption. This is done by analysing samples of sludge taken at intervals for the test substance and carrying out a mass balance for the total sludge content and total liquid content relative to the total added.

C8 Calculation of Biodegradation

For primary biodegradation, the percentage removal is calculated from the following formula:

$$\text{percentage removal} = \frac{CS—CE}{CS} \times 100\%$$

where CS = the concentration of test substance in the sewage
CE = the concentration of test substance in the effluent

For ultimate biodegradability, the dissolved organic carbon levels in the dosed and undosed plants are compared

$$\text{percentage removal} = \frac{CT—(CET—CEB)}{CT} \times 100\%$$

where CT is the estimated carbon concentration in the influent sewage due to test compound,
CET is the dissolved organic carbon content of the dosed plant effluent,
CEB is the dissolved organic carbon content of the undosed plant effluent.

Whether determining primary or ultimate biodegradation, the parameter may be calculated at different sludge retention times. If this is the case, a graph may be drawn of sludge age versus effluent concentration.

C9 Assessment of Results

Although the extent of biodegradation may be calculated as outlined above, the real purpose of the test is to determine the concentration of test substance in the plant effluent under a variety of plant operating conditions. Once the plant has become acclimatised to the test substance and reached steady state, a more or less constant effluent concentration is usually obtained. If sufficient data points are obtained, then it is possible to predict the effluent concentration for any plant operating within the range of conditions studied.

In a complete study, several plants are operated covering the range of SRT's required and these are acclimatised to the test compound. If the effect of the test compound on the overall process is also to be examined then control plants operating at corresponding SRT's are also necessary. This will also be required when studying materials already present in domestic sewage or when using analytical methods which also respond to such materials. In these cases control plant data are essential to allow correction of the observed test plant effluent concentrations.

A typical study produced results as shown in Figure 4. In this case plants were operated at SRT's of 3, 4.5, 6, 7.5 and 9 days with an influent test compound concentration of 10 mg/L. The test plants were acclimatised to the material being studied at 15°C and after a period of steady operation the concentration of the test compound in the effluent was determined on a number of occasions. Different plant operating temperatures were then examined. The temperature was gradually reduced to the new value over a few days and the plants allowed to stabilise at the new temperature before further analysis.

In some cases, eg Curve 3, 5°C the critical SR (see footnote) can be readily evaluated from the graph.

Note: the critical SRT is the value at which all organisms capable of degrading the test compound are lost from the sludge and effluent concentration is equal to the influent concentration.

In cases where this is not possible the critical SRT's may be calculated and approximate values for the maximum specific growth rate and the saturation constant Ks is obtained as outlined below.

The steady state equation can be rearranged to give:—

$$\frac{S_1\theta s}{1 + \theta sKd} = \frac{Ks + S_1}{\hat{u}}$$

If Kd is small, then $(1 + \theta sKd) \approx 1$ and a plot of S_I versus S_IO should give a straight line of stop $\frac{1}{\hat{u}}$ and intercept $\frac{Ks}{\hat{u}}$

Since $SRT_c \approx \frac{1}{\hat{u}}$ then the critical sludge retention time can be calculated where it is not obvious from plotted data.

Clearly such a comprehensive study involves the use of several plants and requires considerable analytical effort. However, experience has shown that the biodegradation of relatively few materials is adversely affected by temperature within the range normally observed in practice. For compounds so far exaxmined in complete studies, preliminary examination at the lowest temperature (5°C) and at the extreme values of the SRT (3 and 9 days) would have given a very clear indication of the likely outcome of the complete study. The cost of this less comprehensive test compares favourably with established simulation tests and provides more information. Simpler and relatively inexpensive sanitary analyses can be used to assess the effect of the material on the overall process and obviate the necessity for other tests eg inhibition of nitrification, and growth of micro-organisms, toxicity to bacteria etc.

C10 References

(1) Methods for the Examination of Waters and Associated Materials. Assessment of Biodegradability 1981 HMSO.

(2) (a) Ammonia in Waters 1980.

(b) Analysis of Surfactants in Waters, Waste Waters and Sludges 1981.

(c) The Instrumental Determination of Total Organic Carbon, Total Oxygen Demand, and Related Determinands, 1979.*

(d) Suspended Matter, Settleable and Total Dissolved Solids in Waters and Effluents 1980.

(e) Biochemical Oxygen Demand 1981.

(f) Chemical Oxygen Demand 1985 (2nd Edn.).

(g) Dissolved Oxygen in Natural and Waste Waters 1979,

All HMSO, in this series.

* A supplement updating this booklet is in preparation at the time of going to press.

Figure 3 Porous pot with SRT control

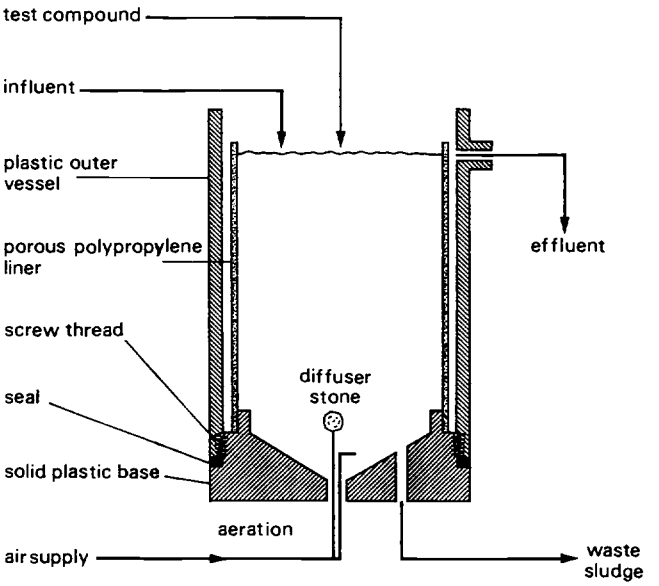
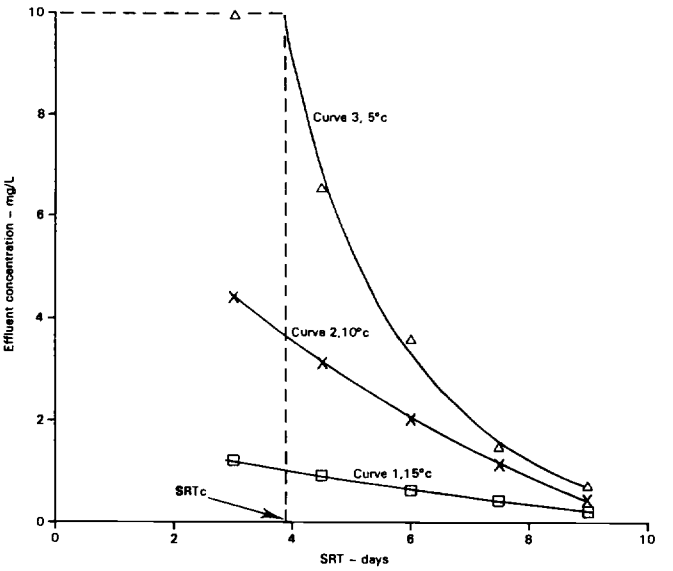


Figure 4



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