ENVIRONMENT AGENCY

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

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

This booklet provides an overview (from a microbiological perspective) of the practices and procedures for the treatment and use of sewage sludge for agricultural purposes and how it impacts on the environment and public health.

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

Part 2 - Practices and procedures for sampling and sample preparation
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 S*almonellae*

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 this 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 on 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

January 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.

Glossary

Biosolids	A diverse range of treated sewage sludges, suitable for soil conditioning, which may be capable of supporting microbial activity
BOD	Biochemical oxygen demand
CCP	Critical control point
Consolidation	Mixing and concurrent thickening of sludge derived from different parts of a sewage treatment process
De-watering	A process whereby the liquid content of sludge is reduced
E. coli	*Escherichia coli*
Fermentability	A property of sludge consequent on nutrient content and toxicity that relates to the quality and quantity of microbial activity promoted under suitable conditions
HACCP	Hazard analysis critical control point
Log reduction	A decrease in numbers (by a factor of ten) that equates to a difference (of one) between the numbers, when transformed to logarithms to the base 10.
MAD	Mesophilic anaerobic digestion
Parasite	Any organism that is dependent on a host, being incapable of independent self-replication
Pathogen	Any micro-organism capable of causing disease in a specific host or group (for example, mammals, primates, cereals etc.) of host organisms
Primary digestion	A treatment process whereby sludge is retained in a tank or reactor under conditions suitable for microbial activity to take place
Secondary digestion	The result of continued microbial activity during storage of sludge following primary digestion
Sewage	The combined flow of wastewater from domestic, social and permitted industrial premises together with infiltration and storm run-off gaining access to the public sewer
Sludge	A liquor containing solids generated as a by-product of sewage treatment
UK	United Kingdom
US EPA	United States Environmental Protection Agency

An overview of the treatment and use in agriculture of sewage sludge in relation to its impact on the environment and public health

1 Introduction

1.1 Scope and objectives

Untreated sewage sludge contains a wide variety of pathogens, including bacteria, viruses, fungi, and cysts and eggs of parasites. These pathogens can potentially pose a risk to the environment, public health and food safety when sludge is applied to agricultural land. Standards for the treatment of sewage sludge are proposed in regulations[1] and guidance on best practice for its beneficial use is described in a code of practice[2] covering the use of sludge in agriculture. The characteristics of sewage sludge will vary according to the nature and source of the raw sewage effluents, season of the year, geographic location and the treatment processes applied. These characteristics will also be subject to continual change over short periods of time.

The aim of this document is to provide guidance to those with responsibility for such matters within the water industry, public health, environment and other agencies and organisations on the significance of pathogens in sewage sludge and on the procedures for reducing their numbers by treatment. Particular emphasis is directed towards the treatment efficacies prior to the beneficial use of sewage sludge, for example, the application, after treatment, of sludge to land for agricultural purposes as a source of nutrients. This document deals with wastewater sludge resulting primarily from human activities, and is not intended to address issues associated with the use of wastes and slurries derived from farm premises.

This document covers the following topics:

- procedures for the treatment of sewage with particular reference to sludge production;
- legislation relating to the application of sewage sludge to land used for agriculture;
- microbiological standards applied to sewage sludge for use on land;
- processes for the treatment, conditioning and consolidation of sewage sludge;
- significance of indicator organisms and pathogens in sewage sludge; and
- effects of sludge treatment processes on indicator organisms and pathogens.

The initial objective has been to provide an introductory context for the application of methods targeted at demonstrating compliance with proposed microbiological standards prescribed in impending UK[1] and European[3] legislation on the use of sewage sludge in agriculture.

Precise details of practices and procedures for sampling, sample preparation, and methods for the isolation and enumeration of *Escherichia coli* including verocytotoxigenic *E. coli* and *Salmonellae* are given elsewhere in this series[4, 5, 6].

1.2 Legislation and sewage sludge disposal

The use of sewage sludge in agriculture is regulated[7] in the UK and amendments[1] to these regulations are proposed, but may be subject to change as currently drafted. These regulations implement the provisions of the EU Directive[3] on the protection of the environment, and in particular soil, when sewage sludge is used in agriculture. The previous regulations[7] were supplemented by guidance contained in a code of practice[8] which has now been incorporated into new guidelines[9]. A revised code of practice[2] (which may be subject to change as currently drafted) will provide supplementary guidance to the proposed regulations[1] which should be consulted before sewage sludge is applied to land. Advice to farmers on the use of sewage sludge

is available from appropriate government and non-government departments such as the Environment Agency, Scottish Environment Protection Agency, Agricultural Development and Advisory Service and the Scottish Agricultural Colleges. The regulations[7] and code of practice[8] were designed to ensure that when sludge is applied to land for agricultural purposes:

- there is no conflict with good agricultural practices;
- the long term viability of agricultural activities is maintained;
- public nuisance and water pollution are avoided; and
- human, animal and plant health is not put at risk.

The proposed regulations[1] will require sludge to be treated before being applied to land for agricultural purposes. The aim is to significantly reduce potential health hazards. Sludge will also need to be classified into one of three categories, namely untreated sludge, conventionally treated sludge or enhanced treated sludge.

Conventionally treated sludge (including septic tank sludge) is defined in the regulations[1] and includes a biological, chemical or heat treatment process to significantly reduce its fermentability and health hazards resulting from its use. This treatment process should ensure a 99 per cent (2 log) reduction in the detectable numbers of *E. coli* present in samples of sludge collected before treatment compared to those numbers detected after treatment, with a maximum allowable concentration of 100000 (10^5) per gram (dry weight). Enhanced treated sludge is defined[1] to ensure a 99.9999 per cent (6 log) reduction in the detectable numbers of *E. coli* before and after treatment with a maximum allowable concentration of 1000 (10^3) per gram (dry weight) and to ensure the absence of *Salmonellae*.

In order to minimise the risks to human, animal or plant health, sludge applications to land for agricultural purposes should be co-ordinated with planting, grazing or harvesting operations. Prior to application, local conditions, such as the potential pollution of surface and ground waters and surface run-off, should be considered and taken into account. Further guidance[9] provides advice and information on the use of sludge in agriculture in relation to the level of treatment and the crop under cultivation. Information is given on crop types and the minimum, acceptable levels of treatment for any sewage sludge-based product that may be applied to land. Scientific support for this approach[9] has been reinforced via the application of modelling techniques to predicted pathogen levels from the consumption of harvested root crops in relation to potential human exposure[10].

Briefly, the proposed regulations[1] place constraints on the disposal of different categories of sludge. Untreated sludge may no longer be applied to any land used for the production of food products, but may be spread on land used for the production of non-food or "industrial" crops until the end of 2005. Conventionally treated sludge may no longer be applied to land used for grazing purposes unless the sludge is injected or ploughed into the soil prior to re-seeding and, in the case of established grassland, where there is a three week period during which grazing or harvesting does not take place. In addition, conventionally treated sludge should not be used on land for growing fruit crops or for horticultural purposes. Enhanced treated sludge may be used for all crop groups, subject to compliance with the proposed regulations including harvesting and grazing limitations.

1.3 Objectives of sewage sludge treatment

Historically, sewage sludge treatment has been directed towards its physical characteristics by stabilising and thickening the sludge to facilitate its transport, storage and disposal. Secondary objectives of sewage sludge treatment relate to improving its physical properties, and to the

reduction of its fermentability and noxious character via digestion treatment processes. Stabilisation implies reduced susceptibility to further microbial breakdown. Microbiological safety in relation to sludge use on land for agriculture is addressed by a multiple barrier approach encompassing treatment, crop type and harvest period.

In relation to the application of sludge to land the focus of previous guidance has been directed to the control of toxic substances, in particular heavy metals, and the potential for their accumulation in soil over prolonged periods of time. It has been recognised that whilst the treatment of sludge reduces the numbers of pathogens present, those organisms surviving might still give rise to potential health hazards after the sludge is applied to land for agricultural purposes. This has lead to the introduction of time intervals between the application of sludge to land and grazing, cropping and harvesting operations that might be carried out following application. The scientific evidence for such controls on the agricultural use of sewage sludge has been reviewed elsewhere[11].

The proposed regulations[1] introduce new microbiological standards and monitoring requirements together with a hazard analysis critical control point (HACCP) approach to the control of sludge treatment processes. This approach derives from contemporary concerns for food safety. The HACCP approach depends on the identification of critical control points (CCPs) in the sludge treatment processes, such as temperature, time and pH and the monitoring requirements ensure that the microbiological standards required for the recycling or disposal route are satisfied.

2 Physical and microbiological characteristics of sewage sludge

2.1 Sources of sewage

Sewages comprise effluents derived from a variety of sources, many of which may contain pathogenic micro-organisms. These sources include effluents from abattoirs, hospitals, and food production premises, which may contain human and animal pathogens, and wastes from vegetable processing plants that may contain plant pathogens. Effluents from these sources augment the daily input of wastewater from domestic and commercial properties containing human faecal and other house-hold wastes. The presence of pathogenic organisms from these sources will relate to the general state of health of the population in the local catchment area, and during periods of heavy rainfall run-off may contribute to the effluent flow by adding contaminated soil, silt and grit at the same time.

2.2 Derivation of sewage sludge during sewage treatment

The primary purpose of sewage treatment processes is to minimise the polluting potential of the liquid waste which comprises the main bulk of sewage effluents. This is usually achieved through a combination of stages designed to remove solid material, and hence reduce the biochemical oxygen demand (BOD) of the resulting effluent. The treated liquid effluent is then discharged either to a nearby local watercourse or into coastal waters. The solid matter removed constitutes the sewage sludge, the production of which is thus an unavoidable consequence of the treatment process. Implementation of the Urban Wastewater Treatment Directive[12] is designed to improve the environment and produce better quality effluents. At the same time, the effect of increasing the proportion of sewage receiving treatment, increasing population and the raising of treatment standards has lead to an increase in the quantity of sludge being generated. In addition, the banning of certain disposal routes, for example disposal at sea, has lead to an increased need for alternative means of disposal[13].

Different sludge fractions are generated during various stages of the sewage treatment process. The sewage effluent enters the treatment works and is usually screened to remove coarse material and

other gross debris. The resulting effluent is then passed to primary settlement tanks where particulate matter settles out to produce primary settled sludge. The primary settlement process reduces the BOD of the liquid phase by removing fermentable solid matter along with inert material. The liquid phase, settled sewage, is then subject to an aerobic biological process that breaks down most of the remaining organic content. This is achieved via a number of processes, for example the filter-bed process or the activated sludge process.

In the filter-bed process, settled sewage is slowly passed over the surface of a substrate, usually consisting solely or in combination of clinker, stones or a synthetic plastic medium. Micro-organisms which grow on the surface of the substrate break down remaining organic matter in the settled sewage to produce a liquid phase possessing an even lower BOD. The thick biologically active mat generated on the surface of the substrate also adsorbs particulate material from the settled sewage and forms humus sludge. Pieces of this sludge continually break away or become detached from the substrate and thus elute out with the liquid phase. A further settlement stage is then used to separate the humus solids from the liquid phase. This secondary settled sludge may then be combined with the primary sludge and subjected to further treatment (see section 3).

In the activated sludge process, settled sewage is aerated to promote the breakdown of organic material by the natural activity of micro-organisms present in the sewage, thereby further reducing the BOD of the settled sewage. When the process works effectively, these micro-organisms form cohesive floccules that adsorb dissolved and suspended organic and particulate material. These flocculent solids (activated sludge) are then separated from the body of treated liquid by a secondary settlement stage. A portion of this activated sludge is retained within the process to provide a source of micro-organisms to help perpetuate, promote and maintain the treatment process. This secondary settled sludge may then be combined with the primary sludge and subjected to further treatment (see section 3).

A wide variety of different arrangements exist for the implementation of the treatment processes and some recent innovations combine biological treatment with fine membrane filtration to produce high quality effluents for discharge. Such arrangements increase the retention of solid material and hence the quantities of sludge produced.

2.3 Rationale of sewage sludge treatment and use on land

The motivation for using sewage sludge on agricultural land lies in its value as a nutrient-rich fertiliser and source of organic material. For several decades, controlled application of sewage sludge to agricultural land has been a major route for disposing of sludge. However, its use in this capacity is traditional, going back centuries. In many circumstances, recycling sludge by application to agricultural land is considered the best practicable environmental option. It is generally considered a beneficial practice, consistent with sustainable economic and environmental imperatives for the future.

Until the end of 1998 it was permissible under EU legislation[3] to dispose of sewage sludge at sea. This was previously the means of disposal of approximately 30 % of sludge produced in the UK, mainly derived from large coastal conurbations[13]. In 1996-7, sewage sludge in the UK was produced at an annual rate of over 1.1 million tonnes, based on dry solids, of which about 0.5 million tonnes (47 %) were applied to agricultural land. By 2005, the quantity of sludge is expected to rise significantly, as a greater proportion of sewage will be treated, as the population increases and more stringent treatment standards are prescribed. Recent predictions suggest that by 2005 the proportion of sludge used on land will rise to 60 %. Other options for dealing with sludge include incineration, gasification and land-filling. It is anticipated that these options may account for the disposal of 40 % of sludge by 2005, provided confidence in agricultural use is maintained. Disposal

to land-fill sites is likely to be the least favoured route since new regulatory constraints[14] will further impact on this practice and limit its application.

2.4 Environmental and public health hazards associated with sludge use

Sewage sludge should not be applied to land where there is a risk that it might pollute watercourses or water sources. The rate of run-off will be increased on frozen or near-saturated ground. In addition, percolation may occur via land drains or in dry, cracked soils, particularly after sludge injection. Sludge should not be stored or applied to land in the immediate vicinity of water supply sources and advice on the prevention of contamination of water is provided elsewhere[15].

The potential risks in relation to food safety arising from the inadequate control of sludge treatment and use are as follows:

- the ingestion of contaminated grass by grazing animals and the subsequent contamination of livestock used for food purposes;
- the contamination of crops such as salad ingredients which, although washed, may be consumed raw; and
- the contamination of adherent soil on root crops that may give rise to hazards in food handling areas.

In addition, facilities should be available for operators to change from or into their working clothes and to be able to wash themselves after working with sludge. Operators should also be encouraged to use such facilities before eating or drinking, and meals should be consumed well away from areas where sludge is handled. Notwithstanding the potential risks, in the UK, there have been no recorded incidents of illnesses resulting from the application of treated sewage sludges to agricultural land since the introduction of the 1989 regulations.

3 Processes for the treatment of sewage sludge

3.1 Introduction

Sludges can be treated in a variety of different ways. In practice, the type and degree of treatment will depend on local economic factors and the legal requirements that apply to the chosen route of usage. For example, economies in transport costs may be forthcoming following de-watering of sludges, odour problems can be controlled by the addition of lime, and heat treatment practices generally result in reductions in the pathogen content of sludges.

3.2 Preliminary treatment of sludge

Untreated sludge may be treated by reducing its water content (in order to reduce its volume) to facilitate further treatment or in preparation for transport. This process, referred to as thickening, can be achieved by pressing or centrifuging the sludge to produce a “cake”. In some cases, thickening is promoted during settlement by the addition of selected polymer resins that then form part of the sludge and affect its physical characteristics. Sludge cake may then be treated with lime, see section 3.3.6.

3.3 Summary of procedures used for treatment of sewage sludge for land use

The following treatment processes, which are not exhaustive, briefly describe procedures considered to be effective using the US EPA criterion of giving a 90 % reduction in numbers of *Salmonellae* and infectivity of *Taenia saginata* eggs to calves[16]. This work is based on published

studies and data from UK sewage treatment works[11].

3.3.1 Sludge pasteurisation

Whilst specific conditions may differ as a result of treatment designs and configurations, sludge is heated at a minimum temperature of 70 ^{o}C for at least 30 minutes or a minimum of 55 ^{o}C for at least 4 hours. Appropriate intermediate conditions may also be used. Whilst heat treatment reduces the pathogen content of sludge, the process does not address sludge fermentability and hence, heat treatment is not used alone. Subsequent treatment, for example by mesophilic anaerobic digestion, is required.

3.3.2 Mesophilic anaerobic digestion

Mesophilic anaerobic digestion is carried out under anaerobic conditions in a digester maintained either at 35 ± 3 ^{o}C for at least 12 days or at 25 ± 3 ^{o}C for at least 20 days. Digesters are usually operated on a near-continual basis employing a "draw and fill" system that minimises short-circuiting on addition of untreated sludge. This process stabilises the sludge, and reduces its fermentability and the potential for odour nuisance problems to occur. When followed by secondary digestion at ambient temperature for periods usually in excess of 14 days, this treatment is effective at reducing numbers of pathogens.

3.3.3 Thermophilic aerobic digestion

Thermophilic aerobic digestion is usually carried out by aeration of the sludge with air or oxygen. This process promotes biological oxidation of the sludge and generates heat. Temperatures in the range 40 - 70 °C can be maintained, and in practice digestion of the sludge should be achieved in about 7 days, i.e. a mean retention time of at least 7 days. For pathogen reduction, the sludge should be held at a minimum of 55 °C for at least 4 hours. Adequate mixing of the sludge is required in order to ensure all the sludge is exposed to the required conditions.

3.3.4 Composting

In this procedure, de-watered sludge is first mixed with bulking products such as straw, wood-chip or sawdust. The organic matter in the sludge is then broken down under aerobic conditions by the action of thermophilic bacteria. This is an exothermic process that generates sufficient heat to significantly reduce the numbers of pathogens in the sludge. Two processes are generally used in the UK, namely the windrow process and aerated static pile process. The compost should be maintained during this period at 40 ^{o}C for at least 5 days with a minimum temperature of 55 ^{o}C being maintained for at least 4 hours. The relatively short thermal stage should be followed by a longer "maturation" stage for completion of the composting process.

3.3.5 Vermiculture

This treatment process which is rarely used in the UK is a form of composting that depends on the activity of worms to breakdown and modify the character of the sewage solids.

3.3.6 Lime stabilisation of liquid and caked sludge

Lime is added to sludge to produce a pH value of at least 12 and these conditions are maintained for at least 2 hours. These conditions produce a bacteriostatic effect, which can reduce during storage over a period of time due to decreasing pH. Thus, it may be not advisable to store the product for more than a few days before being applied to agricultural land. This will depend on specific sites

and sludge properties, as a potential for re-growth or re-infection may arise.

3.3.7 De-watering and storage

In this process, untreated sludge or sludge that has been treated by primary mesophilic anaerobic digestion is first treated, or "conditioned", with lime and/or coagulants. A de-watering process follows in which solid matter is mechanically separated from most of the liquid. De-watering may involve plate pressing or belt pressing, vacuum filtration or centrifugation to produce a sludge "cake". Cake produced in this way from untreated sludge should be stored for at least 3 months, and cake produced from digested sludge may require 14-day storage to achieve treated status.

3.3.8 Liquid sludge storage

Untreated liquid sludge is stored in lagoons, tanks or cold digesters at ambient temperatures for at least 3 months. During the natural microbial process, digestion, competition, predation and degradation processes take place both aerobically and anaerobically and solid material settles out. This process produces sludge of a more stable character, possessing reduced fermentability and pathogen numbers.

3.3.9 Thermal drying

Raw or digested sludge is thickened before being passed through a baffled drying chamber. Whilst in transit through the chamber, the sludge is exposed to air which is heated to temperatures in excess of 300 °C. During this heating process, the sludge, which is dried to a granular consistency possessing a dry matter content of greater than 80 %, exits the drying chamber at temperatures in excess of 80 °C. The moist air mixture, comprising the heated air and the water from the sludge, is separated from the dried sludge after leaving the drying chamber and either re-cycled to the heat exchanger or used for combustion purposes in heater burners. Alternatively the water vapour is condensed. Further processing of the dried sludge is then undertaken to produce a product, in granular or pellet form, of a particular size.

3.4 Microbiological standards applied to treated sewage sludge

The proposed regulations[1] and revised code of practice[2] prescribe and give guidance on microbiological standards for the monitoring of treated sludge products. The regulations propose that sludge producers will be required, in advance of this monitoring, to identify CCPs in the treatment processes used to show these conditions will satisfy the required reductions in the numbers of *E. coli* present in the sludge, see section 1.2. Monitoring requirements will apply to sampling events initially set at monthly intervals. For each sampling event, five random samples should be taken and analysed. For enhanced treated sludge, there is provision for reducing the sampling frequency to quarterly intervals, if results have shown consistent compliance for a period of six months. Sampling practices and procedures are described elsewhere within this series[4]. The code of practice advises that for novel treatment processes, the manufacturer should demonstrate the efficacy of the process in respect of other pathogens such as *Campylobacter*, *Salmonella*, *E. coli* O157:H7, *Listeria*, *Cryptosporidium* and enteroviruses. Initial discussions on revising the EU directive[3] include proposals that the efficacy of certain treatment processes be demonstrated.

3.4.1 Conventionally treated sludge

The proposed regulations require that conventionally treated sludge should not contain *E. coli* above an upper limit of 100000 (10^5) *E. coli* per gram dry weight of sludge based on a percentile criterion, i.e. a permitted maximum number of failed samples is allowed. In addition, a maximum

allowable concentration of 10^7 *E. coli* per gram of dry solids will be required. In future, the maximum allowable concentration will be 10^5 *E. coli* per gram dry weight of sludge.

3.4.2 Enhanced treated sludge

The maximum allowable concentration for *E. coli* in enhanced treated sludge will be 1000 (10^3) per gram of dry solids. In addition, *Salmonella* should not be present (expressed on a dry weight basis) in five random samples (each of 2 g).

3.5 Examples of sludge treatment performance

3.5.1 Summary of performance

The following table shows data obtained at operational treatment facilities based on a reported UK national survey[17]. The table shows the reduction in counts before and after treatment expressed as the mean difference in log-transformed counts before and after treatment and the mean log-transformed count after treatment. Both values are expressed on a dry weight basis.

Sludge treatment	Number of sites	Number of samples	Mean log-transformed Reduced count before and after treatment of *E.coli* per 100 g of sample (based on dry weight)	Mean log-transformed count of *E.coli* per 100 g of sample after treatment (based on dry weight)
Lagooning	2	36	2.65	5.93
MAD, liquid *	14	208	1.39	7.41
MAD, cake	5	93	2.29	6.65
Vermiculture	1	14	5.12	4.50
Composting	2	31	6.71	2.43
Lime addition	3	32	7.10	1.45
Thermal drying	4	70	7.14	1.67

***** After primary digestion

3.5.2 Performance conclusions

The following table shows data from additional studies[18]. Initial *E.coli* counts were in the range 10^5 - 10^8 per g of sample expressed on a dry weight basis.

Sludge treatment	Number of Sites	Log-transformed reduction in *E.coli* per g of sample (based on dry weight)
MAD with secondary digestion	2	2.04 - 7.25
MAD with cake production	1	3.94
MAD with lagooning	1	3.05
Pasteurisation	1	6.91
Lime addition	4	5.72 - 6.57
Thermal drying	1	6.52

4 Significance of indicator organisms and pathogens in sewage sludge

Pathogens, whether derived from humans, animals or crops, including bacteria, fungi, viruses, and cysts and eggs of parasites may enter the sewage treatment system from a range of sources. These sources include infected members of the community, abattoirs, farms, industry, animal and crop processing plants, domestic and wild animals and surface water drainage systems. Sewage always contains pathogenic micro-organisms and the types and numbers present depend on the season and the pattern of their circulation within the community. Any pathogenic organism present in raw sewage may also be present in sewage sludge. Also, the number of organisms in sewage sludge depends on the number of organisms present in the original sewage, and varies according to the characteristics of the sludge treatment processes applied to the sludge and the resistance of the organism to stress caused by the treatment process. Replication of most pathogenic micro-organisms in sewage sludge is unlikely to be significant in the UK environment and impossible for viral and protozoan parasites. After application to land the numbers of pathogenic micro-organisms present can be expected to decrease over time due to a combination of factors such as degradation, predation and starvation within the soil.

Whilst unlikely from a practical point, there exists a theoretical risk that transmission of organisms, with consequent risk of infection and disease, may occur when agricultural practices are used and sufficient numbers of organisms are brought into contact with susceptible hosts or vectors. The range of potential pathogen-host combinations in the environment is likely to be very large. There is, however, limited epidemiological evidence, and observation tends to confirm that the actual risk of disease transmission is significant only for a few types of organisms and in relatively rare circumstances. By careful adherence to the proposed regulations[1] and code of practice[2] the risks of disease are reduced. In addition, consideration should also be given to those activities that bring people into contact with sludge, the use of sludge with appropriate crops and the risk of polluting ground or surface waters when applying sludge to land.

4.1 Use of indicator organisms

Ideally, the analysis of an indicator organism should only be used as a surrogate for the analysis of a specific or individual organism if the indicator organism is:

- of exclusive faecal origin;
- present in greater numbers than faecally transmitted pathogens; and
- equally as robust as the pathogen of concern.

4.1.1 *Escherichia coli* as an indicator organism

The revised code of practice[2] utilises *E. coli* for measurements of the reduction in numbers of organisms before and after treatment and for final product assessment. *E. coli* is present in sewage and untreated sludge in consistently high numbers, is known to behave[18] (with regard to survival during treatment) in a manner equivalent to that of the pathogenic strain *E. coli* O157:H7, and can be analysed relatively easily without the need for stringent containment procedures[19]. Whilst *E. coli* possesses many of the properties of an ideal indicator organism, further research is required to establish greater confidence in how well log reductions of this indicator organism, relate to the wide range of other pathogens that might be present.

4.1.2 Other indicators

In some particular circumstances, especially where reliance is placed on temperature-time relationships (for example to assess the efficacy of treatment processes etc) the detection and

enumeration of more resistant indicator organisms, such as *Clostridium perfringens*, may be more appropriate. *Clostridium perfringens* is an opportunistic pathogen occurring widely in sewage and soil, and possesses a characteristic property of forming heat-resistant spores. This means that the determination of a significant reduction of its numbers present in sludge before and after treatment can provide additional confidence in the effectiveness of the treatment process. However, the number of *Clostridium perfringens* found in sludges is highly variable and it may not be appropriate to use this indicator organism in all treatment processes.

4.2 Human and animal pathogens

The pathogenic bacteria of primary concern that may be present in sewage sludge are the species of *Salmonella*, members of the *Campylobacter* group, *E. coli* O157:H7 and *Listeria monocytogenes*. Assessed against their survival in the environment, the risks from other potentially pathogenic bacteria, such as species of *Aeromonas*, *Shigella*, *Vibrio* and *Yersinia* are considered relatively low. These organisms are not associated with sewage or water-borne infections, in that they are present in the environment in low numbers. Generally, the number of viable pathogenic bacteria in sewage sludges reduces by at least 99 %, (equivalent to two log cycles) during thermophilic aerobic digestion, pasteurisation, lime treatment and composting.

4.2.1 *Salmonella* species

Infection by the non-typhoid *Salmonella* species is a significant cause of gastro-enteritis in humans. These organisms are members of the Enterobacteriaceae family, and are Gram-negative, non-spore-forming rod-shaped organisms which are sub-divided on the basis of somatic and flagellar antigens. The primary modes of transmission are via person-to-person contact and by food ingestion, particularly the consumption of undercooked poultry and eggs. *Salmonella* species have been detected in raw and treated sewage sludge, and on grassland. The organisms are widespread in farm animals, wild animals and birds. Water-borne infections are rarely reported, as the infectious dose is usually high and the number of organisms found in sewage effluents is relatively low. Reduction in numbers found before and after treatment, as a result of the treatment, and through "die-off" on grassland have both been shown[20, 21] to be a significant barrier to the spread of disease caused by these organisms when sludge has been applied to land.

The use of mesophilic anaerobic digestion procedures produces a 90 - 99 % reduction in the numbers of viable *Salmonella* species. Treatment by lime has been found to be effective[22, 23] with reductions in numbers in excess of 6 log cycles[18]. Laboratory-based studies[18] have shown reductions in numbers of *Salmonella* species generally to be in excess of 6 log cycles for pasteurisation processes and in excess of 5 log cycles for composting processes.

Although *Salmonella* species may survive in soil for more than a year, depending on the prevailing conditions, they are rapidly inactivated on herbage[16]. Cattle grazed experimentally[16] on contaminated pastures were not easily infected and the few examples of infections associated with sludge that have been reported indicate that the risks are slight when adequate time intervals are allowed between the application of sewage sludge to land and when grazing has been permitted. Several outbreaks involving accidental contamination of pasture or water courses by sewage effluent or septic tank wastes have been reported[16], but few have been traced to direct grazing of pasture spread with sludge and, where such outbreaks have occurred, it is possible that correct grazing intervals were not observed.

The small number of pathogenic infectious outbreaks (in animals associated with the application of sludge to land) are probably explained by the low numbers of *Salmonellae* that remain viable on pasture after the application of sludge to land and the time interval before grazing is allowed. The

number of such organisms required to initiate illness in calves has been estimated to be between 10^5 - 10^{11} organisms, and for adult cattle and sheep, in excess of 10^8 organisms. Much smaller numbers may, however, initiate natural infections, particularly in animals where resistance has been lowered by "stress" factors or the occurrence of concurrent disease. The most significant modes of environmental transmission in animals are via direct animal-to-animal contact and the consumption of contaminated feed. Cattle may also become infected via consumption of surface water contaminated with sewage and via contact with bird and animal excreta.

4.2.2 Thermotolerant *Campylobacter* species

Thermotolerant *Campylobacter* species, especially *Campylobacter jejuni* and *Campylobacter coli*, are recognised as a common cause of gastro-enteritis in humans. Infection gives rise to a "flu-like" illness, with fever, myalgia and malaise, followed by diarrhoea. *Camplylobacter* species are Gram-negative, spirally curved rods and strictly micro-aerophilic. Whilst *Camplylobacter* species do not multiply in food, the presence of low numbers of organisms is sufficient to cause infection. The consumption of poultry is the most common cause associated with infection. The consumption of raw milk and inadequately treated drinking water have also been responsible for causing outbreaks of disease. *Campylobacter* species are common in the environment and have been detected in sewage and sludge. Both recreational use and consumption of contaminated surface water have been sources of infection.

Campylobacter species are inactivated by aerobic processes, but appear relatively unaffected by anaerobic processes. This is possibly due to their micro-aerophilic nature[24, 25]. The presence of *Campylobacter* species in sewage sludge is relatively insignificant[25]. Laboratory-based studies[18] have shown that for pasteurisation, lime treatment and composting processes, reductions in the numbers of *Campylobacter* species before and after treatment are greater than 5 log cycles.

4.2.3 *Escherichia coli* O157:H7

Escherichia coli O157:H7 is a Gram-negative rod-shaped organism, which is a member of the family Enterobacteriaceae. The organisms are found primarily in cattle where they usually give rise to asymptomatic infection. In humans, the infective dose is low, i.e. low numbers of the organism are required to cause infection which can result in haemorrhagic colitis, and haemolytic uraemic syndrome which causes kidney failure. The main cause of infection is the consumption of meat, meat products and raw milk, although potatoes have been implicated in one outbreak[26]. It is possible that organisms can be transferred to food handling areas via contaminated soil present on root crops, and act as a source of infection. Water-borne outbreaks and infections transmitted by cattle have been reported[25].

To date, there have been no reported outbreaks of *E. coli* O157:H7 associated with the use of sludge on agricultural land but there may be a need for further research[25] to determine the number of organisms in sludge, the effects of treatment and its inactivation in soil. Laboratory-based studies[18] on mesophilic anaerobic digestion have shown that the survival of verocytotoxigenic *E. coli* is similar to that of non-verocytotoxigenic strains.

4.2.4 *Listeria monocytogenes*

Listeria monocytogenes is a Gram-positive, rod shaped organism naturally found in the environment. The organisms have been found in sewage sludge in similar numbers to those found for *Salmonella*[27]. To date, no human or animal infections associated with sludge applications to agricultural land have been reported. Laboratory-based studies[18] have shown log reductions of 1.8 - 2.2 in mesophilic anaerobic digestion processes, 2.4 - 3.1 in composting processes and

approximately 6.7 in lime treatment processes. Complete inactivation of the organism was found during processes involving pasteurisation at 70 °C, but with some survival of the organism at 55 °C, that could have been due to inadequate mixing.

The conditions relating to the application of sludge to agricultural land[9] to protect foods considered "at risk" should not increase the burden of *Listeria* in food[25].

4.2.5 Other pathogenic bacteria

Species of the genera *Leptospira*, *Erysipelothrix*, *Treponema hyodysenteriae*, *Yersinia*, *Clostridium* and *Streptococcus* may be isolated from human and animal wastes. However, the frequency of occurrence, either in the animal population or the environment, indicates that sewage is unlikely to be a significant source of infection. Similarly, although *Bacillus anthracis* and *Brucella abortus* could be present in sludge, both are now so rarely found in the UK, and the latter so susceptible to adverse effects of the environment, that the risk of dissemination of these organisms by sewage sludges is negligible.

4.2.6 Viruses

Human viruses including norovirus (previously known as Norwalk-like virus or small round structured virus), rotavirus, astrovirus and adenovirus 40/41 can cause gastro-enteritis. Since many strains exist, norovirus is a major cause of gastro-enteritis in humans of all ages, however, the other groups primarily cause illness in children. Hepatitis A virus is transmitted by the consumption of faecally contaminated material, infects the liver, and may cause hepatitis. The enterovirus group do not cause gastro-enteritis unless part of a wider systematic illness. Poliovirus is an enterovirus but is not considered a risk for fully vaccinated individuals as its presence in the UK occurs only as the vaccine strain. The infective dose of viruses is low and, in theory, one virus may cause infection.

Enterovirus, Hepatitis A virus, rotavirus and astrovirus have been detected in sewage using cell culture; norovirus is detectable only by molecular techniques. The major mode of transmission for these viruses is via person-to-person contact. Water-borne infections are recognised for norovirus and Hepatitis A virus, but rarely for rotavirus. Water-borne transmission has occurred following consumption of sewage-contaminated drinking water and recreational water.

Cytopathic enteroviruses and rotaviruses can be inactivated by pasteurisation and other thermal processes, mesophilic anaerobic digestion at about 35 ^{o}C and by lime stabilisation[25]. Mesophilic anaerobic digestion at about 25 ^{o}C appears to be less effective. Based on the limited information available it would appear that inactivation of viruses in soil is slow in the UK.

4.2.7 Parasites

Some protozoan and helminth parasites are of significance in the context of water-borne infection. These include *Cryptosporidium parvum, Giardia duodenalis* (*Giardia intestinalis, Giardia lamblia*) and *Cyclospora*, and *Taenia saginata* (the beef tapeworm).

4.2.7.1 *Protozoan parasites*

Cryptosporidium parvum is a coccidian parasite that inhabits the small intestine of humans and mammals. No intermediary host is required as the infectious stage, the sporulated oocyst, is shed in faecal material. Infection results in acute self-limiting diarrhoea except in immuno-compromised individuals where the infection may cause long-term, life threatening diarrhoea. Farm animals, particularly cattle and sheep act as a reservoir for *Cryptosporidium parvum* from which infection

can be transmitted to humans via contact with faeces or consumption of contaminated water. Person-to-person transmission can also occur via the consumption of faecally contaminated material. Oocysts have been detected in sewage and may survive for long periods in cool, moist conditions. Water-borne transmission has been well documented[28] when sewage or animal waste has contaminated drinking water. The oocysts are resistant to chlorine disinfection.

Giardia duodenalis is a flagellated protozoan parasite that inhabits the small intestine of humans and other vertebrates. No intermediary host is required as the infectious form, the cyst, is shed in faecal material and may survive for long periods in the environment. Person-to-person contact is the most commonly reported mode of transmission. Cysts have been detected in sewage and water-borne transmission is recognised. The cysts are more sensitive to chlorine disinfection than the oocysts of *Cryptosporidium parvum*.

Studies[29] have shown that *Cryptosporidium parvum* and *Giardia duodenalis* should be completely inactivated by thermophilic digestion and pasteurisation and that the numbers of both *Cryptosporidium*[30] and *Giardia*[31] are likely to show significant reductions in numbers during the mesophilic anaerobic digestion process. It would appear[16], therefore, that there is not a significant risk from these organisms to public health in the UK when sludge is applied to land.

Cyclospora are coccidian protozoan parasites. The oocysts are spherical and slightly larger than those of *Cryptosporidium parvum*, and require a period of maturation post excretion before becoming infective. The parasites are a cause of watery, profuse diarrhoea that may be prolonged in immuno-deficient hosts. Many infections reported in the UK have originally developed during overseas travel to developing countries. Transmission has occurred via the consumption of contaminated water and fruit. *Cyclospora* are not considered to be endemic in the UK so their detection in sludge is unlikely to occur. As more cases are reported, there may be a need, periodically, to re-assess this organism.

4.2.7.2 *Helminth parasites*

Whilst the helminth parasite, *Taenia saginata* (beef tapeworm) is the most commonly reported[31] indigenous cestode to the UK, reports of *Taenia solium* are rare. The adult tapeworm can live for many years in the human intestine shedding eggs into faeces. If the eggs are ingested as a result of applying sludge to land, cattle may become infected and develop bovine cysticercosis. The eggs of *Taenia saginata* develop within the host until the larval stage migrates to muscle. The consumption by humans of immature larvae (cysts) present in contaminated, undercooked beef completes the cycle of infection. In each year, the number of reported cases of infection in the UK is small, and many of these cases originate as infections caused abroad whilst travelling overseas. Infected individuals may experience slight abdominal discomfort, with either diarrhoea or constipation. The use of pasteurisation, mesophilic anaerobic digestion and lime stabilisation processes have been effective in reducing the infectivity of eggs of *Taenia saginata*[16, 33, 34].

Another indigenous cestode of concern for humans is *Echinococcus granulosus*, the causative agent of hydatid disease. Incidence has been largely confined to mid-Wales and Herefordshire where sheep, which are the main hosts, have become infected by ingesting cysts whilst grazing on pasture infected with the faeces of dogs or foxes. Humans are intermediate hosts, not normally playing a part in the biological cycle of the cestode. There is little clinical or epidemiological information on the diseases caused by this organism.

4.3 Plant pathogens

There is a risk that certain pests and pathogens, particularly those with persistent life stages, can

survive in sewage sludge. There is a further risk, therefore, if sewage sludge is used on agricultural or horticultural land, of introducing new and potentially damaging organisms to such land or of increasing the population, to damaging levels, of such organisms that already exist on the land. The management and disposal of agricultural and horticultural wastes (that may contain plant material infested with pests or infected with pathogens) is described elsewhere[35]. Examples of the type of organisms that may survive in sewage sludge are described below. These organisms may, therefore, pose a risk to crop production,.

4.3.1 Potato cyst nematode

The potato cyst nematodes, *Globodera rostochiensis* and *Globodera pallida* are parasitic nematodes. Another species, *Globodera achilleae*, rarely present in the UK, does not infect potatoes. Cysts of both the potato cyst nematode species are spherical, about 0.5 mm in diameter, with a protruding neck but no posterior protuberance. Each new cyst may contain 200 - 600 juveniles, each coiled within an eggshell. Old cysts may be empty. The juveniles infest the root of the potato with consequential reductions in the yield cultivated. The female of the species forms a cyst that is attached to the root which usually remains in the soil when the potato is removed from the ground. The eggs in the cyst remain viable.

A survey of rural sewage works[16, 36] reported low numbers of cysts in treated sludge following application of sludge to land at recommended rates. These numbers were at levels that would not have been detected in soil by a commonly used test procedure[36]. The report concluded there was a risk due to the prolonged survival of eggs in cysts and the potential for infection of growing potatoes. Consequently, the report[16, 36] recommended that sewage sludges should not be applied to land intended for the growth of seed potatoes, bulbs or other nursery stock. The test procedure commonly used is unable to distinguish between viable and non-viable cysts and the determination of even one non-viable cyst would automatically condemn the sample, and hence, the crop. Hence nurseries with an interest in export markets to potato cyst nematode-sensitive countries are particularly concerned.

4.3.2 Other infections of crops

Brown rot of potatoes is caused by the bacterium *Ralstonia solanacearum*, which occurs mainly in warm climates. Outbreaks have occasionally occurred in tomato plants as well as potatoes in the UK as a result of transmission of the organism through river water used for irrigation. The pathogen can replicate in a secondary host (*Solanum dulcamara*) which grows along river banks. The organism can survive in tubers in cool conditions where further infection can develop. Infection gives rise to the rotting of tubers in fields and after harvesting. Recent experimental evidence[37] suggests that the organism will not survive for more than 48 hours during anaerobic sludge digestion processes.

A number of viruses, namely, cucumber mosaic virus, tomato mosaic virus and tomato bushy stunt virus have been detected[38] in tomato plants growing on dried sewage sludge from tomato seeds which had survived sewage treatment. Tomato bushy stunt virus and other similar plant viruses have also been detected in river water, thought to originate from the liquid effluent from sewage treatment works.

Numerous other plant viruses that are resistant to biological degradation have similarly been isolated from river waters[39]. Many of these viruses undoubtedly survive sewage treatment and, hence, could be transmitted to horticultural crops, if sludge were used on land.

Other organisms of major concern are those with highly resistant resting spores, such as potato wart

disease (*Synchytrium endobioticum*) and strawberry red core (*Phytophthora fragariae*). Both of these organisms can survive in soil, even in the absence of a suitable host, for periods in excess of 20 years. The organisms are subject to control[40] where, upon discovery, appropriate authorities should be notified. In the case of potato wart disease, certain growing conditions apply. The risk of potato wart disease is thus a further factor to be taken into account when considering the use of sludge on land. The occurrence of strawberry red core, whilst relatively widespread in fruiting crops, should be notified to the relevant authorities when found. Hence, it may not be appropriate to use sludge on land in these circumstances.

Sludge containing wastes from imported root vegetables present a further risk of introducing new organisms to land not currently found in the UK. Sugar beet rhizomania disease (caused by beet necrotic yellow vein virus) has been identified in soil associated with imported potatoes destined for processing. The ability of this disease to survive in sludge is currently under investigation[37], but the actual risk is considered low. All processors are expected to comply with control procedures[35].

The use of sludge on land can increase the numbers of organisms which are already widely distributed in soils and which presently are not regarded as a concern. The cyst nematodes (*Heterodera* species) of cereals and sugar beet are present almost everywhere there are suitable host crops grown. Hence, additional cysts surviving sewage treatment and used on land would be an insignificant contribution to the total numbers. However, the presence of other nematodes is relatively uncommon and therefore if present in significant numbers in sludge, and then used on land, their contribution would be significant in a relatively clean area.

Fungal pathogens such as *Olpidium* and *Pythium* would fall into this category. Care should be exercised to avoid the exacerbation of common diseases due to the injudicious use of sludge on some crops when sludge is used on land and certain problems have been encountered with an increased incidence of cavity spot in carrots. However, it is not clear whether this increase resulted from *Pythium* contamination of the sludge, or reflected the additional organic matter in the sludge.

5 Microbiological monitoring of sewage sludge

5.1 Sampling and standards

The HACCP approach should be used to identify CCPs in sludge treatment processes. Also, CCPs should typically focus on process operational control parameters such as temperature, time, dry solids content and lime dose requirements etc. These parameters may also include the monitoring of design and management aspects, for example the positioning of inlet and outlet pipes and the design of safety systems. The proposed regulations[1] require that end-product samples, each consisting of five random samples of not less than 100 ml of liquid sludge or 100 g of dried sludge, should be analysed at monthly intervals to ensure satisfactory operation. Samples should be analysed for *E. coli* in conventionally treated sludge and for *E. coli* and *Salmonellae* in enhanced treated sludge. If results are satisfactory during the first 6 months, then sampling may be reduced to quarterly intervals. More frequent sampling should be undertaken if unsatisfactory results are reported. Details of practices and procedures to be used for sampling and analysing sludge samples are given elsewhere in this series[4, 5, 6].

5.2 Health and safety considerations

Due to the 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 and laboratory analysis, see also page 5, “Warning to

users". 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 not be used, wherever possible[41].

It is the responsibility of those managing and supervising sludge treatment and related activities, including sampling and analysis, to ensure that the appropriate risk assessments[42] are carried out, and that control measures are identified and implemented. Further guidance on the management, design and operation of microbiological containment laboratories[19] and the categorisation of pathogens[43] is given elsewhere. Particular attention should be paid to the disposal of surplus sludge samples and associated cultures. Persons handling sludge samples and associated cultures should be appropriately protected from diseases, for example vaccinated against poliomyelitis and tetanus.

Field operations, including sampling, should be conducted with due regard to the possible occurrence of local hazards and portable safety equipment should be available, if appropriate. In addition, suitable support measures should be considered whenever staff are required to work alone or in isolation. All equipment should be cleaned and disinfected after use, and clothing, including footwear, should be disinfected by appropriate methods if contamination is suspected.

Following an incident, medical personnel should be informed of the microbiological nature of the activity. Some unusual parasites, viruses and other micro-organisms are occasionally encountered in samples and it cannot be over-emphasised that prompt administration of the correct decontamination, or first-aid procedures, can prevent serious illness. Other useful guidance on laboratory safety is provided elsewhere[44, 45].

5.3 Suitability of methods

The methods[5, 6] described in this series are based on widely used routine analytical procedures for environmental, wastewater or drinking water microbiology. However, two methods are based on research experiences in one laboratory. The methods for *E. coli* have been tested in several laboratories and comparative data produced in support of their adoption. It is recognised that additional development work may provide further improvements. All analytical techniques should be fully documented, including reference to organisms used as positive and negative controls in isolation and confirmation procedures as well as quality control tests for assessing media.

As newer methods are developed, older test procedures are often replaced. Thus, it is important that new test procedures are properly validated and their performance established by comparison with the old test procedures. New test procedures should only be adopted after it has been demonstrated that the performance obtained is equivalent to, or better than that shown by the old test procedures. Details of, and data generated for, the new test procedures should be fully documented. Guidance on the validation of microbiological methods[46] and method comparison protocols, based on those described for drinking water[47] are available elsewhere. Both should be suitably adapted for use on sewage sludge.

In view of the different treatment processes used in the production of sludge, sludge matrices vary considerably in nature and character. The sensitivity of methods used to analyse conventionally treated sludge is also different to that of methods used to analyse enhanced treated sludge. Hence, methods suitable for analysing raw or mesophilically-digested sludge may not be suitable, without

adaptation, for analysing lime-treated or thermally dried sludge. These factors should be taken into account, and may be significant when comparing enumerated counts of sludges before and after treatment. Reported results should, therefore, make reference to the methods used and any comparative recovery data.

6 References

1. Proposed regulations: "The Sludge (Use in Agriculture) (Amendment) (England and Wales) Regulations.

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

3. Council Directive on the protection of the environment, and in particular the soil, when sewage sludge is used in agriculture. Council of European Communities, 1986, 86/278/EEC. *Official Journal of the European Communities.* 4.7.86 No.L 181/6-12.

4. Standing Committee of Analysts. 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*, in this series, Environment Agency.

5. Standing Committee of Analysts. The Microbiology of Sewage Sludge (2003) - Part 3 - Methods for the isolation and enumeration of *Escherichia coli*, including verocytotoxigenic *Escherichia coli*. *Methods for the Examination of Waters and Associated Materials*, in this series, Environment Agency.

6. Standing Committee of Analysts. The Microbiology of Sewage Sludge (2003) - Part 4 - Methods for the detection, isolation and enumeration of *Salmonellae*. *Methods for the Examination of Waters and Associated Materials*, in this series, Environment Agency.

7. The Sludge (Use in Agriculture) Regulations 1989, Statutory Instrument No. 1263, as amended by The Sludge (Use in Agriculture) (Amendment) Regulations 1990, Statutory Instrument No. 880.

8. *Code of Practice for Agricultural Use of Sewage Sludg*e. 1989, Department of the Environment.

9. *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.

10. Using event trees to quantify pathogen levels on root crops from land application of treated sewage sludge. *Journal of Applied Microbiology*, P Gale, 2003, **94**, pp35-47.

11. *Progress in Research*. Standing Committee on the Disposal of Sewage Sludge. 1984, Department of the Environment.

12. Council Directive concerning urban waste water treatment. Council of European Communities, 1991, 91/271/EEC. Official Journal of the European Communities No. L 135/40-52.

13. Raising the Quality: Treatment and Disposal of Sewage Sludge. 1998, Department for Environment Food and Rural Affairs.

14. EU Council Directive 1999/31/EC of 26 April 1999 on the Landfill of Waste.

15. Code of good agricultural practice for the protection of water. 1998, PB0585. Ministry of Agriculture, Fisheries and Food.

16. *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.* 1998, Department of the Environment, Transport and Regions.

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

18. Pathogens in biosolids - The Fate of Pathogens in Sewage Treatment. 2002, UKWIR Report Reference No. 02/SL/06/6. See also Methods for the detection and Enumeration of Pathogens in Biosolids. 2000, UKWIR Report Reference No. 00/SL/06/5.

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

20. Viable but non-culturable salmonellas in soil. *Journal of Applied Bacteriology*, P E Turnpin, K A Maycroft, C L Rowlands E M H Wellington, 1993, 74, pp421-427.

21. The effect of *Streptomyces* spp. on the survival of *Salmonella* in Soil. *FEMS Microbiology Ecology*, P E Turpin, V K Dhir, K A Maycroft, C L Rowlands and E M H Wellington, 1992, 101, pp271-280.

22. Lime stabilised sludge: its stability and effect on agricultural land. Nat. Env. Research Centre, C A Counts and A J Shuckrow, Prog. Element IBB043, 1975, pp14-20.

23. "The effectiveness of chemical disinfection of faecal bacteria in sludge", A F Godfree, F Jones, M Satchwell and D C Watson in "Sewage Sludge Stabilisation and Disinfection", (Eds) A Bruce and E Horwood, 1984.

24. Epidemiological aspects of thermophilic *Campylobacter* in water related environments: a review. *Water Environment Research*, P M F J Koenraad, F M Rombouts and S H W Notermans, 1997, 69, pp52-63.

25. *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.* 1998, Department of the Environment, Transport and Regions.

26. First recognised community outbreak of haemorrhagic colitis due to verotoxin producing *Escherichia coli* O157: H7 in the UK. *Epidemiology and Infection*, G M Morgan, C Newman, S R Palmer, J B Allen, W Shepherd, A M Rampling, R E Warren, R J Gross, S M Scotland and N R Smith, 1988, 101, pp83-91.

27. Isolation and enumeration of *Listeria monocytogenes* from sewage, sewage sludge and river water. *Journal of Applied Bacteriology*, J Watkins and K P Sleath, 1981, 50, pp1-9.

28. Standing Committee of Analysts. Isolation and identification of *Cryptosporidium* oocysts and *Giardia* cysts in waters 1999. *Methods for the Examination of Waters and Associated Materials*, in this series, Environment Agency.

29. Disinfection and oocysts. In *Cryptosporidiosis*, D A Blewitt in *Proceedings of the First International Workshop,* (Eds) K W Angus and D A Blewitt, Animal Diseases Research Association, Edinburgh, 1989, pp107-111.

30. *Fate of Cryptosporidium During Sewage Treatment*. T N Whitmore, 1992, *(EHA 9036)*. Report to the Department of the Environment, WRc report No. DoE 3265, WRc Medmenham, Marlow.

31. Inactivation of *Giardia* by anaerobic digestion of sludge. *Water Research*, P D Gavaghan, J L Sykora, W Jakubowski, C A Sorber, A M Sninsky, M D Lichtem and G Keleti, 1993, 27, pp111-114.

32. Surveillance of taeniasis in England and Wales: laboratory reports 1990-98. Anon, *CDR Weekly*, 1999, 9, No. 41, pp365.

33. The effects of pasteurisation and stabilisation of sludge on *Taenia saginata* eggs, D L Hughes, I J Norrington, D L Morris and W M Waite in *Inactivation of micro-organisms in sewage sludge by stabilisation processes*, (Eds) D Stauch, A H Havelaar and P l'Hermite, Elsevier Applied Science Publishers, London, 1985, pp126-134.

34. Inactivation of parasitic ova during disinfection and stabilisation of sludge, E B Pike and E G Carrington in *Processing and Use of Organic Sludges and Liquid Agricultural Wastes*, (Ed) P l'Hermite, D Reidel Publishing Company, Dordrecht, 1985, pp198-209.

35. Code of Practice for the Management of Agricultural and Horticultural Waste. 1998, Department for Environment Food and Rural Affairs.

36. Effects of various sewage sludge treatments on the survival of potato-cyst nematodes. (*Globodera* spp.) and the implications for disposal. *Water Science and Technology*, A M Spaull, D M McCormack and E B Pike, 1988, 21, pp909-916.

37. Additional UKWIR Funded Biosolids Research. In the Proceedings of the 6th European Biosolids and Organic Residuals Conference, Pre-conference Workshop - Pathogens in Biosolids and their Significance in Beneficial Use Programmes, C Rowlands, 2001.

38. Studies on the occurrence of Tomato bushy stunt virus in English rivers. *Annals of Applied Biology*, J A Tomlinson and E M Faithfull, 1984, 104, pp485-495.

39. Plant viruses in rivers and lakes. *Advances in Virus Research*, R Koenig, 1986, 31, pp321-333.

40. Plant Health Order (as amended) 1993, Statutory Instrument 1993 No 1320.

41. 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.

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

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

44. Safety in Microbiological Laboratories in the Water Industry. National Joint Health and Safety Committee for the Water Service (1983). Health and Safety Guidelines No.5. A Report by the NWC Microbiology Working Group Sub-Committee on Laboratory Safety.

45. The Prevention of Laboratory Acquired Infection, Safety procedures: notes for guidance, 4th edition, 1993, Public Health Laboratory Service Monograph 6, HMSO, London.

46. ISO TR 13843:2001 – Water Quality – Guidance on validation of microbiological methods.

47. 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.

Address for correspondence

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.

Standing Committee of Analysts
Environment Agency (National Laboratory Service)
Wheatcroft Office Park
Landmere Lane, Edwalton
Nottingham, NG12 4DG
www.environment-agency.gov.uk/nls

Standing Committee of Analysts
Members assisting with this booklet

R A Barrell
P Boyd
K Chadwick
S Cole
A Davies
R Down
P Finch
A Gawler
A Hockin
A Jonas
J V Lee
E McDonnell
R Pitchers
C Rowlands
D Sartory
J Sellwood
D Taylor
K C Thompson
J Watkins
J Watson

CONTACTS:

ENVIRONMENT AGENCY HEAD OFFICE

Rio House, Waterside Drive, Aztec West, Almondsbury, Bristol BS32 4UD
Tel: 01454 624 400 Fax: 01454 624 409

www.environment-agency.gov.uk
www.environment-agency.wales.gov.uk

ENVIRONMENT AGENCY REGIONAL OFFICES

ANGLIAN
Kingfisher House
Goldhay Way
Orton Goldhay
Peterborough PE2 5ZR
Tel: 01733 371 811
Fax: 01733 231 840

MIDLANDS
Sapphire East
550 Streetsbrook Road
Solihull B91 1QT
Tel: 0121 711 2324
Fax: 0121 711 5824

NORTH EAST
Rivers House
21 Park Square South
Leeds LS1 2QG
Tel: 0113 244 0191
Fax: 0113 246 1889

NORTH WEST
PO Box 12
Richard Fairclough House
Knutsford Road
Warrington WA4 1HG
Tel: 01925 653 999
Fax: 01925 415 961

SOUTHERN
Guildbourne House
Chatsworth Road
Worthing
West Sussex BN11 1LD
Tel: 01903 832 000
Fax: 01903 821 832

SOUTH WEST
Manley House
Kestrel Way
Exeter EX2 7LQ
Tel: 01392 444 000
Fax: 01392 444 238

THAMES
Kings Meadow House
Kings Meadow Road
Reading RG1 8DQ
Tel: 0118 953 5000
Fax: 0118 950 0388

WALES
Rivers House Plas-yr-Afon
St Mellons Business Park
Fortran Road
St Mellons
Cardiff CF3 0EY
Tel: 029 2077 0088
Fax: 029 2079 8555

ENVIRONMENT AGENCY
GENERAL ENQUIRY LINE
0845 9 333 111

ENVIRONMENT AGENCY
FLOODLINE
0845 988 1188

ENVIRONMENT AGENCY
EMERGENCY HOTLINE
0800 80 70 60

ENVIRONMENT AGENCY