ENVIRONMENT AGENCY

The Microbiology of Sewage Sludge (2003) - Part 2 - Practices and procedures for sampling and sample preparation

Methods for the Examination of Waters and Associated Materials

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Methods for the Examination of Waters and Associated Materials

This booklet contains details of the practices and procedures that should be adopted for taking samples and for preparing samples for microbiological analysis in the laboratory.

Within this series there are separate booklets dealing with different topics concerning the microbiology of sewage sludge. Other booklets include

Part 1 - An overview of the treatment and use in agriculture of sewage sludge in relation to its impact on the environment and public health
Part 3 - Methods for the isolation and enumeration of *Escherichia coli*, including verocytotoxigenic *Escherichia coli*
Part 4 - Methods for the detection, isolation and enumeration of *Salmonellae*

Whilst specific commercial products may be referred to in this document this does not constitute an endorsement of these particular materials. Other similar materials may be suitable and all should be confirmed as such by validation of the method.

Contents

About this series

Introduction

This booklet is part of a series intended to provide authoritative guidance on methods of sampling and analysis for determining the quality of drinking water, ground water, river water and sea water, waste water and effluents as well as sewage sludges, sediments, soil (including contaminated land) and biota. In addition, short reviews of the most important analytical techniques of interest to the water and sewage industries are included.

Performance of methods

Ideally, all methods should be fully evaluated with results from performance tests. These methods should be capable of establishing, within specified or pre-determined and acceptable limits of deviation and detection, whether or not any sample contains concentrations of parameters above those of interest.

For a method to be considered fully evaluated, individual results from at least three laboratories should be reported. The specifications of performance generally relate to maximum tolerable values for total error (random and systematic errors) systematic error (bias) total standard deviation and limit of detection. Often, full evaluation is not possible and only limited performance data may be available.

In addition, good laboratory practice and analytical quality control are essential if satisfactory results are to be achieved.

Standing Committee of Analysts

The preparation of booklets within the series "Methods for the Examination of Waters and Associated Materials" and their continuing revision is the responsibility of the Standing Committee of Analysts. This committee was established in 1972 by the Department of the Environment and is now managed by the Environment Agency. At present, there are nine working groups, each responsible for one section or aspect of water quality analysis. They are

1 General principles of sampling and accuracy of results
2 Microbiological methods
3 Empirical and physical methods
4 Metals and metalloids
5 General non-metallic substances
6 Organic impurities
7 Biological methods
8 Biodegradability and inhibition methods
9 Radiochemical methods

The actual methods and reviews are produced by smaller panels of experts in the appropriate field, in co-operation with the working group and main committee. The names of those members principally associated with this booklet are listed at the back of the booklet.

Publication of new or revised methods will be notified to the technical press. An index of methods and details on how to obtain copies are available from the Agency's internet web-page (www.environment-agency.gov.uk/nls) or from the Secretary.

Every effort is made to avoid errors appearing in the published text. If, however, any are found, please notify the Secretary.

Dr D Westwood
Secretary

April 2003

Warning to users

The analytical procedures described in this booklet should only be carried out under the proper supervision of competent, trained analysts in properly equipped laboratories.

All possible safety precautions should be followed and appropriate regulatory requirements complied with. This should include compliance with the Health and Safety at Work etc Act 1974 and all regulations made under the Act, and the Control of Substances Hazardous to Health Regulations 1999 (SI 1999/437). Where particular or exceptional hazards exist in carrying out the procedures described in this booklet, then specific attention is noted.

Numerous publications are available giving practical details on first aid and laboratory safety. These should be consulted and be readily accessible to all analysts. Amongst such publications are; "Safe Practices in Chemical Laboratories" and "Hazards in the Chemical Laboratory", 1992, produced by the Royal Society of Chemistry; "Guidelines for Microbiological Safety", 1986, Portland Press, Colchester, produced by Member Societies of the Microbiological Consultative Committee; and "Safety Precautions, Notes for Guidance" produced by the Public Health Laboratory Service. Another useful publication is "Good Laboratory Practice" produced by the Department of Health.

Practices and procedures for sampling and sample preparation

1 Introduction

The sampling of sewage sludge and associated materials should be carried out with care, as it should be assumed that pathogenic organisms might be present. Only suitably trained or experienced persons should carry out sludge sample collection.

The aim of this booklet is to highlight those issues relevant to the sampling of sewage sludge for microbiological analysis. General principles of sampling apply and are well described in other booklets published within this series[1, 2, 3] and elsewhere[4].

Taking a sample in a suitable container, in a correct manner and storing it appropriately until commencement of analysis are vital parts of any microbiological analysis. The sample submitted to the laboratory should (within the constraints of any heterogeneity of the material) be representative of all of the material to be assessed, and should be collected in such a way as to avoid risks of contaminating the sample. The storage and transport of samples should be undertaken in a manner designed to minimise any change in the composition of the sample, including deterioration, and avoid contamination of the sample. The maintenance of sample integrity via representative sub-sampling, homogenisation and accurate dilution using appropriate aseptic techniques all contribute to the overall reproducibility of the result obtained.

2 Principles and scope

2.1 General principles

The procedures described in this booklet are intended to facilitate the sampling of all types of sludges for microbiological analysis, regardless of the purpose to which the results of analyses are to be used. It is recognized that samples may be taken for a variety of purposes, including compliance with respect to regulatory requirements[5], operational performance monitoring, investigations, research or problem-solving objectives. Documented protocols should be available for the purposes of training, and to ensure consistent approaches are adopted, particularly where data are generated and then compared.

The application and interpretation of microbiological standards for sludge depend on a sound understanding of the sludge treatment processes involved, and the factors affecting the distribution and survival of organisms in different sludge matrices. An overview of microbiological aspects of sewage sludge and sludge treatment processes is described elsewhere in this series[6]and in other publications[7, 8]. Representative samples are crucial if an accurate reflection of the microbiological condition of the sludge is required. Micro-organisms are unlikely to be evenly distributed within a sample of sludge and it will always be necessary to undertake some form of pre-treatment and preparation of the sample to ensure the sludge is as homogenous as possible in order to obtain representative counts.

2.2 Legislative context

The recycling of sewage sludge via agricultural use is considered a beneficial and sustainable practice. Legislation within the United Kingdom[5] and European Union[9] is designed to encourage the recycling of sludge to agricultural land whilst ensuring that treatment processes and application practices are controlled and monitored to prevent potentially adverse environmental and public health consequences. In particular, the regulations incorporate measures (to allay the concerns of food agencies and other organisations) to prevent pathogens entering the food chain.

In the UK, the proposed regulations[5] implement an EU Directive[9] and a code of practice[10] complements the regulations and describes advice on "best practice". The code also supplements the information contained in the "Safe Sludge Matrix"[11] with its cropping and grazing guidance.

2.2.1 Regulatory requirements

The regulations[5] introduce definitions for "conventionally treated sludge " and "enhanced treated sludge" that specifically relate to microbiological standards, and describe requirements (which may change as currently drafted) for monitoring and control.

Specifically, a conventional treatment process is one that:

- is designed so as to reduce the amount of *E. coli* present in sludge by not less than 99 per cent (2 $\log_{10}$ reduction);
- is monitored and controlled in accordance with the regulations; and
- satisfies end-product tests for *E. coli*.

Similarly, an enhanced treatment process is one that:

- is designed so as to reduce the amount of *E. coli* present in sludge by not less than 99.9999 per cent (6 $\log_{10}$ reduction);
- is monitored and controlled in accordance with the regulations; and
- satisfies end-product tests for *Salmonellae* and *E. coli*.

Schedule 3 to the regulations[5] identifies units for expressing *E. coli* test results (as colony-forming units per gram dry weight of sludge) and states that the presence or absence of *Salmonella* species shall be determined by reference to 2 g of dry weight of sludge. A monthly sampling frequency is prescribed with a set of five samples being taken at random from a batch of sludge. Samples of not less than 100 ml of liquid sludge and 100 g of dried sludge are specified and each of the five samples is to be analysed. The schedule describes the end-product standards that apply and allows for a reduced sampling frequency for enhanced treated sludge that consistently meets the standards.

The regulations[5] require the monitoring and control of the treatment process via a critical control point (CCP) approach[10], and provide for inspection, audit and enforcement by the Environment Agency.

2.2.2 Code of practice

The code of practice[10] details additional guidance on microbiological issues, and describes a hazard analysis critical control point (HACCP) approach[12, 13] as the framework for improving microbial quality of sludge via the control of treatment processes. Additionally, further guidance on sampling and analysis is described, but may be subject to change as currently drafted.

The HACCP approach should be used to identify CCPs within sludge treatment processes. Examples of process control parameters that may need to be monitored include temperature, pH, time, percentage dry solids content of thermally dried sludge and lime dose requirements etc. The CCPs identified within the treatment processes should be monitored and controlled to ensure that the required indicator organism reduction is achieved, as well as producing a final end-product sludge material that satisfies the required standard.

In order to show compliance with the HACCP requirement, a generic protocol[14] has been developed between sludge treatment undertakers and the Environment Agency. This protocol describes guidance for demonstrating 2 (or 6) $\log_{10}$ reduction across sludge treatment processes

required for conventionally treated sludge (and enhanced treated sludge) respectively. The aim of the protocol is to provide a recognised approach for specific sites, setting down the description of relevant process control conditions and identification of associated CCPs.

Sampling and analysis requirements[14] are described and are based on a pragmatic approach, recognising inherent difficulties of homogeneity and temporal variation within the treatment process. Specifically, samples of untreated sludge are taken at the inlet to the treatment process, as are end-product samples. For continuous treatment processes, replicate sub-samples are taken for each composite sample after thorough mixing. Each of the sub-samples is then analysed. Composite samples of discontinuous or bulk storage processes, including cakes or liquids are obtained by combining five random samples. The results are then submitted to the regulator as the mean and standard deviation of the analytical results and $\log_{10}$ reduction where:

$$\log_{10} \text{reduction} = \log_{10} \text{mean (inlet)} - \log_{10} \text{mean (end-product)}$$

For example, if the mean enumerated count of an untreated sludge at the inlet is 3.162×10^7 *E. coli* per gram dry weight of sludge, and the mean enumerated count of the end-product secondary digested sludge is 7.943×10^4 *E. coli* per gram dry weight of sludge, then

$$\begin{aligned} \log_{10} \text{reduction} &= \log_{10} 3.162 \times 10^7 - \log_{10} 7.943 \times 10^4 \\ &= 7.5 - 4.9 \\ &= 2.6 \end{aligned}$$

In the context of the protocol, this treatment process would demonstrate a log reduction of at least 2 and further indicate an end-product of less than 10^5 *E. coli* per gram dry weight of sludge.

The regulations[5] require ongoing monitoring of the end-product on a periodic basis to provide continuing evidence of compliance, and that the CCPs are correctly monitored and operated. In this context, *E. coli* is chosen as the key indicator organism rather than specifically enumerating selected pathogens. This is because *E. coli* is present in sewage in consistently high numbers and its behaviour in sludge treatment processes is similar to that of the pathogenic strain, *E.coli* O157.

Guidance is provided[10] on remedial action to be taken , including increased sampling frequencies in the event of sludge samples failing the relevant end-product standard.

2.3 Definitions

In the context of sludge sampling, ‘grab’ or ‘composite’ samples can be taken. ‘Grab’ samples are also known as ‘discrete’, ‘spot’ or ‘instantaneous’ samples, and ‘composite’ samples are also known as ‘pooled’ or ‘combined’ samples.

***Grab sample*:** A discrete sample taken instantaneously either at random or at a specific location.

***Composite sample*:** A sample produced by combining a number of grab samples.

For a continuously operated process, a composite sample can be taken as a proportion of the flow passing a specific location over a defined period of time, or for the duration of the whole production batch.

The regulations[5] relating to the sampling of treated sewage sludge require that five random ‘samples’ should be taken and analysed.

The following definitions have been adapted[15] from definitions relating to food sampling, but may be used in the context of sludge sampling and are given for illustrative purposes only:

Batch: The whole quantity of treated sludge produced, ideally, under identical conditions. For regulatory purposes, the sampling of each batch of sludge relates to plant operation defined within the HACCP assessment.

Sample: The amount of sludge taken as an individual portion from a batch of sludge. Where more than one such sample is taken, these form part of a sample set. For example, a 100 g portion of sludge, of which (for regulatory purposes) there are five in a sample set.

Sample set: The total number of samples taken at random from a batch. For example, five random samples are required for regulatory purposes.

Sub-sample: The portion of sludge taken from a sample, for the purpose of separate analysis.

Analytical unit: The amount of sludge withdrawn from the sample or sub-sample and used in the analytical determination. For example, for the enumeration of *E. coli*, 10 g wet weight of sludge are used in the determination; and for *Salmonella*, 2 g dry weight of sludge are used to determine the presence or absence of the organism.

2.4 Expression of results

Analytical results for sludge may be expressed volumetrically or gravimetrically. Where the result is expressed on the dried weight of sludge, the sludge needs to be analysed for its percent dry solids content[16] and the appropriate calculation performed. In some cases, it may be appropriate to make allowance for the contribution made during sample preparation, for example, by the addition of lime, or acid in the neutralisation of lime, following lime treatment.

Enumerated counts for *E. coli* are typically reported as colony-forming units or most probable number of colonies per gram dry weight of sludge. The reporting of the presence or absence of an organism should be referenced to the actual quantity of sludge material tested. Where a test result is to be reported as being absent, the intended meaning is that the organism was not detected in the quantity of material tested. A result of zero (in the quantity examined) should be recorded, as a result implying a value less than a specific value is, in microbiological terms, meaningless. Where enumeration is to be made on solid media or on membrane filters, and these counts exceed the accepted countable range, typically 20-80 colonies, then the result should be reported as being greater than a specific value together with an appropriate reference to any dilution. The maximum number of colonies that should be counted from a single membrane filter is approximately 100. For multiple tube most probable number techniques, if all the tubes or containers for a particular dilution series exhibit characteristic growth within the medium, i.e. are regarded as positive, it is usual practice to report the results as being greater than a specific value. Further guidance is provided elsewhere[17] in this series.

3 Health and safety considerations

Due to the inherent nature of sewage sludge, the circumstances under which sampling takes place and the likelihood that a biohazard exists, particular attention should be paid to health and safety considerations when undertaking sampling or analysis, see page 5, “Warning to users”. In addition, some sample preparation and pre-treatment procedures may result in the generation of aerosols and

this should be taken into account when carrying out risk assessments.

Sewage and sewage sludge samples can contain hazardous and flammable substances. They may also contain pathogenic organisms and are liable to undergo biological action. Consequently, these samples should be handled with care. Gases that can be produced by microbiological activity are potentially flammable and once generated within the sample container will cause the container to become pressurised. Infectious material and/or pathogenic aerosols will, therefore, be of concern and may be potentially hazardous if containers explode. Glass containers should be avoided, wherever possible[18].

Appropriate risk assessments are required under health and safety legislation[19] and control measures identified should be implemented. Guidance on the management, design and operation of microbiological containment laboratories has been provided[20] as has the categorisation of pathogens[21].

Particular attention should be paid to the disposal of un-used quantities of sludge samples and their associated cultures. Staff who handle sludge samples and associated cultures should be appropriately protected, for example, with up-to-date vaccinations for poliomyelitis, tetanus and possibly immunisation against Hepatitis A. Consideration should also be given to the provision of specific immunisation, where available, for particularly hazardous organisms under study.

Field operations, including sampling, should be conducted with due regard to the possibility of local hazardous situations and portable safety equipment may be required. Appropriate support measures should be considered wherever staff are required to work unaided or alone, whether in the laboratory or located on-site. All equipment used should be cleaned and disinfected after use. In addition, if contamination is known or suspected, clothing, including footwear, should be appropriately disinfected.

4 Sample containers and sample size

4.1 Sample containers

For most sludge sampling purposes, it is usually sufficient to use suitably sized clean containers, and proof of sterility may not be essential owing to the nature of the material being sampled. However, where sludges that have received enhanced treatment are sampled, the use of sterile containers may be advisable. In any event, such containers should be dry, and the cap, typically, screw-threaded or of a "snap on" variety, should provide a good seal in order to prevent leakage.

Glass containers (jars or bottles) should not be used because of inherent dangers associated with their use, either on-site or in the laboratory. This may also apply to certain polystyrene containers. For most sludge sampling requirements, new, disposable plastic containers (typically, 150-250 ml capacity) that are free from inhibitory or toxic substances and pre-sterilised if required, are sufficient. Sludge samples are typically submitted to the laboratory in quantities of at least 100 ml or 100 g. Samples for operational or non-regulatory purposes may be submitted as single samples or comprise replicate numbers of samples. Depending on the sampling protocol, samples may represent three or more sub-samples from a composite sample or batch of sludge, or a sample set may comprise five or more random samples from a batch of sludge.

If pre-sterilised containers are used, they should be supplied with adequate records of their sterility and the manufacturer's expiry or use-by date. Where non-sterile containers are used it may be appropriate to include a proportion of blank control samples to demonstrate that the containers do not make a significant contribution to the result. Ideally, containers should be capable of showing

whether samples have been tampered with. For ease of sample collection, a wide-necked sample container is to be preferred. Where sample containers are to be re-used, they need to be easy to clean and disinfect. In addition, the container material should be able to be autoclaved.

The container should be clearly labelled to allow unambiguous identification of the contents to be made. Sufficient sample should also be submitted to the laboratory to enable all determinations to be carried out and any un-used sample should be stored in a refrigerator until after completion and reporting of the analysis.

4.2 Container labelling and sampling records

Self-adhesive labels are suitable for labelling containers. Labels may be pre-printed or bar-coded, and information entered thereon, or subsequently added, should be permanently recorded. The information provided should clearly identify the sample and the purpose for which the sample was taken. All this information should also be available if requested, for example, on a sampling worksheet or record or in a log-book, and should, for example include the following details:

- a unique reference number or code;
- the date and time of sampling;
- the exact position of the sampling point location;
- the type of material being analysed (for example digested sludge, sludge cake, processed sludge etc);
- the reason for taking the sample (for example audit, regulatory, routine or operational etc);
- the identity of the person taking the sample; and
- the analyses required.

All information should be recorded at the time of sampling.

5 Sampling Techniques

5.1 General principles

The nature and composition of sludge makes the application of aseptic techniques difficult or impractical for sampling purposes. Nevertheless, cleanliness together with good hygiene practices and the use of correct sampling techniques are essential requirements in order to obtain representative sludge samples.

All equipment used should be kept clean, and attention given to the order of taking samples at each site. For example, if possible, it is advisable to prioritise the sampling of sludge by sampling sludge that has undergone the most treatment before sampling sludge that has undergone limited or no treatment. Since sludge that is un-treated or has received the least treatment may contain higher numbers of pathogens, it is advisable to sample this sludge towards the end of the sampling process. Knowledge of the treatment process is therefore important if the correct or most appropriate choice of sampling location is to be made with a view to obtaining a representative sample. For regulatory samples, this needs to be pre-defined from the CCP identification process. For other sampling purposes, knowledge of process operations such as “fill and draw” or continuous feed and mixing characteristics, including potential “short circuits”, may be required. Sampling may also need to take into account the length of time it takes for sludge to undergo treatment between the process inlet and outlet locations, and this may dictate the time and order of sampling.

An appropriate supply of protective clothing should be available, and all equipment to be used

should be prepared and made ready before sampling begins. It may also be appropriate to have available a supply of alcohol-based disinfectant disposable cleaning tissues. In addition to sample containers, a variety of sampling aids, including disposable items such as spoons, spatulas and durable plastic or stainless steel implements such as buckets, beakers, augurs, ladles and corers may be required. Sampling poles may also be required to enable the sampling of liquid surfaces etc to be undertaken. Items to be re-used should be thoroughly washed and then rinsed in clean water and dried. This may be followed by sterilisation involving autoclaving, or disinfection using chlorine-based disinfectants. Before use, traces of residual disinfectant should be washed off using clean water.

The sampling process should take account of the recording of CCP observations, and readings or measurements such as temperature, pH, dry solids content and lime dosage requirements etc. Preparation should take account of the type of sample to be taken and includes whether a "grab" or "composite" sample is required.

5.2 Liquid sludges

Liquid sludges can readily be taken from taps or valves situated on outlet pipe-work. For example, this might apply to digested or secondary digested sludges, and possibly raw sludges if collected from tanks. Where this option is available and enables an acceptably mixed and representative sample to be taken, the pipe-work should be flushed clear of old (static or stagnant) sludge prior to taking a sample. The period of time required to flush the pipe-work will depend on the length and diameter of the pipe, the available "head" (i.e. the pressure exerted by the bulk of sludge stored above the sample line) and, if a pump is fitted, the pump rate.

In other circumstances, it may be necessary to take dipped samples from a sludge storage tank. Practical and safety considerations may dictate the point at which such samples can be obtained, but wherever possible, operational characteristics should be considered in order to obtain an adequately mixed and representative sample. Under "fill and draw" operational conditions, the timing of the operational cycle may be critical and may need to be considered when taking a sample.

Composite samples should be derived from pooled material comprising aliquots of equivalent size. These should be thoroughly mixed in a separate container, for example beaker or bucket prior to dispensing into a sample container.

5.3 Thickened sludge, sludge cake and composted sludge

Sludge of thicker consistency containing greater solids content, may be sampled at the output of the thickening or de-watering process, for example as the sludge emerges from a sludge press or from a conveyor belt system.

Alternatively, for caked sludge and compost where its storage over a period of time may form part of the treatment process, then appropriate samples should be taken from the stored material. Samples should be taken from below the surface of the stored sludge using for example, a spade, auger or 'corer' device. The surface layer comprising approximately 100 mm of 'crust' should be discarded, and the sample taken at a depth of approximately 100-300 mm. To be representative of all of the stored material, several samples should be taken randomly from within the sludge and the results of analysis compared. The regulations[5] require samples of sludge to be taken from at least 5 randomly selected locations of the batch to be tested.

In certain circumstances, for example operational or investigation purposes, practical and safety factors may need to be considered and a systematic approach adopted. Samples may need to be

taken at locations equidistant along the width or circumference of the stored material.

Taking composite samples can be more difficult due to inadequate integration or mixing of the sludge to be analysed. Whilst adequate mixing may be achieved using a mechanical blending device, attempts at manual mixing are likely to be less effective. The kneading of material, for example in a plastic bag, should be avoided since there may be a risk that sharp objects might be present in the sludge.

5.4 Lime-treated sludge cake

Depending on the treatment process, lime-treated sludge samples may be collected at the end of the liming process or from the stored material after the appropriate storage time interval. Recording of on-site readings, measurements or determinations is important for CCP monitoring. For example, the treatment specification may dictate that sampling or batch release approval should not occur until after the sludge has cooled to below 40 °C. When the correct temperature has been attained, the principles of sampling lime-treated sludge cakes are similar to those described in section 5.3.

5.5 Thermally dried sludge

Thermally dried sludges generally possess very low water contents and sampling and storage procedures should take this into account in order to ensure that the product does not change significantly after treatment. Thermal drying processes generally produce a product that is of a pellet or granular character and is often bagged or packaged for transport in quantities of up to a tonne. Usually, it is possible to sample the sludge directly into a sample container from an in-line hopper or hatch in the pipe-work leading to the bagging silo. The layout of such treatment processes is, generally, location-specific, and for regulatory purposes, the sampling point should be selected on the basis of the HACCP assessment.

Composite samples of thermally dried sludges are not difficult to obtain and may, for example, comprise time-interval grab samples or quantities extracted from different bags or packages. For this purpose, a clean dry bucket or similar vessel may suffice for the initial collection, and a suitable container, for example a plastic bag, may be required for mixing the samples. The efficacy of mixing to produce an homogenised composite sample is difficult to assess, but with care, it can reasonably be assumed that adequate mixing is achievable.

6 Transport and storage of samples

Samples for microbiological analysis should be stored (immediately after being taken) in the dark and at temperatures between 2 - 8 °C. Samples should be transported to the laboratory in for example, insulated cool boxes, small refrigerators or refrigerated vans. These boxes etc should be used exclusively for this purpose and should be kept clean and dry, particularly the inside surfaces, and should be regularly disinfected. Van storage racks or boxes should be treated similarly. Samples should be delivered to the laboratory without delay so that, wherever possible, analysis can commence the same day.

In general, samples should be analysed as soon as practicable, preferably on the day of collection. In exceptional circumstances, for example, if there is a delay, storage under the above conditions should not exceed 24 hours before commencement of the analysis. If samples are not analysed immediately on receipt within the laboratory, they should be kept at temperatures between 2 - 8 °C, in the dark until analysis can begin. It may be necessary to note details of the storage time and temperature as well as the time the analysis commences.

To date, there are no published data on the effects of storage (in terms of time and temperature) and commencement of analysis on sludge microbial populations. Many sludge samples contain large and diverse populations of micro-organisms. In certain cases, the microbial populations to be enumerated may have been exposed to intensely antagonistic environments, and consequently may be in a poor physiological condition. The potential on-going disinfectant activity of lime treatment on sludge is typical of these environmental conditions. There is, therefore, the potential for changes to occur within the sludge sample between the time the sample is taken and the commencement of the analysis. These changes may be influenced by a variety of factors. Clearly, the longer the delay between sampling and commencement of analysis, the more likely that changes will occur within the sample.

It may be necessary to store samples until after the analysis is complete and the results have been reported. If a repeat analysis of a retained sample is required, the enumerated counts obtained for samples stored in this way may not be comparable to those obtained in the originally submitted sample. The number of organisms in a sample is liable to change over a period of time and under the conditions of storage. If results of a retained sample are to be reported, supportive data should be available to demonstrate comparability of results.

For thermally dried sludges that contain very low water contents (i.e. dry solids contents greater than 90 %) it may be that samples are sufficiently stable when stored in air-tight containers at temperatures between 20 ± 5 °C. Deterioration can reasonably be expected to be minimal provided moisture is excluded. However, the validity of this assumption would need to be demonstrated before applying it to individual sludges.

7 Choice of analytical methodolgy

Factors, such as the purpose of the analysis, the magnitude of the enumerated count expected and the character and dry solids content of the sludge may all have a bearing on the choice of methodology to use. These factors should be clearly understood since they affect the amount of sample, the method of sub-sampling and procedures for homogenisation and dilution.

Crude, primary settled and thickened sludges, and some conventionally treated sludges yielding limited microbial reduction might be expected to contain *E. coli* counts of up to 10^6 per 100 g wet weight of sample. For these types of sample, a membrane filtration technique or defined substrate most probable number technique may be applicable. For enhanced treated sludges where low counts of *E. coli* (up to 100 cfu per 100 g dry weight of sample) might be anticipated, a multiple tube most probable number technique capable of accommodating the presence of large amounts of solids may be more appropriate.

For membrane filtration, the presence of large amounts of solids in the initial homogenised suspension or homogenate and in lower dilutions may inhibit or obscure colonies growing on the membrane filter. However, this should not be a problem with higher dilutions that may also be tested.

Where sludges have been treated with chemicals such as lime, or are exceptionally dry, as are sludges produced by the thermal drying process, sample pre-treatment and preparation such as lime-neutralisation or re-hydration, may impose additional constraints on the procedures followed. In addition, for analyses requiring an indication of the presence or absence of particular organisms, for example *Salmonella*, it may be necessary to adopt a different approach during sample pre-treatment and preparation.

Another important factor for consideration in the choice of methodology is the physiological state

of the target organism in the sludge. In particular, a method may need to be specifically designed with reference to maximising the recovery of sub-lethally damaged organisms. Sludge that has been subjected to treatment involving heating, drying and chemical treatment may give rise to sub-lethally damaged organisms, thereby requiring the use of resuscitation media. Recovery of sub-lethally damaged *E. coli* is more likely to be enhanced in a liquid medium, such as lauryl tryptose broth (in the multiple tube method) as opposed to growth on the surface of a membrane filter. Similarly, the use of pre-enrichment media for *Salmonella* and *E. coli* O157 promotes the recovery of these injured organisms. In multiple tube most probable number methods, the presence of inhibitory or toxic substances in treated sludge may give rise to situations whereby growth is inhibited in the lower dilutions but not in the higher dilutions.

8 Sample pre-treatment and preparation

8.1 Introduction

Sludge matrices are diverse in character and samples may originate from conventional biological treatment processes or from enhanced treatment processes. Conventional biological treatment processes include mesophilic stabilisation, anaerobic mesophilic digestion, aerobic thermophilic digestion and composting, and enhanced treatment processes, typically, include the mixing of sludge with slaked lime or quick lime, or non-chemical treatment processes using heat and drying treatments. Micro-organisms are not uniformly distributed in sludge, and particular attention is therefore required to select techniques, appropriate to the matrix, that disperse and homogenise the sludge to produce a stable suspension for subsequent examination. The use of one or more of these techniques is essential for accurate and reproducible enumeration of counts in sludges.

Some of the practical problems that can be expected with particular sludge matrices include the aggregation or clumping of organisms in association with solid matter, the need to neutralise lime with acid and the re-hydration of thermally dried sludges. To some extent, organisms in the centre of aggregates, granules or pellets may receive a measure of protection in some treatment processes. Suitable procedures for specific micro-organisms are included in appropriate booklets in this series.

Laboratory surfaces should be kept clean at all times and, ideally, to avoid cross-contamination, separate areas should be used for the preparation of samples and for analytical work. Since sewage sludge may contain pathogenic micro-organisms it is vital that any spillages are dealt with immediately using recommended laboratory protocols.

8.2 Sub-sampling for analysis

Sub-sampling for analysis is the process by which the analytical unit (amount of sample to be used for analysis) is obtained from the sample submitted to the laboratory. After thorough mixing, the required quantity of sample (analytical unit) is weighed as soon as possible into a sterile container. Transfer of the sample can be accomplished using a sterile implement such as a pipette, spoon or spatula. A known amount of medium or diluent, usually maximum recovery diluent is then added to the container which is then sealed or capped. The sludge suspension is then thoroughly mixed and homogenised. If the same spoon or spatula is to be used for different samples, it should be sterilised, for example by dipping it into alcohol or industrial methylated spirits, and flamed and then allowed to cool before being used on a different sample. In addition, a sufficient quantity of homogenised suspension or homogenate should be prepared to enable all tests to be carried out.

Depending on the nature of the samples, there may be substantial variation in the distribution of the test organism within each sample. Since only a small amount of the whole sample is required for

microbiological analysis, it is important that the sample is prepared for analysis in such a way as to maximise the possibility of obtaining a representative result.

For semi-liquid samples, the material should be thoroughly mixed, typically, by shaking or stirring, to re-suspend any settled or floating material before removing the amount of sample (analytical unit) required for analysis. Effective mixing will only be achieved if the sampling container is sufficiently large, allowing adequate air space above the sample. If the original container is too full, consideration should be given to transferring the sample to a more suitable, larger container. This container should then be resealed and the sample thoroughly mixed before removing the analytical unit. Care should be taken to avoid any spillage or leakage when carrying out this procedure. In addition, allowance may need to be made for any dilution of the sample that is made, if the original smaller, sample container is washed and rinsed with diluent and the washings transferred to the larger container.

Wet sludges may be mixed effectively by stirring the whole sample thoroughly with a sterilised spatula. Alternatively, a homogenised suspension or homogenate may be produced by blending the sample, aseptically, for example, in an appropriate blender, at 5000 rpm for 30 seconds.

Once thoroughly mixed, liquid and semi-liquid samples may be poured, pipetted or transferred using a sterile spoon or spatula. The appropriate quantity of sample (for example 10 g wet weight of sludge) should be weighed (as soon as possible after mixing is completed) into a sterile container. As directed in the specific method, an amount of medium or diluent, usually maximum recovery diluent should be added to the container, which is then sealed or capped and well shaken and homogenised. The weighing procedure should be carried out aseptically whether a weighing boat is used or the sample is weighed directly into a stomacher bag or other suitable container. Normally, a calibrated balance capable of weighing accurately to ± 0.1 g is required.

For solid samples, including caked, thickened, thermally dried sludges and composts, it may be that a variety of measures are required in order to achieve adequate initial mixing. Dried and caked sludges vary in nature, and whilst some are relatively moist and friable, others are hard and unyielding. Composts are typically moist and friable, but may contain extraneous materials such as twigs and hard plant matter. For soft (semi-solid) and hard caked sludges and composts it is important that the entire sample is homogenised, not just a fraction of it. Soft caked sludges and composts may be transferred to a stomacher bag placed inside another stomacher bag and crushed using a suitable heavy object to aid the homogenisation process. Hard caked sludges, pressed sheet sludges and granules and pellets may need to be aseptically macerated, crushed or ground to facilitate homogenisation. When a laboratory mill is used, the manufacturer's instructions should be followed to ensure that mechanical damage to organisms is minimised.

Typically, enumerated sludge counts require a reported result to be expressed as the number of colony forming units of the test organism per g dry weight of sample. When ascertaining the appropriate quantity of sample (analytical unit) to use, it may be useful to know the typical dry solids content of each sludge matrix for each site regularly sampled. This may be particularly useful, where the equivalent of 2 g of dried sludge may be required for analysis, but where only a wet sample can be provided.

8.3 Pre-treatment

Pre-treatment of most samples will simply involve thorough mixing and homogenisation of the analytical unit of sludge with the appropriate volume of medium or diluent. Typically, this involves the aseptic addition of 90 ml of sterile medium or diluent to 10 g (analytical unit) of sample in a suitable container. This is often referred to as the initial 1 in 10 dilution, (or -1 dilution).

For certain matrices such as thermally dried sludges, it may be useful to allow the sludge to remain in contact with the medium or diluent for a period of time in order to re-hydrate the sludge prior to homogenisation. In such cases, the procedure would involve soaking the sludge in medium or diluent for approximately 30-60 minutes prior to homogenisation.

Lime, in some form, may be added to sludge as part of the treatment process in order to reduce microbial numbers or to dry the sludge by chemical means. Such treated sludge may still be alkaline, typically possessing a pH value of about 12, when sampled. Analytical methods for *E. coli* often depend on the demonstration of the production of acid from lactose, during incubation. This process may be masked or inhibited altogether, if the lime is not neutralised to a pH value of 7.0 ± 0.2 prior to commencement of the method. Neutralisation may be carried out after the initial dilution and homogenisation process (see section 8.5).

8.4 Homogenisation

8.4.1 General

A variety of physical treatment processes to achieve homogenisation are available and several of the more frequently used and emerging techniques are briefly described. The technique used should be the gentlest but effective technique commensurate with the nature of the sample and the need to produce a homogenised matrix for analysis. The approach adopted in most methods described in this series is based on the use of the stomaching process. Techniques involving ultra-sonication, blending and shaking are also capable of de-sorbing micro-organisms from particles of sludge. These techniques, with varying degrees of efficiency, facilitate the break up of the sludge into smaller particles. No single technique is better or worse than another, as all possess advantages and disadvantages. The choice of technique will depend on practical considerations for the particular sludge matrix under test. Where novel approaches are required, each laboratory should establish defined and reproducible conditions (supported by validation data) for homogenising the sludge to ensure uniform recovery of targeted micro-organisms.

Some of the techniques, particularly ultra-sonciation, may be harmful to micro-organisms and in addition, generate a heating effect within the sample. Hence, excessive application of the technique may cause injury or even death to the organisms. Optimum conditions should be established.

The addition of detergents, for example Tween 80, may facilitate dispersion of particles and micro-organisms within the sludge by preventing the aggregation or clumping of particles once treated by the physical process. The addition of other chemical agents, such as ethylenediaminetetraacetic acid may also ensure microbial populations remain stable by overcoming for example toxic or inhibitory effects due to heavy metals present in the sludge. The efficacy of such procedures should, however, be fully validated.

8.4.2 Manual and mechanical mixing

Manual or simple mechanical mixing is often the most accessible and universally applicable technique available for homogenising samples prior to microbiological analysis. This approach has however severe limitations for most sludge matrices where some form of aggregation of material is usual. Mixing, by these methods, should be carried out in suitably robust sealed containers. In addition, the sample may foam during mixing and particular care is required when removing container lids. Furthermore, aerosols may be generated and consideration should be given to any additional precautions that may need to be taken to prevent exposure to biological hazards, for example by inhalation.

Manual mixing involves shaking the sample, or diluted sample, in a suitable sealed container. To achieve reproducibility, a defined period of time and number of inversions of the container may need to be specified.

Mechanical mixing involves automated or semi-automated agitation of the sample in a sealed container. For example, proprietary laboratory equipment such as shakers, orbital shakers and vortex mixers may be used. Manufacturer's instructions should be followed at all times and, as with manual mixing, protocols may need to specify instrument settings such as speed and duration of action.

8.4.3 Stomaching

Stomacher instruments are commercially obtainable and several types are available. The basic principle by which these instruments operate is to pulp or mash the diluted material in a strong sterile plastic bag. The bag and its contents are repeatedly squashed and mixed. The instruments available generally differ in the speed and timing options available. However, lower speeds for short periods of time have been found suitable. Manufacturer's instructions should be followed, where applicable.

For certain sludges, it may be useful to enclose the sample in more than one stomacher bag. This may be appropriate when samples contain extraneous matter for example, grit, twigs and other sharp objects etc that might rupture or damage the bag, if only one was used. The use of two bags reduces the risk that sludge and other liquid will be spilled and the sample lost.

Protocols for transferring the analytical unit of sludge to the stomacher bag vary[22]. For example, the amount of sludge (analytical unit) should be weighed into a sterile container and from a total volume of 90 ml of medium or diluent (usually maximum recovery diluent) approximately 50 ml of medium or diluent should be added to the container and the contents thoroughly mixed. The mixture should then be transferred to a stomacher bag. (The container may then be rinsed with a small volume of the remaining medium or diluent and the washings transferred to the bag.) After appropriate stomaching, the homogenate should be transferred back to the original container and the bag rinsed with the remaining amount of medium or diluent and the washings transferred to the container, and mixed. This suspension constitutes the initial 1 in 10 dilution (or -1 dilution).

Alternatively, the analytical unit may be weighed directly into a stomacher bag and 90 ml of medium or diluent added to the bag, prior to stomaching. If necessary, it may be beneficial, especially if the sludge is particularly hard, to allow the macerated sludge to soak in the medium or diluent for 30-60 minutes prior to stomaching. After stomaching, the bag should be removed from the stomacher and, if necessary, supported to prevent the contents from spilling out before transferring the homogenised suspension to a sterile container. This suspension constitutes the initial 1 in 10 dilution (or -1 dilution).

Different styles of stomacher bags are available and some enable larger particles to be strained out after homogenisation is complete. The use of stomacher bags containing an internal mesh insert are beneficial in membrane filtration methods[23] for enumerating *Salmonella* and verocytotoxigenic *E. coli*. This insert helps to remove particulate matter that might otherwise interfere with subsequent procedures. This type of bag may also reduce the risks of the bag puncturing, for example where samples contain sharp objects or similar materials.

8.4.4 Blending

Laboratory blenders consist of wide-mouthed, robust vessels, typically stainless steel, with lids. Incorporated into the vessel are internal, sharp, rotating blades that are activated by placing the vessel on an external base unit. The base unit contains an electrical motor that provides the driving force for rotation. Blenders can provide a rapid and effective means of homogenising sludge samples containing particulate, aggregated and fibrous material. Stainless steel blender-vessels should be sterilised between use when different samples are homogenised. The vessel should first be thoroughly washed and then autoclaved or totally immersed in boiling water for 5 minutes. The blender speed and blending time should be varied to suit individual samples, and settings should be validated to establish optimum conditions for the sludge matrix under test.

8.4.5 Pulsification

A "pulsifier" is a recently devised sample preparation instrument that uses a metal ring to apply a high frequency vibration to a bag containing diluted sample. In contrast to the stomaching technique, the "pulsifier" does not pulp or crush the sample, but operates by a combination of shock waves and intense stirring. This process releases micro-organisms into suspension in a way that causes little destruction of the sample matrix. Data[24] for food matrices suggests that recovery of organisms using this technique is comparable to the recovery of organisms using the stomaching technique. Potential advantages of the "pulsifier" technique include less suspended debris and fewer problems with puncturing of sampling bags. To date, the technique has not been used widely for sewage sludge and there is some evidence that foaming, emulsification and fine particle suspension may be a cause for concern and lead to poor recoveries for some sludge matrices[23].

8.5 Dilution

For the enumeration of *E.coli* in sewage sludge by membrane filtration, dilution (with sterile medium or diluent, usually maximum recovery diluent) of the initial homogenised 1 in 10 (-1 dilution) suspension will be necessary. The volumes and dilutions of samples should be chosen so that the number of colonies to be counted on the membrane filter lies, if possible, between 20-80. Decimal dilutions should be prepared by transferring, for example 1 ml of the initial (1 in 10) homogenised suspension to 9 ml of medium or diluent. This diluted suspension is then thoroughly mixed. This procedure is repeated, producing further similar dilutions, to give a dilution series that is considered sufficient to accommodate the expected enumerated count. A fresh, clean sterile pipette should be used between each dilution step when preparing the dilution series. The same apparatus, and pipettes, may however be used for the membrane filtration procedure provided the diluted suspensions are tested in increasing order of concentration, i.e. the most dilute suspension is processed first. Occasionally, it may be useful to prepare intermediate dilutions, such as a 1 in 2 dilution, to enable counts in the expected range to be determined correctly. For example, aseptically add 5 ml of one diluted suspension to 5 ml of sterile medium or diluent.

For multiple tube most probable number methods, it is essential to prepare a dilution series where some of the tubes exhibit characteristic growth within the medium and other tubes do not, i.e. results that are regarded as positive and negative, rather than being all positive or all negative. The "best" estimate (in statistical terms) will be given when approximately half the tubes exhibit characteristic growth within the medium, i.e. are positive. For sample quantities of less than 0.1 ml or 0.1 g, serial dilutions may be prepared from the initial homogenised suspension.

Lime-treated sludge should be neutralised before the dilution series is prepared. Neutralisation may be carried out, after the initial 1 in 10 homogenised suspension has been prepared, using the stomaching technique[25] described in section 8.4.3.

Using a calibrated pH meter and a thoroughly cleaned probe that has been wiped with alcohol-based disinfectant, measure the pH of the homogenised suspension. Record this value, which may be used with the amount of added lime (if known) to determine the quantity of acid required for neutralisation. In small aliquots, carefully, add 2N hydrochloric acid to the homogenised suspension. Thoroughly mix (by vigorous shaking) the homogenised suspension and measure the pH again. Repeat this procedure until the pH value is 7.0 ± 0.2. If excess acid is added and the pH falls below 7.0 ± 0.2, the pH can be adjusted to 7.0 ± 0.2 using small aliquots of 2N sodium hydroxide. It may be useful for later reference to record the volume of acid required. Further details on the preparation of diluted suspensions are given in specific methods in this series.

9 References

1. Standing Committee of Analysts, The sampling and initial preparation of sewage and waterworks' sludges, soils, sediments, plant materials and wildlife prior to analysis 1986 (Second Edition), *Methods for the Examination of Waters and Associated Materials*, in this series, London, ISBN 0117518832.

2. General principles of sampling waters and associated materials (Second Edition) 1996; Estimation of flow and load (1996). *Methods for the Examination of Waters and Associated Materials*, in this series, London, ISBN 011752364X.

3. Standing Committee of Analysts, The Microbiology of Drinking Water (2002) - Part 2 - Practices and procedures for sampling, *Methods for the Examination of Waters and Associated Materials*, in this series, Environment Agency.

4. BS EN ISO 5667 - 13: 1998 Water Quality - Sampling - Part 13: Guidance on sampling of sludges from sewage and water treatment works.

5. Proposed regulations, The Sludge (Use in Agriculture) (Amendment) (England and Wales) Regulations.

6. Standing Committee of Analysts, The Microbiology of Sewage Sludge (2003) - Part 1 - An overview of the treatment and use in agriculture of sewage sludge in relation to its impact on the environment and public health, *Methods for the Examination of Waters and Associated Materials*, in this series, Environment Agency.

7. *Review of the Scientific Evidence relating to the Controls on the Agricultural Use of Sewage Sludge. Part 1. The Evidence Underlying the 1989 Department of the Environment Code of Practice for Agricultural Use of Sludges and the Sludge (Use in Agriculture) Regulations.* DETR, 1998.

8. *Review of the Scientific Evidence relating to the Controls on the Agricultural Use of Sewage Sludge. Part 2. Evidence since 1989 Relevant to Controls on the Agricultural Use of Sewage Sludge.* DETR, 1998.

9. Council Directive on the protection of the environment, and in particular the soil, when sewage sludge is used in agriculture. 86/278/EEC. *Official Journal of the European Communities.* 4.7.86 No.L 181/6-12.

10. Proposed Code of Practice: "*Code of Practice for the Agricultural Use of Sewage Sludge".* Department for Environment Food and Rural Affairs, in preparation.

11. *The Safe Sludge Matrix*. Guidelines for the Application of Sewage Sludge to Agricultural Land. 2001, Agricultural Development and Advisory Service, Water UK and the British Retail Consortium.

12. The HACCP concept and microbiological hazard categories. *Food Technology*, H E Bauman, 1974, **28** (9), pp30-34.

13. HACCP: Concept, development and application. *Food Technology*, H E Bauman, 1990, **44** (5), pp156-158.

14. HACCP Group - Draft Procedures for Demonstrating 2/6 Log Reduction across a Sludge Treatment Process. Water UK, 5 April 2001.

15. Micro-organisms in Foods. Volume 2. Sampling for Microbiological Analysis: 'Principles and Specific Applications', Second edition, Blackwell Scientific Publications, Oxford, ICMSF, 1986.

16. Standing Committee of Analysts, The Conditionability, Filterability, Settleability and Solids Content of Sludges (1984) A Compendium of Methods. *Methods for the Examination of Waters and Associated Materials*, in this series, London, ISBN 0117517879.

17. Standing Committee of Analysts, The Microbiology of Drinking Water (2002) - Part 3 - Practices and procedures for laboratories, *Methods for the Examination of Waters and Associated Materials*, in this series, Environment Agency.

18. This text is based on Resolution 74 by CEN TC 292 - Wastes - Working Group 5, the agreed text of which was adopted by CEN TC 308 - Characterisation of sludges - for the section on "General Hazards" associated with sludge material and waste.

19. The Control of Substances Hazardous to Health Regulations 1999, Statutory Instrument 1999 No. 437.

20. The management, design and operation of microbiological containment laboratories, 2001, Advisory Committee on Dangerous Pathogens.

21. Categorisation of Pathogens According to Hazard and Categories of Containment, Fourth edition (1995) and supplement (1998) Advisory Committee on Dangerous Pathogens.

22. *E. coli* in UK Mesophilically Anaerobically Digested Sludges. UKWIR Report Reference No. 99/SL/06/4, 1999.

23. Methods for the detection and Enumeration of Pathogens in Biosolids. UKWIR Report Reference No. 00/SL/06/5, 2000.

24. The Pulsifier®: A New Instrument for Preparing Food Suspensions for Microbiological Analysis. *Journal of Rapid Methods and Automation in Microbiology*, D Y C Fung, A N Sharpe, B C Hart and Y Liu, 1998, **6**, pp43-49.

25. A Survey of E. coli in UK Sludges. UKWIR Report Reference No. 99/SL/06/3, 1999.

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However well procedures may be tested, there is always the possibility of discovering hitherto unknown problems. Analysts with such information are requested to contact the Secretary of the Standing Committee of Analysts at the address given below.

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