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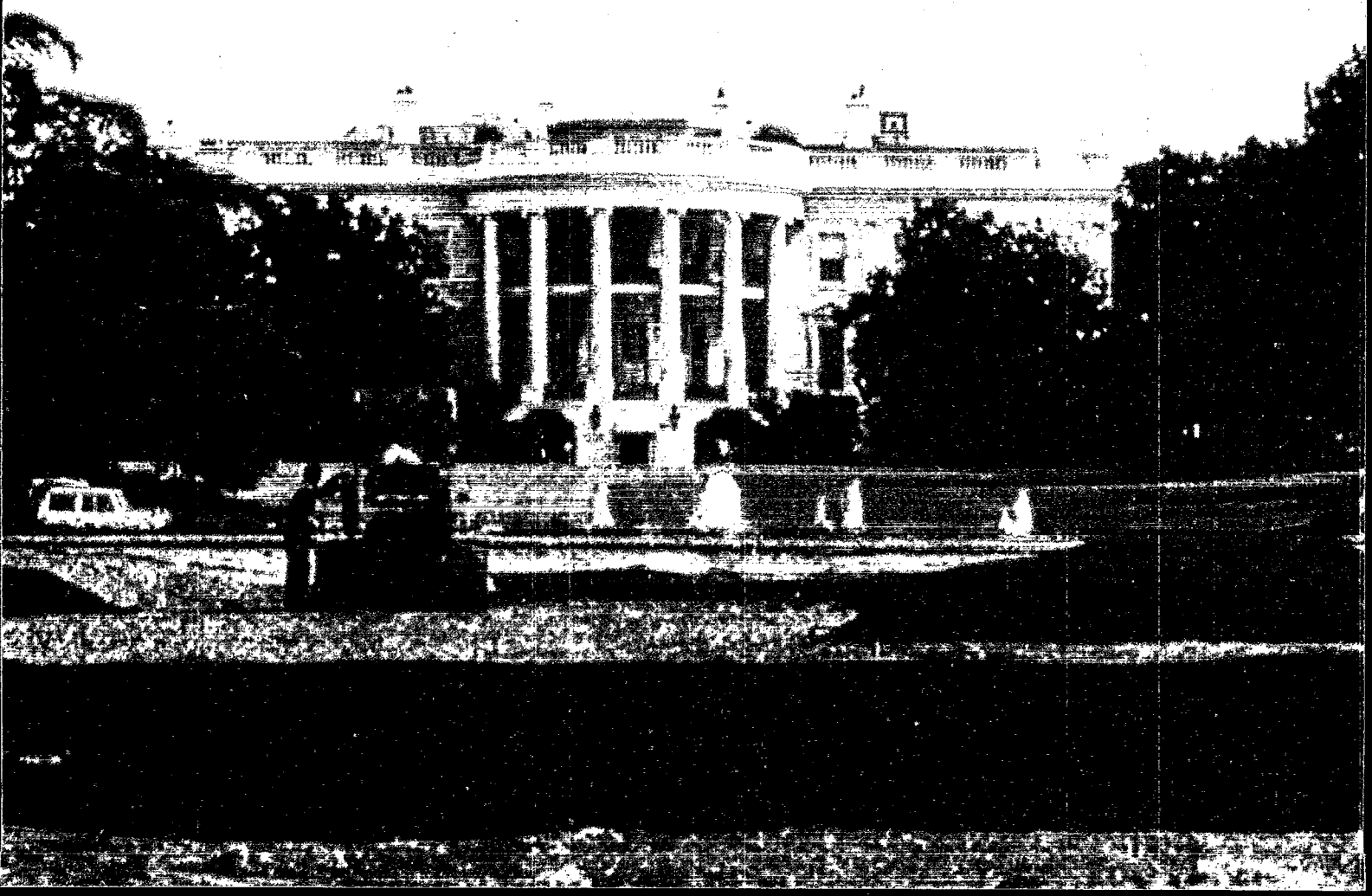
EPA

Environmental Regulations and Technology

Control of Pathogens and Vector Attraction in Sewage Sludge



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Environmental Regulations and Technology

Control of Pathogens and Vector Attraction in Sewage Sludge (Including Domestic Septage) Under 40 CFR Part 503

This guidance was prepared by

U.S. Environmental Protection Agency

Office of Research and Development
Office of Science Planning and Regulatory Evaluation
Center for Environmental Research Information
Cincinnati, OH 45268

Office of Water
Office of Science and Technology
Sludge Risk Assessment Branch
Washington, DC 20460

This document was produced by the U.S. Environmental Protection Agency (EPA's) Pathogen Equivalency Committee (PEC), consisting of Robert Bastian, Joseph Farrell, G. Shay Fout, Walter Jakubowski, Norman Kowal, Mark Meckes, and J.E. Smith, Jr. Joseph Farrell contributed significantly to the document's preparation. Among his contributions were data and other information and the writing of Chapters 7 and 8 and Appendices C, D, and E. Mark Meckes contributed the explanation of the fecal coliform test. G. Shay Fout and Daniel R. Dahling wrote Appendix H, with contributions from colleagues in the Virology Branch, Microbiology Research Division, of EPA's Environmental Monitoring and Support Laboratory. The contributions of Robert M. Southworth of EPA's Office of Water, who critically reviewed the document, are especially appreciated. Jan Connery of Eastern Research Group, Inc., in Lexington Massachusetts, prepared and edited the document under the committee's direction and from information and data supplied by the committee. Other EPA regional, office, and laboratory personnel also contributed information and suggestions for improving this document. Their assistance is sincerely appreciated.

This report has been reviewed by the U.S. Environmental Protection Agency and approved for publication. The process alternatives, trade names, or commercial products are only examples and are not endorsed or recommended by the U.S. Environmental Protection Agency. Other alternatives may exist or may be developed.

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COVER PHOTOGRAPH: Application of sewage sludge compost to the White House lawn.

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Abbreviations and Acronyms

BOD	biological oxygen demand
°C	degrees centigrade
CFR	Code of Federal Regulations
CFU	colony-forming unit
cm	centimeter(s)
EPA	U.S. Environmental Protection Agency
°F	degrees Fahrenheit
FFSR	fractional fixed solids reduction
FFVSR	fractional fixed volatile solids reduction
FR	*Federal Register*
FVSR	fractional volatile solids reduction
g	gram(s)
gpm	gallons per minute
hr	hour(s)
kg	kilogram(s)
L	liter(s)
log	logarithm
m^3	cubic meter(s)
m.b.	mass balance
mg	milligram(s)
MGD	million gallons/day
min	minute(s)
mL	milliliter(s)
MPN	most probable number
no.	number
O_2	oxygen
OWEC	EPA Office of Wastewater Enforcement and Compliance
PEC	EPA Pathogen Equivalency Committee
PFRP	process to further reduce pathogens
PFU	plaque-forming unit
psig	pounds per square inch gauge
PSRP	process to significantly reduce pathogens
RSC	EPA Regional Sludge Coordinator
SM	"Standard Methods for the Examination of Water and Wastewater," 18th edition
SOUR	specific oxygen uptake rate
sp.	species
SRAB	EPA Sludge Risk Assessment Branch
SSC	State Sludge Coordinator
TS	total solids
TSS	total suspended solids
VS	volatile solids
VSS	volatile suspended solids

Chapter 1
Introduction

1.1 What Is Sewage Sludge?

Sewage sludge—the residue generated during treatment of domestic sewage (Figure 1-1)—is used as a soil conditioner and partial fertilizer in the United States and many other countries. It is applied to agricultural land (pastures and cropland), disturbed areas (mined lands, construction sites, etc.), plant nurseries, forests, recreational areas (parks, golf courses, etc.), cemeteries, highway and airport runway medians, and home gardens (see photographs on pages 3 and 4). Certain publicly owned treatment works (POTWs) own or have access to land dedicated solely to disposal of sewage sludge—a practice referred to as surface disposal. The U.S. Environmental Protection Agency (EPA), the primary federal agency responsible for sewage sludge management, encourages the beneficial use of sewage sludge (Figure 1-2). A 1988 survey found that as much as 33% of the sewage sludge generated in the United States was being applied to land (EPA, 1988).[1]

Sewage sludge has beneficial plant nutrients and soil conditioning properties; however, it may also contain bacteria, viruses, protozoa, parasites, and other microorganisms that can cause disease. Land application and surface disposal of sewage sludge create a potential for human exposure to these organisms through direct and indirect contact. To protect public health from these organisms and from the pollutants that some sludges[2] contain, many countries now regulate the use and disposal of sewage sludge.

1.2 U.S. Regulation of Sewage Sludge

In the United States, the use and disposal of sewage sludge (including domestic septage) are regulated under 40 CFR Part 503.[3] This regulation, promulgated on February 19, 1993, was issued under the authority of the Clean Water Act as amended in 1977 and the 1976 Resource Conservation and Recovery Act (RCRA). For most sewage sludge,[4] the new regulation replaces

[1]Domestic septage—the material removed from septic tanks and other on-site treatment systems that receive only domestic sewage—is a form of sewage sludge and therefore may also be applied to the land or disposed in a surface disposal site.
[2]In this document, the term "sludge" always refers to sewage sludge.
[3]Because domestic septage is a form of sewage sludge, any use of the term "sewage sludge" or "sludge" in this document includes domestic septage.
[4]Sewage sludge generated at an industrial facility during the treatment of domestic sewage commingled with industrial wastewater in an industrial wastewater treatment facility is still covered under 40 CFR Part 257 if the sewage sludge is applied to the land.

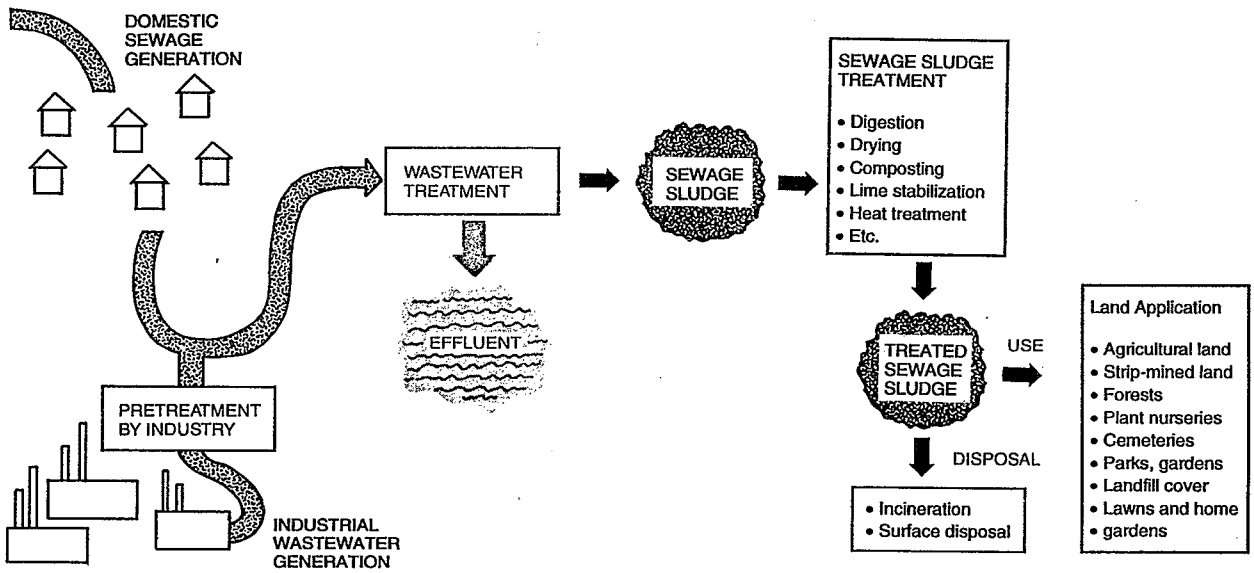


Figure 1-1. Generation, treatment, use, and disposal of sewage sludge.

The U.S. Environmental Protection Agency (EPA) will actively promote those municipal sludge management practices that provide for the beneficial use of sludge while maintaining or improving environmental quality and protecting human health. To implement this policy, EPA will continue to issue regulations that protect public health and other environmental values. The Agency will require states to establish and maintain programs to ensure that local governments utilize sludge management techniques that are consistent with federal and state regulations and guidelines. Local communities will remain responsible for choosing among alternative programs; for planning, constructing, and operating facilities to meet their needs; and for ensuring the continuing availability of adequate and acceptable disposal or use capacity.

Figure 1-2. EPA policy on sludge management. Source: EPA, 1984.

40 CFR Part 257—the original regulation governing the use and disposal of sewage sludge—in effect since 1979.

Protection of Public Health and the Environment

In the judgment of the Administrator of EPA, Subpart D of the Part 503 regulation protects public health and the environment through requirements designed to reduce the potential for contact with the disease-bearing microorganisms (pathogens) in sewage sludge applied to the land or placed on a surface disposal site. These requirements are divided into:

- Requirements designed to control and reduce pathogens in sewage sludge.
- Requirements designed to reduce the ability of the sewage sludge to attract vectors (insects and other living organisms that can transport sludge pathogens away from the land application or surface disposal site).

Subpart D includes both performance- and technology-based requirements. It is designed to provide a more flexible approach than Part 257, which required sewage sludge to be treated by specific listed or approved treatment technologies. Under Part 503, treatment works may continue to use the same processes they used under Part 257, but they now also have the freedom to modify conditions and combine processes with each other, as long as the treated sewage sludge meets the applicable requirements.

Environmental Effects of Pathogens in Sewage Sludge

A major environmental concern (other than effects on public health) associated with land application of sewage sludge is the effect of pathogens on animals. Certain human pathogens can cross species lines and infect animals, particularly warm-blooded animals. Little information is available on whether these pathogens pose a risk to wildlife. Available information on the impact of sludge pathogens on grazing animals suggests that the Part 503 Subpart D requirements for pathogen control (which include restrictions on grazing) protect grazing animals (EPA, 1992).

1.3 What's in This Document?

This document describes the federal requirements concerning pathogens in sewage sludge applied to land or placed on a surface disposal site, and it provides guidance concerning those requirements. The document is intended for:

- Owners and operators of treatment works treating domestic sewage.
- Developers or marketers of sewage sludge treatment processes.
- Groups that distribute and market sewage sludge products.
- Individuals involved in applying sewage sludge to land.
- Regional, state, and local government officials responsible for implementing and enforcing the Part 503 Subpart D regulation.
- Consultants to these groups.
- Anyone interested in understanding the federal requirements concerning pathogens in sewage sludge.

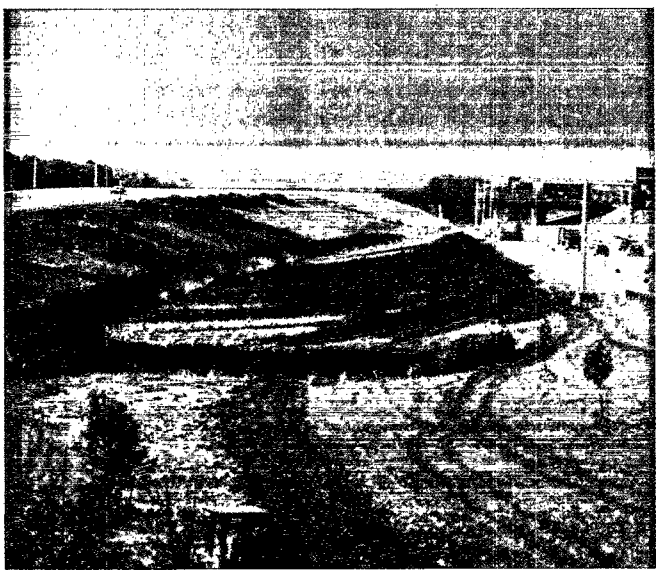
Chapter 2 of this document discusses why pathogen control is necessary to protect public health and the environment, and Chapters 3 through 6 describe the current federal requirements under Subpart D of Part 503. Chapters 7 and 8 discuss sampling and analysis to meet the quantitative requirements of Part 503. Chapters 9 and 10 describe the sewage sludge treatment processes listed under Part 503. Chapter 11 discusses the kind of support EPA's Pathogen Equivalency Committee can provide to permitting authorities.

The appendices provide additional information on:

- Determination of volatile solids and residence time for digestion.
- Sample preparation and analytical methods for meeting the Part 503 pathogen reduction requirements.
- Tests for demonstrating reduced vector attraction.

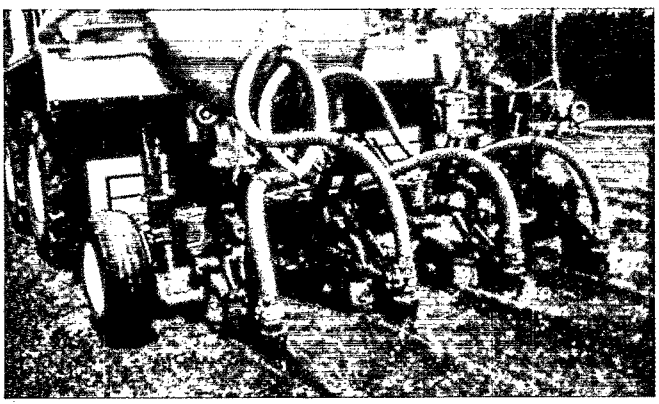
Also, Appendix A lists EPA and state sludge coordinators, and Appendix B contains Subpart D of the Part 503 regulation.

Many sewage sludges also contain heavy metals that may pose public health and environmental concerns. The federal regulation under 40 CFR Part 503 includes requirements designed to limit the amount of heavy metals in sewage sludge applied to land or placed on a surface disposal site. This document focuses on pathogen-related requirements and does not discuss the heavy metal requirements.



Highway median strip in Illinois after land application of dried sludge. (Photo courtesy of Metropolitan Water Reclamation District of Greater Chicago)

Flower beds amended with sludge compost in Tulsa, Oklahoma. (Photo courtesy of City of Tulsa, Oklahoma)

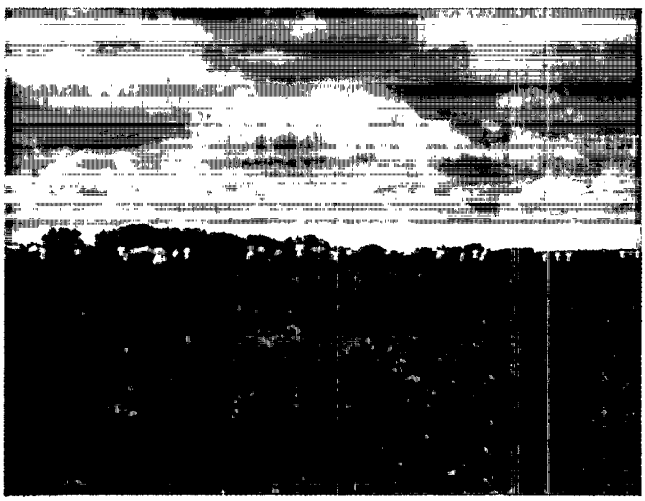


Injection of liquid sludge into sod.

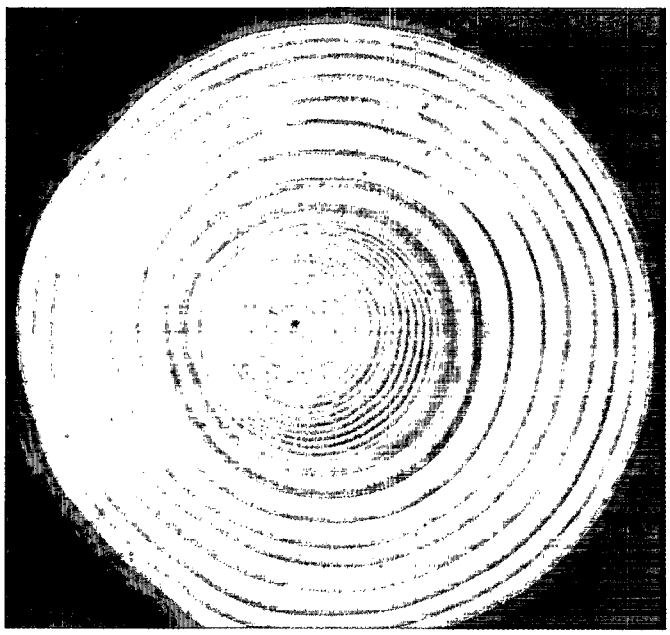
Oat field showing sludge-treated (right) and untreated (left) areas. (Photo courtesy of City of Tulsa, Oklahoma)

Mine spoil land before sludge treatment. Note sparse, weedy growth incapable of supporting grazing cattle. (Photo courtesy of City of Tulsa, Oklahoma)

Corn grown on sludge-treated soil (right) and untreated soil (left).



Mine spoil land after sludge treatment. Note lush vegetative cover on reclaimed soil which will support grazing. (Photo courtesy of City of Tulsa, Oklahoma)



Cross-section of Douglas fir tree showing how sludge application increases tree growth. Note increased size of outer rings indicating more rapid growth after sludge application. (Photo courtesy of Metro Silvigrow)

Chapter 2
Protection of Public Health and the Environment from Sewage Sludge Pathogens

2.1 What Are the Pathogens of Concern?

Municipal wastewater generally contains four major types of human pathogenic (disease-causing) organisms: bacteria, viruses, protozoa, and helminths (parasitic worms) (EPA, 1985). The actual species and density of pathogens present in wastewater from a particular municipality (and the sewage sludge produced when treating the wastewater) depend on the health status of the local community and may vary substantially at different times. The level of pathogens present in sewage sludge also depends on the reductions achieved by the wastewater and sewage sludge treatment processes.

The pathogens in wastewater are primarily associated with insoluble solids. Primary wastewater treatment processes concentrate these solids into sewage sludge, so untreated or raw primary sewage sludges have higher densities of pathogens than the incoming wastewater. Biological wastewater treatment processes such as lagoons, trickling filters, and activated sludge treatment may substantially reduce the number of pathogens in the wastewater (EPA, 1989).

Nevertheless, the resulting biological sludges may still contain sufficient levels of pathogens to pose a public health and environmental concern.[1] Table 2-1 lists some principal pathogens of concern that may be present in wastewater and/or sewage sludge. These organisms and other pathogens can cause infection or disease if humans and animals are exposed to sufficient levels of the organisms or pathogens. The levels—called infectious doses—vary for each pathogen and each host.

Some of the common pathogens of concern that appear in municipal wastewater and sludge are shown in the photographs on pages 9 and 10. These include ascarids (*Ascaris lumbricoides* and *Toxocara*), whipworms (*Trichuris* sp.), tapeworms (*Hymenolepis* sp. and *Taenia* sp.), amoeba (*Entamoeba coli*), and giardia (*Giardia lamblia*). As shown in these photographs, several color staining procedures are needed to identify the organisms and the different structures within the organisms. The photograph of *Giardia lamblia* depicts specimens stained with Lugol's iodine solution, showing two nuclei, a median body, and axonemes in each. In addition, scientists use a blue filter when photographing the pathogenic organisms through a microscope. This filter is necessary to show the natural color of the organisms.

[1]As mentioned in Chapter 1, a major environmental concern (other than effects on public health) is the potential effect of some human pathogens on animals.

2.2 How Does Exposure Occur?

When sewage sludge is applied to land or placed on a surface disposal site, humans and animals can be exposed to pathogens directly by coming into contact with the sewage sludge, or indirectly by consuming drinking water or food contaminated by sewage sludge pathogens. Insects, birds, rodents, and even farm workers can contribute to these exposure routes by transporting sewage sludge and sewage sludge pathogens away from the site. Potential routes of exposure include:

Direct Contact

- Inadvertent contact with sewage sludge.
- Walking through an area—such as a field, forest, or reclamation area—shortly after sewage sludge application.
- Handling soil and raw produce from fields or home gardens where sewage sludge has been applied.
- Inhaling microbes that become airborne (via aerosols, dust, etc.) during sewage sludge spreading or by strong winds, plowing, or cultivating the soil after application.

Indirect Contact

- Consumption of pathogen-contaminated crops grown on sewage sludge-amended soil or of other food products that have been contaminated by contact with these crops.
- Consumption of pathogen-contaminated milk or other food products from animals grazing in pastures or fed crops grown on sewage sludge-amended fields.
- Ingestion of drinking water or recreational waters contaminated by runoff from nearby land application sites or by organisms from sewage sludge migrating into ground-water aquifers.
- Consumption of inadequately cooked or uncooked pathogen-contaminated fish from water contaminated by runoff from a nearby sewage sludge application site.

- Contact with sewage sludge or pathogens transported away from the land application or surface disposal site by rodents, insects, or other vectors, including grazing animals.

Table 2-1. Principal Pathogens of Concern in Municipal Wastewater and Sewage Sludge

Organism	Disease/Symptoms
Bacteria	
Salmonella sp.	Salmonellosis (food poisoning), typhoid fever
Shigella sp.	Bacillary dysentery
Yersinia sp.	Acute gastroenteritis (including diarrhea, abdominal pain)
Vibrio cholerae	Cholera
Campylobacter jejuni	Gastroenteritis
Escherichia coli (pathogenic strains)	Gastroenteritis
Enteric Viruses	
Hepatitis A virus	Infectious hepatitis
Norwalk and Norwalk-like viruses	Epidemic gastroenteritis with severe diarrhea
Rotaviruses	Acute gastroenteritis with severe diarrhea
Enteroviruses	
Polioviruses	Poliomyelitis
Coxsackieviruses	Meningitis, pneumonia, hepatitis, fever, cold-like symptoms, etc.
Echoviruses	Meningitis, paralysis, encephalitis, fever, cold-like symptoms, diarrhea, etc.
Reovirus	Respiratory infections, gastroenteritis
Astroviruses	Epidemic gastroenteritis
Caliciviruses	Epidemic gastroenteritis
Protozoa	
Cryptosporidium	Gastroenteritis
Entamoeba histolytica	Acute enteritis
Giardia lamblia	Giardiasis (including diarrhea, abdominal cramps, weight loss)
Balantidium coli	Diarrhea and dysentery
Toxoplasma gondii	Toxoplasmosis
Helminth Worms	
Ascaris lumbricoides	Digestive and nutritional disturbances, abdominal pain, vomiting, restlessness
Ascaris suum	May produce symptoms such as coughing, chest pain, and fever
Trichuris trichiura	Abdominal pain, diarrhea, anemia, weight loss
Toxocara canis	Fever, abdominal discomfort, muscle aches, neurological symptoms
Taenia saginata	Nervousness, insomnia, anorexia, abdominal pain, digestive disturbances
Taenia solium	Nervousness, insomnia, anorexia, abdominal pain, digestive disturbances
Necator americanus	Hookworm disease
Hymenolepis nana	Taeniasis

Source: Kowal (1985) and EPA (1989).

2.3 How Well Do Pathogens Survive in the Environment?

The potential for exposure diminishes over time as environmental conditions such as heat, sunlight, desiccation, and other microorganisms destroy pathogens that may be present in sewage sludge. Table 2-2 summarizes the survival rates of four types of pathogenic organisms on soil and on plants. Because protozoan cysts on soil and plants are rapidly killed by environmental factors, the threat to public health and animals from protozoa in sewage sludge is minimal. Bacteria, viruses, and helminths (particularly helminth eggs, which are the hardiest part of the helminth life cycle) are of much greater concern. For this reason, Part 503 contains requirements for the reduction of bacteria, viruses, and helminths in sewage sludge, but does not contain requirements for the reduction of protozoa.

Regrowth of Bacteria

Some bacteria are unique among sewage sludge pathogens in their ability to regrow. Even very small populations of certain bacteria can rapidly proliferate under the right conditions, e.g., in sewage sludges where the bacterial populations have been essentially eliminated through treatment (see Section 2.4). Viruses, helminths, and protozoa cannot regrow outside their specific host organism(s). Once reduced by treatment, their populations stay reduced. Part 503 contains specific requirements designed to ensure that regrowth of bacteria has not occurred prior to use or disposal.

2.4 How Can the Public and Animals Be Protected?

Public health and animals can be protected from sewage sludge pathogens in several ways:

- Reduce the number of pathogens in sewage sludge through treatment and/or environmental attenuation.
- Reduce transport of pathogens by reducing the attractiveness of the sewage sludge to disease vectors (insects, birds, rodents, and other living organisms that can transport pathogens).
- Limit human and animal contact with the sewage sludge through site restrictions to allow natural die-off to reduce pathogen levels to low levels.

Part 503 uses a combination of all these approaches (see Chapters 3 through 6 for a description of the requirements).

Pathogen Reduction

Reduction in the number of pathogens can be achieved technologically—by adequately treating sewage sludge prior to use or disposal—and through environmental attenuation (see Section 2.3 above). Many sewage sludge treatment processes are available that use a variety of approaches to reduce pathogens and alter the sewage sludge so that it becomes a less effective medium for microbial growth and vector attraction (Table 2-3). They vary significantly in their effectiveness. For example, some processes (e.g., high pH conditions) may com-

Table 2-2. Survival Times of Pathogens in Soil and on Plant Surfaces[a]

	Soil		Plants	
Pathogen	Absolute Maximum[a]	Common Maximum	Absolute Maximum[b]	Common Maximum
Bacteria	1 year	2 months	6 months	1 month
Viruses	1 year[c]	3 months	2 months	1 month
Protozoan cysts[d]	10 days	2 days	5 days	2 days
Helminth ova	7 years	2 years	5 months	1 month

[a]For survival rates, see Sorber and Moore (1986).
[b]Greater survival time is possible under unusual conditions such as consistently low temperatures or highly sheltered conditions (e.g., helminth ova below the soil in fallow fields) (Jakubowski, 1988).
[c]Sobsey and Shields, 1987.
[d]Little, if any, data are available on the survival times of *Giardia* cysts and *Cryptosporidum* oocysts.
Source: Kowal, 1985.

Table 2-3. General Approaches to Controlling Pathogens and Vector Attraction in Sewage Sludge

Approach	Effectiveness	Process Examples[a]
Kill pathogens with high temperatures (temperatures may be generated by chemical, biological, or physical processes).	Depends on time and temperature. Sufficient temperatures maintained for sufficiently long time periods can reduce bacteria, viruses, protozoan cysts, and helminth ova to below detectable levels. Helminth ova are the most resistant to high temperatures.	•Composting (uses biological processes to generate heat). •Heat drying and heat treatment (use physical processes to generate heat, e.g., hot gases, heat exchangers). •Pasteurization (physical heat, e.g., hot gases, heat exchangers). •Aerobic digestion (biological heat).[b] •Anaerobic digestion (physical heat).[b]
Kill pathogens with radiation.	Depends on dose. Sufficient doses can reduce bacteria, viruses, protozoan cysts, and helminth ova to below detectable levels. Viruses are most resistant to radiation.	•Gamma and high-energy electron beam radiation.
Kill pathogens using chemical disinfectants.	Substantially reduces bacteria and viruses and vector attraction. Probably reduces protozoan cysts. Does not effectively reduce helminth ova unless combined with heat.	•Superchlorination. •Lime stabilization.
Inhibit pathogen growth by reducing the sewage sludge's volatile organic content (the microbial food source).	Reduces bacteria. Reduces vector attraction.	•Aerobic digestion. •Anaerobic digestion. •Composting.[b]
Inhibit pathogen survival by removing moisture from the sludge.	Reduces viruses and bacteria. Reduces vector attraction as long as the sewage sludge remains dry. Probably effective in destroying protozoan cysts. Does not effectively reduce helminth ova unless combined with other processes such as high temperature.	•Air or heat drying.

[a]See Chapters 9 and 10 for a description of these processes. Many processes use more than one approach to reduce pathogens.
[b]Effectiveness depends on design and operating conditions.

pletely destroy bacteria and viruses but have little or no effect on helminth eggs. The effectiveness of a particular process can also vary depending on the conditions under which it is operated. For example, the length of time and the temperature to which sewage sludge is heated is critical to the effectiveness of heat-based treatment processes.

Part 503 lists sewage sludge treatment technologies that are judged to produce sewage sludges with pathogens sufficiently reduced to protect public health and animals. The regulation also allows the use of any other technologies that produce a sewage sludge with adequately reduced pathogens as demonstrated through microbiological monitoring. The Part 503 requirements also include site restrictions to allow environmental factors to further reduce pathogens in treated sewage sludge that is used or disposed.

2.5 How Can Pathogen Reduction Be Measured?

Under Part 503, microbiological analysis of sewage sludge (see photographs on pages 9 and 10) is an important means of determining the effectiveness of a sewage sludge treatment

process in reducing pathogens. Methods have not yet been developed to detect all pathogens that may occur in sewage sludge, and it would be impractical to run all the tests that do exist. For this reason, Part 503 requires monitoring for representative pathogens and nonpathogenic indicator organisms, as described below.

Nonpathogenic Indicators

As detailed in Chapters 4 and 5, some of the Part 503 requirements call for monitoring of fecal coliform bacteria. These bacteria are commonly used as indicators of the potential presence of pathogens in sewage sludges. They are abundant in human feces and therefore are always present in untreated sewage sludges. They are easily and inexpensively measured. Although fecal coliforms themselves are usually not harmful to humans, their presence indicates the presence of fecal waste which may contain pathogens.

Direct Monitoring for Pathogens

Part 503 also requires direct monitoring for the three more common types of pathogens—bacteria, viruses, and viable helminth ova. For viable helminth ova, a single test is available that monitors for *Ascaris* ova and thereby serves as an indicator for several other helminth species (*Toxocara, Trichuris*, and *Hymenolepis*—see photographs on pages 9 and 10). The *Analytical Method for Viable Helminth Ova*, provided in Appendix I, involves extraction, concentration, and incubation of recovered ova versus control *Ascaris* ova to determine viability, rather than the traditional staining techniques or membrane and dilution tube culture techniques used for many other pathogens. For viruses, a test is available that simultaneously monitors for several enterovirus species (a subset of enteric viruses—see Table 2-1), which are presumed to be good indicators for other types of enteric viruses. No such test is available for bacteria. When direct monitoring of pathogenic bacteria is important, Part 503 requires monitoring of *Salmonella* sp. Salmonellae are bacteria of great concern in sewage sludge. They are also good indicators of reduction of other bacterial pathogens because they are typically present in higher densities than are other bacterial pathogens and are at least as hardy.

2.6 What Units Are Used to Measure Microorganism Density Under Part 503?

Use of Units of Mass Versus Units of Volume

Density of microorganisms in Part 503 is defined as *number of microorganisms per unit mass of total solids (dry weight)*. Ordinarily, microorganism densities are determined as number per 100 milliliters of wastewater or sewage sludge. While the use of units of volume is sensible for wastewater, it is less sensible for sewage sludge. The microorganisms in sewage sludge are associated with the solid phase. When sewage sludge is diluted, thickened or filtered, the number of microorganisms per unit volume changes markedly, whereas the number per unit mass of solids remains almost constant. This argues for reporting their densities as the number present per unit mass of solids, which would require that sewage sludge solids content always be determined when measuring microorganism densities.

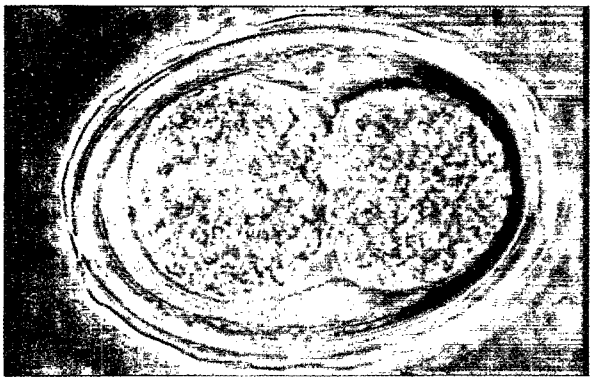
A second reason for reporting densities per unit mass of solids is that sewage sludge application to the land is typically measured and controlled in units of mass of dry solids per unit area of land. If pathogen densities are measured as numbers per unit mass of solids, the rate of pathogen application to the land is thus directly proportional to the mass of dry sewage sludge solids applied.

Different Methods for Counting Microorganisms

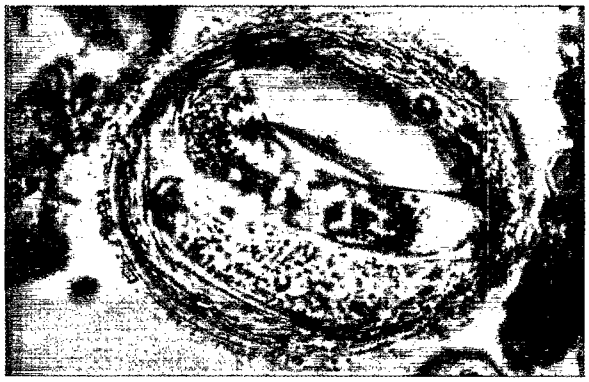
The methods and units used to count microorganisms vary depending on the type of microorganism. Viable helminth ova are observed and counted as individuals under a microscope. Viruses are usually counted in plaque-forming units (PFU). Each PFU represents an infection zone where a single infectious virus has invaded and infected a layer of animal cells. For bacteria, the count is in colony-forming units (CFU) or most probable number (MPN). CFU is a count of colonies on an agar plate or filter disk. Because a colony might have originated from a clump of bacteria instead of an individual, the count is not necessarily a count of separate individuals. MPN is a statistical estimate of numbers in an original sample. The sample is diluted at least once into tubes containing nutrient medium; there are several duplicates at each dilution. The original bacterial density in the sample is estimated based on the number of tubes that show growth.

Part 503 Density Limits

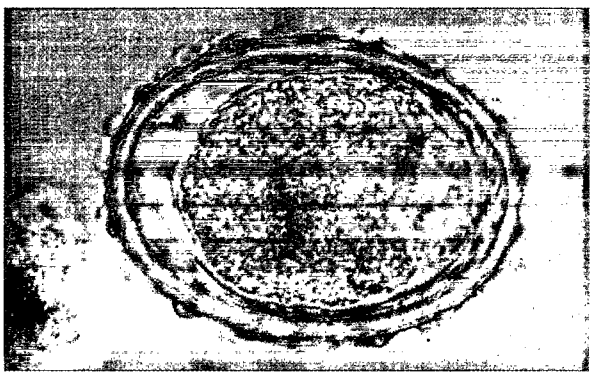
Under Part 503, the density limits for the pathogens are expressed as numbers of PFUs, CFUs, or MPNs *per 4 grams* total solids sewage sludge (see Section 4). This terminology came about because most of the tests started with 100 mL of sewage sludge which typically contained 4 grams of sewage sludge solids. Also, expressing the limits on a "per gram" basis would have required the use of fractions (i.e., 0.25/g or 0.75/g). Density limits for fecal coliform, however, are given on a "per gram" basis because these organisms are much more numerous than pathogens.



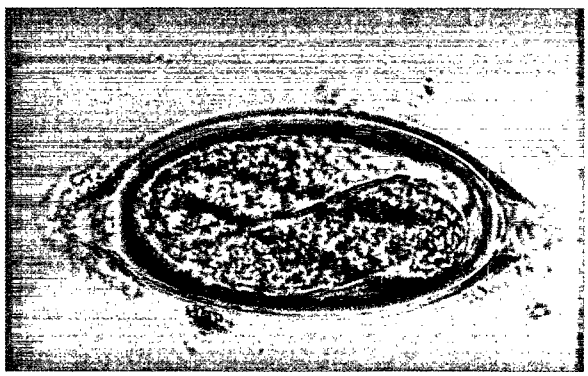
***Ascaris lumbricoides* (or var. *suum*) eggs, 65 μm, from anaerobically digested sludge. Two-cell stage.** (Photos on this page courtesy of Fox et al., 1981)



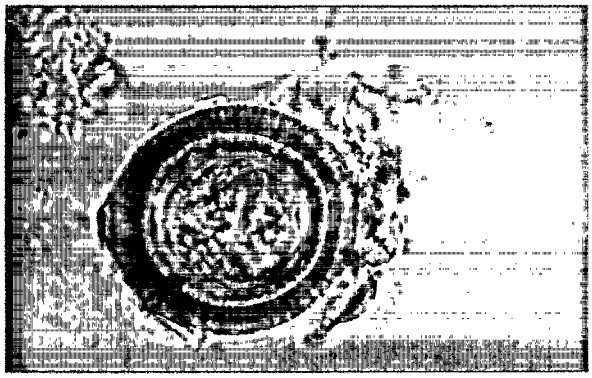
***Toxocara* sp. egg, 90 mμ, from raw sewage.**



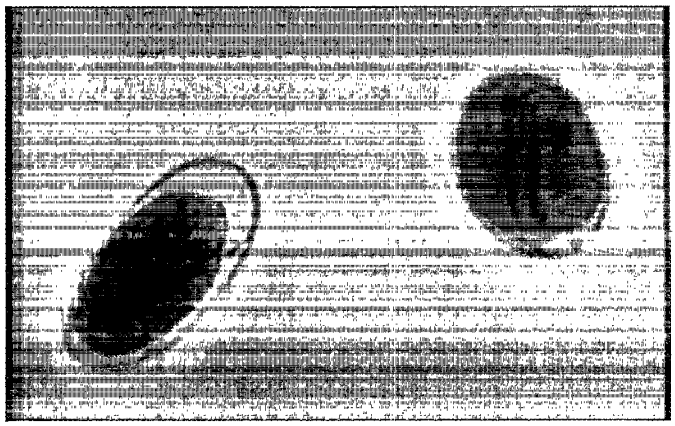
***Ascaris lumbricoides* (or var. *suum*) eggs, 65 μm, from anaerobically digested sludge.**



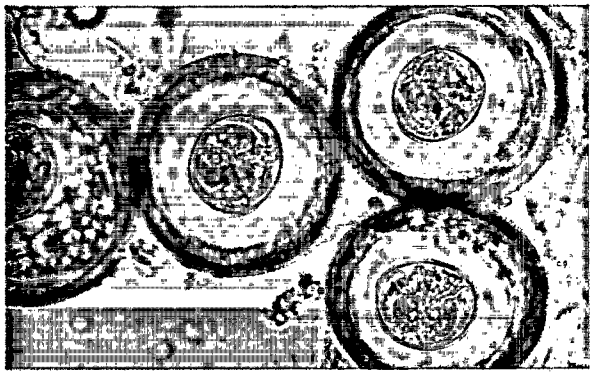
***Trichuris* sp egg, 80 μm, from anaerobically digested sludge.**



***Taenia* sp. ovum.** (Photo courtesy of Fox et al., 1981)



***Giardia lamblia* cysts.** (Photo courtesy of Frank Schaefer, U.S. EPA, Risk Reduction Engineering Laboratory, Cincinnati, Ohio)



***Hymenolepis* (tapeworm) ova.** (Photo courtesy of Fox et al., 1981)

Preparing compost for pathogen analysis. (Photo courtesy of U.S. Department of Agriculture, Beltsville, Maryland)

***Entamoeba coli* cyst, 15 μm, from anaerobically digested sludge.** (Photo courtesy of Fox et al., 1981)

Chapter 3
Overview of Part 503 Subpart D Requirements, Their Applicability, and Related Requirements

3.1 Introduction

The Subpart D (pathogen and vector attraction reduction) requirements of the 40 CFR Part 503 regulation apply to sewage sludge (both bulk sewage sludge and sewage sludge that is sold or given away in a bag or other container for application to the land) and domestic septage applied to the land or placed on a surface disposal site. The regulated community includes persons who generate or prepare sewage sludge for application to the land, as well as those who apply it to the land. Included is anyone who:

- Generates sewage sludge that is land applied or placed on a surface disposal site.
- Derives a material from sewage sludge.
- Applies sewage sludge to the land.
- Owns or operates a surface disposal site.

A sewage sludge cannot be applied to land or placed on a surface disposal site unless it has met the two basic types of requirements in Subpart D:

- Requirements to ensure reduction of pathogens.
- Requirements to reduce the potential of the sewage sludge to attract vectors (rodents, birds, insects, and other organisms that can transport pathogens).

These two types of requirements are separated in Part 503 (they were combined in Part 257), which allows flexibility in how they are achieved. Compliance with the two types of requirements must be demonstrated separately. Therefore, demonstration that a requirement for reduced vector attraction has been met does *not* imply that a pathogen reduction requirement also has been met, and vice versa.

This chapter provides an overview of these requirements, their applicability, and the requirements related to frequency of monitoring and recordkeeping. Where relevant, the titles of the sections in this chapter include the number of the Subpart D requirement discussed in the section. Chapters 4 through 6 provide detailed information on the pathogen and vector attraction reduction requirements.

3.2 Pathogen Reduction Requirements

Sewage Sludge [503.32(a) and (b)]

The pathogen reduction requirements for sewage sludge are divided into two categories: Class A and Class B. These requirements use a combination of technological and microbiological requirements to ensure reduction of pathogens.

The implicit goal of the Class A requirements is to reduce the pathogens in sewage sludge (including enteric viruses, pathogenic bacteria, and viable helminth ova) to below detectable levels. The implicit goal of the Class B requirements is to ensure that pathogens have been reduced to levels that are unlikely to pose a threat to public health and the environment under the specific use conditions. For Class B sewage sludge that is applied to land, site restrictions are imposed to minimize the potential for human and animal contact with the Class B sewage sludge for a period of time following land application until environmental factors have further reduced pathogens. Class B sludges cannot be sold or given away in bags or other containers for application to the land. There are no site restrictions for Class A sewage sludge.

Domestic Septage [503.32(c)]

As mentioned in Chapter 1, domestic septage is a form of sewage sludge. The requirements for domestic septage vary depending on how it is used or disposed. Domestic septage applied to a public contact site, lawn, or home garden must meet the same requirements as other forms of sewage sludge. Separate, less complicated requirements for pathogen reduction apply to domestic septage applied to agricultural land, forests, or reclamation sites. These requirements include site restrictions to reduce the potential for human contact and to allow for environmental attenuation, or pH adjustment with site restrictions only on harvesting crops. No pathogen requirements apply if domestic septage is placed on a surface disposal site.

3.3 Vector Attraction Reduction Requirements [503.33]

Subpart D specifies 12 options to demonstrate reduced vector attraction. These are referred to in this document as Options 1 through 12. Table 6-1 summarizes these options, and Chapter 6 provides more detailed information.

Reduction Through Treatment

Options 1 through 8 apply to sewage sludge that has been treated in some way to reduce vector attraction (e.g., aerobic or anaerobic digestion, composting, alkali addition, drying). These options consist of operating conditions or tests to demonstrate that vector attraction has been reduced in the treated sludge.

Option 12 is a requirement to demonstrate reduced vector attraction in domestic septage through elevated pH. This option applies only to domestic septage.

Reduction Through Barriers

Options 9 through 11 are "barrier" methods. These options require the use of soil as a physical barrier (i.e., by injection, incorporation, or as cover) to prevent vectors from coming in contact with the sewage sludge. They include injection of sewage sludge below the land surface, incorporation of sewage sludge into the soil, and placement of a cover over the sewage sludge. Options 9 through 11 apply to both sewage sludge and domestic septage. Option 11 may only be used at surface disposal sites.

3.4 Applicability of the Requirements [503.15]

The applicability of the pathogen and vector attraction reduction requirements is covered in Part 503.15. Tables 3-1 to 3-3 summarize the applicability of the Subpart D requirements to sewage sludge and domestic septage.

3.5 Frequency of Monitoring

Sewage Sludge [503.16(a)and 503.26(a)]

The Class A and Class B pathogen requirements and the vector attraction reduction Options 1 through 8 (the treatment-related methods) all involve some form of monitoring. The minimum frequency of monitoring for these requirements is given in Part 503.16(a) for land application and Part 503.26(a) for surface disposal. The frequency depends on the amount of sewage sludge used or disposed annually (see Table 3-4). The larger the amount used or disposed, the more frequently monitoring is required.

Domestic Septage [503.16(b) and 503.26(b)]

One of the options that can be used for demonstrating both pathogen reduction and vector attraction reduction in domestic septage is to elevate pH to ≥12 for 30 minutes (see Sections 5.6 and 6.13). When this option is used, each container of domestic septage (e.g., each tank truck load) applied to the land or placed on a surface disposal site must be monitored for pH.

3.6 Recordkeeping Requirements [503.17 and 503.27]

Recordkeeping requirements are covered in Part 503.17 for land application and Part 503.27 for surface disposal. Records are required for both sewage sludge and domestic septage. All records must be retained for 5 years except when the cumulative pollutant loading rates in Part 503 Subpart B (Land Application) of Part 503 are used.[1] In that case, certain records must be kept indefinitely. Some records must be reported to the permitting authority (see Section 3.7).

Table 3-1. Subpart D Requirements for Bulk Sewage Sludge[1]

	Land Application		
	Applied to Agricultural Land, a Forest, a Public Contact Site,[2] or a Reclamation Site[3]	Applied to a Lawn or Home Garden	Surface Disposal
Pathogen Requirements	Class A *or* Class B with site restrictions	Class A[4]	Class A *or* Class B excluding the site restrictions[5] *unless* the unit is covered at the end of each operating day, in which case no pathogen requirements apply[6]
Vector Attraction Reduction Requirements	Options 1-10[7]	Options 1-8[7,8]	Options 1-11[7]

[1]*Bulk sewage sludge* is sewage sludge that is *not* sold or given away in a bag or other container for application to the land.
[2]*Public contact site* is land with a high potential for contact by the public, e.g., public parks, ball fields, cemeteries, plant nurseries, turf farms, and golf courses.
[3]*Reclamation site* is drastically disturbed land (e.g., strip mine, construction site) that is reclaimed using sewage sludge.
[4]The regulation does not permit use of a sludge meeting Class B requirements on lawns or home gardens, because it would not be feasible under these circumstances to impose the site restrictions that are an integral part of the Class B requirements.
[5]Class B site restrictions are excluded here because the management practices in Part 503 Subpart C already impose similar site restrictions to reduce exposure to pollutants in sewage sludge.
[6]No pathogen requirement applies when daily cover isolates the sewage sludge and allows the environment to reduce the pathogens in the sewage sludge.
[7]See Chapter 6 for a description of these options.
[8]The two vector attraction reduction requirements that cannot be met when bulk sewage sludge is applied to a lawn or a home garden are injection of the bulk sewage sludge below the land surface and incorporation of bulk sewage sludge into the soil. Implementation of these requirements for bulk sewage sludge applied to a lawn or a home garden would be difficult, if not impossible.

Land Application

Records must be kept to ensure that the sewage sludge meets the applicable pollutant limits, management practices,[2]

[1]Cumulative pollutant loading rates are not related to pathogen control and therefore are not covered in this document.
[2]Pollutant limits and management practices are not related to the pathogen requirements and therefore are not covered in this document.

Table 3-2. Subpart D Requirements for Sewage Sludge Sold or Given Away in a Bag or Other Container

	Land Application	Surface Disposal
Pathogen Requirements	Class A[1]	N/A
Vector Attraction Reduction Requirements	Options 1-8[2]	N/A

[1]Class B requirements do not apply to sewage sludge that is sold or given away because it is not feasible to impose the Class B site restrictions when sewage sludge is widely distributed in bags or other containers.
[2]Only the treatment-related options for vector attraction reduction apply to sewage sludge that is sold or given away in bags or other containers for application to the land, because enforcement of the barrier options, which are implemented at the site of application, would be impossible. See Chapter 6 for a description of these options.

Table 3-3. Subpart D Requirements for Domestic Septage Applied to Agricultural Land, a Forest, or a Reclamation Site[1] or Placed on a Surface Disposal Site

	Application to Agricultural Land, a Forest, or a Reclamation Site[2]	Surface Disposal
Pathogen Requirements	Class B site restrictions only *or* a pH adjustment (pH ≥12 for 30 minutes) plus restrictions concerning crop harvesting	No pathogen requirement[3]
Vector Attraction Reduction Requirements	Options 9,10,12[4]	Options 9-12[4]

[1]For application to all other types of land, domestic septage must meet the same requirements as other forms of sewage sludge (see Tables 3-1 and 3-2).
[2]*Reclamation site* is drastically disturbed land (e.g., strip mine, construction site) that is reclaimed using sewage sludge.
[3]There is no pathogen requirement for domestic septage placed on a surface disposal site because site restrictions for grazing of animals, public access, and crop growing are already imposed by the Part 503 Subpart C management practices to reduce exposure to pollutants in domestic septage placed on a surface disposal site.
[4]See Chapter 6 for a description of these options.

one of the pathogen requirements, one of the vector attraction reduction requirements and, where applicable, the site restrictions associated with land application of Class B sludge. When sewage sludge is applied to land, the person preparing the sewage sludge for land application and the person applying bulk sewage sludge must keep records.[3,4] The person applying sewage sludge that was sold or given away does not have to keep records. Table 3-5 summarizes the recordkeeping requirements for land application.

[3]*Person* as defined under Part 503.9 may be an individual, association, partnership, corporation, municipality, state or federal agency, or an agent or employee of a state or federal agency.
[4]When sewage sludge is prepared by one person, and another person who places it in a bag or other container for sale or give-away for application to the land changes the quality of that sewage sludge, both persons must keep the records required of preparers (see Table 3-5).

Table 3-4. Frequency of Monitoring for Land Application and Surface Disposal

Amount of Sewage Sludge[1] (metric tons dry solids per 365-day period)	Frequency
Greater than zero but less than 290[2]	Once per year
Equal to or greater than 290 but less than 1,500[2]	Once per quarter (four times per year)
Equal to or greater than 1,500 but less than 15,000[2]	Once per 60 days (six times per year)
Equal to or greater than 15,000[2]	Once per month (12 times per year)

[1]*Either* the amount of bulk sewage sludge applied to the land, *or* the amount of sewage sludge received by a person who prepares sewage sludge that is sold or given away in a bag or other container for application to the land (dry weight basis), *or* the amount of sewage sludge (excluding domestic septage) placed on a surface disposal site.
[2]290 metric tons = 320 tons (approximately 0.9 tons/day for a year)
1,500 metric tons = 1,653 tons (approximately 4.5 tons/day for a year)
15,000 metric tons = 16,534 tons (approximately 45 tons/day for a year)

Surface Disposal

When sewage sludge is placed on a surface disposal site, the person preparing the sludge and the owner/operator of the surface disposal site must keep records. In the case of domestic septage applied to agricultural land, forest, or a reclamation site or placed on a surface disposal site, the person applying the domestic septage and the owner/operator of the surface disposal site may be subject to pathogen-related recordkeeping requirements, depending on which vector attraction reduction option was used. Table 3-6 summarizes the pathogen-related recordkeeping requirements for surface disposal.

Certification Statement

In every case, recordkeeping involves signing a certification statement that the requirement has been met. Parts 503.17 and 503.27 of the regulation contain the required certification language.

3.7 Reporting Requirements for Sewage Sludge [503.18 and 503.28]

Reporting requirements for sewage sludge are found in Part 503.18 for land application and Part 503.28 for surface disposal. These requirements apply to Class I sludge management facilities[5] and to publicly owned treatment works either with a design flow rate equal to or greater than 1 million gallons per day and/or that serve 10,000 or more people. These facilities must submit to the permitting authority the records they are required to keep as "preparers" of sewage sludge (see Tables 3-5 and 3-6) and/or as the owner/operators of surface disposal sites (see Table 3-6) on February 19 of each year. There are no reporting requirements associated with the use or disposal of domestic septage.

[5]A *Class I sludge management facility* is any publicly owned treatment works (POTW) required to have an approved pretreatment program under 40 CFR 403.8(a) [including any POTW located in a state that has assumed local program responsibilities under 40 CFR 403.10(e)] and any treatment works treating domestic sewage classified as a Class I sludge management facility by EPA or the state sludge management program because of the potential for its sewage sludge use or disposal practices to adversely affect public health and the environment.

Table 3-5. Summary of Pathogen-Related and Vector Attraction Reduction-Related Recordkeeping Requirements for Land Application of Sewage Sludge[1]

Who Must Keep Records?	Required Records: Description of How Class A Pathogen Requirement Was Met	Description of How Class B Pathogen Requirement Was Met	Description of How the Class B Site Restrictions Were Met at Each Site Where Sewage Sludge Was Applied	Description of How Pathogen Requirement for Domestic Septage Applied to Agricultural Land, a Forest, or a Reclamation Site Was Met	Description of How Vector Attraction Reduction Requirement Was Met	Certification Statement That the Requirement Was Met
Sewage Sludge—Pathogen Requirements						
Person preparing Class A bulk sewage sludge	✔					✔
Person preparing Class A sewage sludge for sale or give away in a bag or other container	✔					✔
Person preparing Class B sewage sludge		✔				✔
Person applying Class B sewage sludge			✔			✔
Sewage Sludge—Vector-Attraction Reduction Requirements						
Person preparing sewage sludge that meets one of the treatment-related vector attraction reduction requirements (Options 1-8)					✔	✔
Person applying sewage sludge if a barrier-related option, (Options 9-11) is used to meet the vector attraction reduction requirement					✔	✔
Domestic Septage						
Person applying domestic septage to agricultural land, a forest, or a reclamation site				✔	✔	✔

[1]Other recordkeeping requirements, not covered in this document, apply to pollutant limits and management practices.

3.8 Permits and Direct Enforceability [503.3]

Permits

Under Part 503.3(a), the requirements in Part 503 may be implemented through (1) permits issued to treatment works treating domestic sewage by EPA or by states with an EPA-approved sludge management program, and (2) by permits issued under Subtitle C of the Solid Waste Disposal Act; Part C of the Safe Drinking Water Act; the Marine Protection, Research, and Sanctuaries Act of 1972; or the Clean Air Act. Treatment works treating domestic sewage should submit a permit application[6] to the approved state program, or, if there is no such program, to the EPA Regional Sludge Coordinator (see Appendix A).

Direct Enforceability

Under Part 503.3(b), the requirements of Part 503 automatically apply and are directly enforceable even when no permit has been issued.

3.9 Compliance Period [503.2]

Compliance with the Part 503 requirements must be achieved as expeditiously as possible. Full compliance must be

[6]See 40 CFR Parts 122, 123, and 501; 54 FR 18716/May 2, 1989; and 58 FR 9404/February 19, 1993, for regulations establishing permit requirements and procedures, as well as requirements for states wishing to implement approved sewage sludge management programs as either part of their NPDES programs or under separate authority.

Table 3-6. Summary of Pathogen-Related and Vector Attraction Reduction-Related Recordkeeping Requirements for Surface Disposal of Sewage Sludge[1]

Who Must Keep Records?	Required Records: Description of How Class A or B Pathogen Requirement Was Met	Required Records: Description of How Vector Attraction Reduction Requirement Was Met	Required Records: Certification Statement That the Requirement Was Met
Sewage Sludge—Pathogen Requirements			
Person preparing the sewage sludge	✔		✔
Sewage Sludge—Vector Attraction Reduction Requirements			
Person preparing sewage sludge that meets one of the treatment-related vector attraction reduction requirements (Options 1-8)		✔	✔
Owner/operator of the surface disposal site if a barrier-related option (Options 9-11) is used to meet the vector attraction reduction requirement		✔	✔
Domestic Septage			
Person who places domestic septage on the surface disposal site if the domestic septage meets Option 12 for vector attraction reduction		✔	✔
Owner/operator of the surface disposal site if a barrier-related option (Options 9-11) is used to meet the vector attraction reduction requirement		✔	✔

[1]Other recordkeeping requirements, not covered in this document, apply to pollutant limits and management practices.

achieved no later than February 19, 1994, unless compliance requires construction of new pollution control facilities, in which case full compliance must be achieved no later than February 19, 1995. Monitoring, recordkeeping, and reporting requirements related to pathogens are effective on July 20, 1993.

Chapter 4
Class A Pathogen Requirements

4.1 Introduction

This chapter discusses the Class A pathogen requirements in Subpart D of the 40 CFR Part 503 regulation. Sewage sludge that is sold or given away in a bag or other container for application to land must meet these requirements (see Section 3.4). Bulk sewage sludge applied to a lawn or home garden also must meet these requirements. Bulk sewage sludge applied to other types of land may meet these requirements.

There are six alternative requirements for demonstrating Class A pathogen reduction. Two of these alternatives provide continuity with 40 CFR Part 257 by allowing use of Processes to Further Reduce Pathogens (PFRPs) and equivalent technologies (see Sections 4.8 and 4.9). Any one of these six alternatives may be met for the sewage sludge to be Class A with respect to pathogens. The implicit objective of all these requirements is to reduce pathogen densities to below detectable limits which are:

Salmonella sp.	less than 3 per 4 grams total solids sewage sludge
enteric viruses[1]	less than 1 per 4 grams total solids sewage sludge
viable helminth ova	less than 1 per 4 grams total solids sewage sludge

One of the vector attraction reduction requirements (see Chapter 6) also must be met when sewage sludge is applied to the land or placed on a surface disposal site.

This chapter discusses the Class A pathogen requirements. These include:

- A requirement concerning the relationship of pathogen reduction to reduction of vector attraction (Section 4.2).
- The six alternatives for Class A pathogen reduction (Sections 4.4 to 4.9), each of which includes a requirement to monitor for regrowth (Section 4.3).

The title of each section provides the number of the Subpart D requirement discussed in the section. The exact regulatory language can be found in Appendix B, which reproduces Subpart D. Chapters 7 and 8 provide guidance on the sampling and analysis needed to meet the Class A microbiological monitoring requirements.

4.2 Vector Attraction Reduction to Occur With or After Class A Pathogen Reduction [503.32(a)(2)]

The order of Class A pathogen reduction in relation to the reduction of vector attraction is important when certain vector attraction reduction options are used. Part 503.32(a)(2) requires that Class A pathogen reduction be accomplished *before or at the same time as* vector attraction reduction, except for vector attraction reduction by alkali addition [503.33(b)(6)] or drying [503.33(b)(7) and (8)] (see Chapter 6).

This requirement is necessary because Class A sludges have very low bacterial densities (below detectable levels). Bacterial regrowth (see Section 2.3) is possible unless a deterrent remains in the sewage sludge after the pathogen reduction process. Drying and alkali addition provide such a deterrent. So do the nonpathogenic bacterial populations left in the sewage sludge when the pathogen reduction process and the vector attraction reduction process occur at the same time, and when pathogen reduction occurs before vector attraction reduction.

4.3 Monitoring of Fecal Coliform or Salmonellae to Detect Regrowth [503.32(a)(3)-(8)]

The potential for regrowth of pathogenic bacteria in Class A sludges makes it important to ensure that substantial regrowth has not occurred. For this reason, all the Class A pathogen requirement alternatives require that:

- *Either* the density of fecal coliform in the sewage sludge be less than 1,000 MPN[2] per gram total solids (dry weight basis),
- *Or* the density of *Salmonella* sp. bacteria in the sewage be less than 3 MPN per 4 grams of total solids (dry weight basis).

[1]Enteric viruses are monitored using a method that detects several enterovirus species—a subset of enteric viruses. This method is presumed to be a good indicator of enteric viruses. Since the objective of the regulation is to reduce all enteric viruses to less than 1 per 4 grams total solids sewage sludge, this document refers to "enteric viruses" when discussing this requirement, although, in reality, the detection method enumerates only enteroviruses.

[2]The membrane filter method is not allowed here because, at the low fecal coliform densities expected, the filter would have too high a loading of sludge solids to permit a reliable count of the number of fecal coliform colonies.

This requirement must be met either:

- At the time of use or disposal,[3] *or*
- At the time the sewage sludge is prepared for sale or give away in a bag or other container for land application, *or*
- At the time the sewage sludge or material derived from the sewage sludge is prepared to meet the requirements in 503.10(b), 503.10(c), 503.10(e), or 503.10(f).[4]

In each case, the timing represents the last practical monitoring point before the sewage sludge is applied to the land or placed on a surface disposal site. Sewage sludge that is sold or given away cannot be monitored just prior to actual use or disposal; instead monitoring is required as it is prepared for sale or give away. Sewage sludge that meets the 503.10(b, c, d, or e) requirements is of very high quality with regard to both pollutants and pathogens and is therefore not subject to further control. For this reason, the regrowth requirement must be met at the time the sewage sludge is prepared to meet the 503.10 requirements, which in most cases is the last time the sewage sludge is controlled with respect to the Part 503 requirements.

The fecal coliform requirement is based on experimental work by Yanko (1987) which shows that this level of fecal coliform correlates with a very low level of salmonellae detection in composted sludge (EPA, 1992). Anecdotal reports suggest that some composting facilities may have difficulty meeting this requirement even when salmonellae are never detected. This might be expected under several circumstances. For example, very severe thermal treatment of sewage sludge during composting can totally eliminate salmonellae yet leave residual fecal coliforms. If the product has been poorly composted and thus is a good food source, fecal coliforms may have regrown after the compost cooled down from thermophilic temperatures. Because the salmonellae are absent, they cannot regrow. An even more probable circumstance could occur if the sewage sludge had been treated with lime before composting. Lime effectively destroys salmonellae in sewage sludge and leaves surviving fecal coliforms (Farrell et al., 1974). Under conditions favorable for regrowth, the fecal coliforms can regrow to levels higher than 1,000 MPN per gram. For this reason, all the Part 503 Class A alternatives allow use of a test (in lieu of the fecal coliform test) to determine that *Salmonella* sp. are below detectable limits.

4.4 Alternative 1: Thermally Treated Sewage Sludge [503.32(a)(3)]

This alternative may be used when the pathogen reduction process uses specific time-temperature regimes to reduce pathogens. Under these circumstances, time-consuming and expensive tests for the presence of specific pathogens can be avoided. It is only necessary to demonstrate that:

- *Either* fecal coliform densities are below 1,000 MPN per gram of total solids (dry weight basis), *or Salmonella* sp. bacteria are below detection limits (3 MPN per 4 grams total solids [dry weight basis]) at the time the sewage sludge is used or disposed, at the time the sewage sludge is prepared for sale or give away in a bag or other container for land application, or at the time the sewage sludge or material derived from the sewage sludge is prepared to meet the requirements in 503.10(b), 503.10(c), 503.10(e), or 503.10(f).
- *And* the required time-temperature regimes are met.

Microbiological Requirement

The microbiological portion of the requirement is designed to ensure that the microbiological reductions expected as a result of the time-temperature regimes have actually been attained. This requirement uses the low level of fecal coliform or nondetection of salmonellae as an indicator of the destruction of pathogenic bacteria, enteric viruses, and helminths (based on research by Lee et al. [1989], Yanko [1987], and Martin et al. [1990], and discussed by Farrell [1992]). The microbiological requirement also ensures (as described in Section 4.3) that regrowth of bacterial pathogens has not occurred.

Time-Temperature Requirement

Four different time-temperature regimes are given in Alternative 1. Each regime is based on the percent solids of the sewage sludge and on operating parameters of the treatment process. Experimental evidence (EPA, 1992) demonstrates that these four time-temperature regimes reduce the pathogenic organisms to below detectable levels.

The four time-temperature regimes are summarized in Table 4-1. They involve two different time-temperature equations. The equation used in Regimes A through C results in requirements that are more stringent than the requirement obtained using the equation in Regime D. For any given time, the temperature calculated for the Regime D equation will be 3°C (37°F) lower than the temperature calculated for the Regimes A through C equation.

A more conservative equation is required for sewage sludges with 7% or more solids (i.e., those covered by Regimes A and B) because these sewage sludges form an internal structure that inhibits the mixing that contributes to uniform distribution of temperature. The more stringent equation is also used in Regime C (even though this regime applies to sewage sludges with less than 7% solids) because insufficient information is available to apply the less stringent equation for times less than 30 minutes.

The time-temperature requirements apply to every particle of sewage sludge processed. Time at the desired temperature is readily determined for batch operations, turbulent flow in pipes, or even laminar flow in pipes (time of contact is one-half the contact time calculated from the bulk throughput rate). Time of contact also can be calculated for a number of completely mixed reactors in series, but for the very large reductions

[3] Minus the time needed to test the sewage sludge and obtain the test results prior to use or disposal (see Section 7.3).
[4] The 503.10(b)(c)(e) and (f) requirements are not discussed in this document.

Table 4-1. The Four Time-Temperature Regimes for Alternative 1 (Thermally Treated Sewage Sludge) [503.32(a)(3)]

Regime	Part 503 Number	Applies to:	Required Time-Temperature Relationship[1]
A	503.32(a)(3)(ii)(A)	Sewage sludge with at least 7% solids (except those covered by Regime B)	$D = 131{,}700{,}000/10^{0.1400t}$ $t \geq 50°C$ (122°F)[2] $D \geq 0.0139$ (i.e., 20 minutes)[3]
B	503.32(a)(3)(ii)(B)	Sewage sludge with at least 7% solids that are small particles heated by contact with either warmed gases or an immiscible liquid[4]	$D = 131{,}700{,}000/10^{0.1400t}$ $t \geq 50°C$ (122°F)[2] $D \geq 1.74 \times 10^{-4}$ (i.e., 15 seconds)[5]
C	503.32(a)(3)(ii)(C)	Sewage sludge with less than 7% solids treated in processes with less than 30 minutes contact time	$D = 131{,}700{,}000/10^{0.1400t}$ 1.74×10^{-4} (i.e., 15 seconds) $\leq D \leq 0.021$ (i.e., 30 minutes)[6]
D	503.32(a)(3)(ii)(D)	Sewage sludge with less than 7% solids treated in processes with at least 30 minutes contact time	$D = 50{,}070{,}000/10^{0.1400t}$ $t \geq 50°C$ (122°F)[2] $D \geq 0.021$ (i.e., 30 minutes)[7]

[1]D = time in days; t = temperature.
[2]The restriction to temperatures of at least 50°C (122°F) is imposed because information on the time-temperature relationship at lower temperatures is uncertain.
[3]A minimum time at 20 minutes is required to ensure that the sewage sludge has been uniformly heated.
[4]Two examples of sewage sludge to which this requirement applies are:
— Sewage sludge cake that is mixed with previously dried solids to make the entire mass a mixture of separate particles, and is then dried by contact with a hot gas stream in a rotary drier.
— Sewage sludge dried in a multiple-effect evaporator system in which the system sludge particles are suspended in a hot oil that is heated by indirect heat transfer with condensing steam.
[5]Time-at-temperature of as little as 15 seconds is allowed because, for this type of sewage sludge, heat transfer between particles and the heating fluid is excellent. Note that the temperature is the temperature achieved by the sewage sludge particles, not the temperature of the carrier medium.
[6]Time-at-temperature of as little as 15 seconds is allowed because heat transfer and uniformity of temperature is excellent in this type of sewage sludge. The maximum time of 30 minutes is specified because a less stringent regime (D) applies when time-at-temperature is 30 minutes or more.
[7]Time-at-temperature of at least 30 minutes is required because information on the effectiveness of this time-temperature regime for reducing pathogens at temperatures of less than 30 minutes is uncertain.

required to reduce densities to below detection limits, this type of processing would require so many reactors in series as to be totally impractical.

4.5 Alternative 2: Sewage Sludge Treated in a High pH–High Temperature Process (Alkaline Treatment) [503.32(a)(4)]

This alternative describes conditions of a particular high temperature–high pH process that has proven effective in reducing pathogens to below detectable levels. The process conditions required by the regulation are:

- Elevating pH to greater than 12 for more than 72 hours.
- Maintaining the temperature above 52°C (126°F) for at least 12 hours during the period that the pH is greater than 12.
- Air drying to over 50% solids after the 72-hour period of elevated pH.

The hostile conditions of high pH and high temperature for prolonged time periods allow a variance to a less stringent time-temperature regime than for the thermal requirements under Alternative 1. The pH of the sludge is measured at 25°C (77°F) or an appropriate correction is applied (see Section 7.7).

As with all the Class A alternatives, microbiological monitoring for fecal coliforms or salmonellae is required (see Section 4.3) to ensure that pathogens have been reduced and regrowth has not occurred.

4.6 Alternative 3: Sewage Sludge Treated in Other Processes [503.32(a)(5)]

This alternative applies to sewage sludge produced by processes that do not meet the process conditions required by Alternatives 1 and 2. This requirement relies on comprehensive monitoring of bacteria, enteric viruses, and viable helminth ova to demonstrate adequate reduction of pathogens:

- *Either* the density of fecal coliform in the sewage sludge must be less than 1,000 MPN per gram of total solids (dry weight basis), *or* the density of Salmonella sp. bacteria in sewage sludge must be less than 3 MPN per 4 grams of total solids (dry weight basis) at the time the sewage sludge is used or disposed, at the time the sewage sludge is prepared for sale or give away in a bag or other container for land application, or at the time the sewage sludge or material derived from the sewage sludge is prepared to meet the requirements in 503.10(b), 503.10(c), 503.10(e), or 503.10(f).
- The density of enteric viruses in the sewage sludge after pathogen treatment must be less than 1 PFU per 4 grams of total solids (dry weight basis).

- The density of viable helminth ova in the sewage sludge after pathogen treatment must be less than 1 per 4 grams of total solids (dry weight basis).

Testing for enteric viruses and viable helminth ova can be complicated by the fact that they are sometimes not present in the untreated sewage sludge. In this case, an absence of the organisms in the treated sewage sludge does not demonstrate that the process can reduce them to below detectable limits. *For this reason, Alternative 3 requires that the feed sewage sludge be analyzed for enteric viruses and viable helminth ova.* If these organisms are not detected in the feed sewage sludge, the sewage sludge is presumed to be acceptable as a Class A material until the next monitoring episode. Monitoring is continued until enteric viruses and/or viable helminth ova are detected in the feed sewage sludge (i.e., the density of enteric viruses is greater than or equal to 1 PFU per 4 grams total solids [dry weight basis] *and/or* the density of viable helminth ova is greater than or equal to 1 per 4 grams of total solids [dry weight basis]). At this point, the treated sewage sludge is then analyzed to see if these organisms survived treatment. If enteric viruses densities are below detection limits, the sewage sludge meets Class A requirements for enteric viruses and will continue to do so *as long as the treatment process is operated under the same conditions that successfully reduced the enteric virus densities.* If the viable helminth ova densities are below detection limits, the process meets the Class A requirements for enteric viruses and will continue to do so *as long as the treatment process is operated under the same conditions that successfully reduced the viable helminth ova densities.* Thus, it is essential to monitor and document operating conditions until adequate enteric virus and viable helminth ova reduction have been successfully demonstrated. Samples of untreated and treated sewage sludge must correspond (see Section 7.4).

Tests for enteric viruses and viable helminth ova take substantial time: 4 weeks to determine whether helminth ova are viable, and 2 weeks or longer for enteric viruses. The treatment works operator does not know whether the feed sewage sludge has enteric viruses or helminth ova until at least 2 to 4 weeks after the first samples for testing feed densities are taken. This problem can be overcome by sampling both the feed and the treated sewage sludge during each monitoring episode and preserving the treated sewage sludge samples until the results of the feed analysis indicate whether analysis of the treated sewage sludge is necessary. For enteric viruses, the sewage sludge should be stored frozen, unless the sample can be processed within 24 hours, in which case the samples may be stored at 4°C (39°F). For viable helminth ova, the sewage sludge should be stored at about 4°C (39°F) (see Section 8.6).

4.7 Alternative 4: Sewage Sludge Treated in Unknown Processes [503.32(a)(6)]

This requirement is similar to Alternative 3, except there is no option to substitute monitoring of effective operating parameters for microbiological monitoring. The sewage sludge must meet the following limits at the time the sewage sludge (or material derived from sludge) is used or disposed, at the time the sewage sludge is prepared for sale or give away in a bag or other container for land application, or at the time the sewage sludge or material derived from the sewage sludge is prepared to meet the requirements in 503.10(b), 503.10(c), 503.10(e), or 503.10(f):

- *Either* the density of fecal coliform in the sewage sludge must be less than 1,000 MPN per gram of total solids (dry weight basis), *or* the density of *Salmonella* sp. bacteria in sewage sludge must be less than 3 MPN per 4 grams of total solids (dry weight basis).

- The density of enteric viruses in the sewage sludge must be less than 1 PFU per 4 grams of total solids (dry weight basis).

- The density of viable helminth ova in the sewage sludge must be less than 1 per 4 grams of total solids (dry weight basis).

This requirement applies in the following situations:

- A sewage sludge treatment process is unknown.

- The sewage sludge was produced with the process operating at conditions less stringent than the operating conditions at which the sewage sludge could qualify as Class A under other alternatives.

- The past history of the sewage sludge is not completely known.

The requirements for enteric viruses and viable helminth ova may be modified by the permitting authority. An example of this situation would be a pile of sewage sludge that had been stored for many years. If fecal coliform densities are sufficiently low, enteric virus survival is unlikely. In such a case, the permitting authority may reduce the requirement to test for enteric viruses, but would probably insist on measuring viable helminth ova densities.

4.8 Alternative 5: Use of PFRP [503.32(a)(7)]

Alternative 5 provides continuity with the 40 CFR Part 257 regulation. This alternative states that sewage sludge is considered to be Class A if:

- It has been treated in one of the Processes to Further Reduce Pathogens (PFRPs) listed in Appendix B of the regulation, *and*

- *Either* the density of fecal coliform in the sewage sludge is less than 1,000 MPN per gram total solids (dry weight basis), *or* the density of Salmonella sp. bacteria in the sewage sludge is less than 3 MPN per 4 grams total solids (dry weight basis) at the time the sewage sludge is used or disposed, at the time the sewage sludge is prepared for sale or give away in a bag or other container for land application, or at the time the sewage sludge or material derived from the sewage sludge is prepared to meet the requirements in 503.10(b), 503.10(c), 503.10(e), or 503.10(f).

To meet this requirement, the sewage sludge treatment processes must be operated according to the conditions listed in Appendix B of the regulation.

The Appendix B list of PFRPs is reproduced in Table 4-2. This list is very similar to the PFRP technologies listed in 40 CFR Part 257, with two major differences:

- All requirements related to vector attraction reduction have been removed.
- All the "add-on" processes listed in Part 257 are now full-fledged PFRPs.

Under this alternative, treatment processes classified as PFRP under 40 CFR Part 257 can continue to be operated; however, microbiological monitoring must now be performed to ensure that the pathogen density levels are below detection limits and to ensure that regrowth of *Salmonella* sp. bacteria does not occur between treatment and use or disposal.

Table 4-2. Processes to Further Reduce Pathogens (PFRPs) Listed in Appendix B of 40 CFR Part 503[1]

1. Composting

Using either the within-vessel composting method or the static aerated pile composting method, the temperature of the sewage sludge is maintained at 55°C (131°F) or higher for 3 days.

Using the windrow composting method, the temperature of the sewage sludge is maintained at 55°C (131°F) or higher for 15 days or longer. During the period when the compost is maintained at 55°C (131°F) or higher, there shall be a minimum of five turnings of the windrow.

2. Heat Drying

Sewage sludge is dried by direct or indirect contact with hot gases to reduce the moisture content of the sewage sludge to 10% or lower. Either the temperature of the sewage sludge particles exceeds 80°C (176°F) or the wet bulk temperature of the gas in contact with the sewage sludge as the sewage sludge leaves the dryer exceeds 80°C (176°F).

3. Heat Treatment

Liquid sewage sludge is heated to a temperature of 180°C (356°F) or higher for 30 minutes.

4. Thermophilic Aerobic Digestion

Liquid sewage sludge is agitated with air or oxygen to maintain aerobic conditions and the mean cell residence time (i.e., the solids retention time) of the sewage sludge is 10 days at 55°C (131°F) to 60°C (140°F).

5. Beta Ray Irradiation

Sewage sludge is irradiated with beta rays from an electron accelerator at dosages of at least 1.0 megarad at room temperature (ca. 20°C [68°F]).

6. Gamma Ray Irradiation

Sewage sludge is irradiated with gamma rays from certain isotopes, such as Cobalt 60 and Cesium 137, at dosages of at least 1.0 megarad at room temperature (ca. 20°C [68°F]).

7. Pasteurization

The temperature of the sewage sludge is maintained at 70°C (158°F) or higher for 30 minutes or longer.

[1]Chapter 10 provides a detailed description of these technologies.

4.9 Alternative 6: Use of a Process Equivalent to PFRP [503.32(a)(8)]

The 40 CFR Part 257 regulation allowed any treatment process to be determined *equivalent to* a PFRP. Under Alternative 6, sewage sludge is considered to be a Class A sewage sludge if:

- It is treated by any process equivalent to a PFRP, *and*
- *Either* the density of fecal coliform in the sewage sludge is less than 1,000 MPN per gram total solids (dry weight basis), *or* the density of *Salmonella* sp. bacteria in the sewage sludge is less than 3 MPN per 4 grams total solids (dry weight basis) at the time the sewage sludge is used or disposed, at the time the sewage sludge is prepared for sale or give away in a bag or other container for land application, or at the time the sewage sludge or material derived from the sewage sludge is prepared to meet the requirements in 503.10(b), 503.10(c), 503.10(e), or 503.10(f).

Processes Already Recommended as Equivalent

Table 4-3 lists some of the processes recommended to be equivalent to PFRP under Part 257. Since these processes have

Table 4-3. A Partial List of Processes Recommended as Equivalent to PFRP Under Part 257[1]

Operator	Process Description
Scarborough Sanitary District Scarborough, Maine	Static pile aerated "composting" operation that uses fly ash from a paper company as a bulking agent. The process creates pile temperatures of 60°C to 70°C (140°F to 158°F) within 24 hours and maintains these temperatures for up to 14 days. The material is stockpiled after 7 to 14 days of "composting" and then marketed.
Mount Holly Sewage Authority Mount Holly, New Jersey	Zimpro 50-gpm low-pressure wet air oxidation process. The process involves heating raw primary sewage sludge to 177°C to 204°C (350°F to 400°F) in a reaction vessel under pressures of 250 to 400 psig for 15 to 30 minutes. Small volumes of air are introduced into the process to oxidize the organic solids.
Miami-Dade Water and Sewer Authority Miami, Florida	Anaerobic digestion followed by solar drying. Sewage sludge is processed by anaerobic digestion in two well-mixed digesters operating in series in a temperature range of 35°C to 37°C (95°F to 99°F). Total residence time is 30 days. The sewage sludge is then centrifuged to produce a cake of between 15% to 25% solids. The sewage sludge cake is dried for 30 days on a paved bed at a depth of no more than 46 cm (18 inches). Within 8 days of the start of drying, the sewage sludge is turned over at least once every other day until the sewage sludge reaches a solids content of greater than 70%. The PFRP recommendation was conditional on the microbiological quality of the sewage sludge.

[1]These processes were all recommended for site-specific equivalency (see Section 11.1).

already been recommended as equivalent, the sewage sludges produced by these processes should meet the Class A pathogen requirements as long as they meet the microbiological requirements.

An Approach to Determining Equivalency

One procedure likely to be used for determining equivalency under Part 503 is Alternative 3. This alternative enables a treatment works to demonstrate, through microbiological monitoring, that a sewage sludge treatment process effectively reduces enteric viruses and viable helminth ova to below detectable levels. Once these reductions have been demonstrated, no further enteric virus or viable helminth ova monitoring is required as long as the process continues to be operated under the conditions that produced the reduction (see Section 4.6). The only further monitoring that must be conducted is the fecal coliform or salmonellae monitoring required of all Class A alternatives. Thus, for all practical purposes, a process that successfully demonstrates all the requirements in Alternative 3 can be considered equivalent to a PFRP.

Who Determines Equivalency?

Part 503 gives the permitting authority responsibility for determining equivalency under Alternative 6. The EPA's Pathogen Equivalency Committee (PEC) is available as a resource to provide guidance and recommendations on equivalency determinations to both the permitting authority and the regulated community (see Chapter 11).

Chapter 5
Class B Pathogen Requirements and Requirements for Domestic Septage Applied to Agricultural Land, a Forest, or a Reclamation Site

5.1 Introduction

Class B pathogen requirements can be met in three different ways. The implicit objective of all three alternatives is to ensure that pathogenic bacteria and enteric viruses are adequately reduced in density, as demonstrated by a fecal coliform density in the treated sludge of 2 million MPN or CFU per gram total solids sewage sludge (dry weight basis).[1] Viable helminth ova are not necessarily reduced in a Class B sludge.

Unlike a Class A sludge, which is essentially pathogen-free, a Class B sludge contains some pathogens. For this reason, site restrictions that restrict crop harvesting, animal grazing, and public access for a certain period of time until environmental factors have further reduced pathogens have to be met when a Class B sewage sludge is applied to land. Where appropriate, these restrictions are designed to ensure sufficient reduction in viable helminth ova—the hardiest of pathogens—since these pathogens may not have been reduced during sludge treatment.

The Class B requirements apply to bulk sewage sludge that is applied to agricultural land, a forest, a public contact site, or a reclamation site. Sewage sludge that is placed on a surface disposal site also must meet the Class B pathogen requirement, unless the active sewage sludge unit on which the sewage sludge is placed is covered at the end of each operating day (see Table 3-1).

The requirements for pathogens in domestic septage applied to agricultural land, forest, or a reclamation site are different from the Class B requirements for sewage sludge applied to those types of land. Domestic septage applied to other types of land (e.g., a public contact site) must meet the pathogen requirements and site restrictions for sewage sludge. No pathogen-related requirements apply to domestic septage placed on a surface disposal site.

[1]Farrell et al. (1985) have shown that if a processed sewage sludge has a fecal coliform density of 2 million MPN or CFU per gram, pathogenic viruses and bacteria are reduced. A comparison of suspended solids densities in entering wastewater to suspended solids densities in treated sewage sludge shows that this density of fecal coliform in treated sludge represents a 100-fold (2-log) reduction in fecal coliform density, and is expected to correlate with an approximately 1.5-log (approximately 32-fold) reduction in salmonellae density and an approximately 1.3-log (20-fold) reduction in the density of enteric viruses.

Class B sewage sludge and domestic septage also must meet a vector attraction reduction requirement (see Chapter 6).

Sections 5.2 to 5.4 discuss the three alternative Class B pathogen requirements for sewage sludge. Section 5.5 discusses the site restrictions and Section 5.6 presents the requirements for domestic septage applied to agricultural land, forests, or reclamation sites. The title of each section provides the number of the Subpart D requirement discussed in the section. The exact regulatory language can be found in Appendix B, which reproduces the Subpart D regulation. Chapters 7 and 8 provide guidance on the sampling and analysis necessary to meet the Class B microbiological requirements.

5.2 Sewage Sludge Alternative 1: Monitoring of Fecal Coliform [503.32(b)(2)]

Alternative 1 requires that seven samples of treated sewage sludge be collected at the time of use or disposal, *and* that the geometric mean fecal coliform density of these samples be less than 2 million CFU or MPN per gram of sewage sludge solids (dry weight basis).

This approach uses fecal coliform density as an indicator of the average density of bacterial and viral pathogens. Over the long term, fecal coliform density is expected to correlate with bacterial and viral pathogen density in sewage sludge treated by biological treatment processes (EPA, 1992).

This alternative requires analysis of multiple samples during each monitoring episode because the methods used to determine fecal coliform density (membrane filter methods and the MPN dilution method) have poor precision and sewage sludge quality varies. Use of at least seven samples is expected to reduce the standard error to a reasonable value.

The standard deviation can be a useful predictive tool. A relatively high standard deviation for the fecal coliform density indicates a wide range in the densities of the individual samples. The wider this range (i.e, the higher the standard deviation), the less the treatment process can be relied on to consistently produce sewage sludge that will meet the requirement. A high standard deviation (e.g., a log standard deviation > 0.3) can therefore alert treatment workers of the potential need for process modifications to improve consistency and reliability.

5.3 Sewage Sludge Alternative 2: Use of PSRP [503.32(b)(3)]

Class B Alternative 2 provides continuity with the 40 CFR Part 257 regulation. Under this alternative, sewage sludge is considered to be Class B if it is treated in one of the "Processes to Significantly Reduce Pathogens" (PSRPs) listed in Appendix B of Part 503. The listed PSRPs are reproduced in Table 5-1 and described in detail in Chapter 9. They are similar to the PSRPs listed in the Part 257 regulation, except that all conditions related to reduction of vector attraction have been removed. Under this alternative, sewage sludge treated by processes that are PSRPs under 40 CFR Part 257 are Class B with respect to pathogens. Unlike the comparable Class A requirement (see Section 4.8), this Class B alternative does not require microbiological monitoring.

Table 5-1. Processes to Significantly Reduce Pathogens (PSRPs) Listed in Appendix B of 40 CFR Part 503[1]

1. Aerobic Digestion

Sewage sludge is agitated with air or oxygen to maintain aerobic conditions for a specific mean cell residence time (i.e., solids retention time) at a specific temperature. Values for the mean cell residence time and temperature shall be between 40 days at 20°C (68°F) and 60 days at 15°C (59°F).

2. Air Drying

Sewage sludge is dried on sand beds or on paved or unpaved basins. The sewage sludge dries for a minimum of 3 months. During 2 of the 3 months, the ambient average daily temperature is above 0°C (32°F).

3. Anaerobic Digestion

Sewage sludge is treated in the absence of air for a specific mean cell residence time (i.e., solids retention time) at a specific temperature. Values for the mean cell residence time and temperature shall be between 15 days at 35°C to 55°C (131°F) and 60 days at 20°C (68°F).

4. Composting

Using either the within-vessel, static aerated pile, or windrow composting methods, the temperature of the sewage sludge is raised to 40°C (104°F) or higher and remains at 40°C (104°F) or higher for 5 days. For 4 hours during the 5-day period, the temperature in the compost pile exceeds 55°C (131°F).

5. Lime Stabilization

Sufficient lime is added to the sewage sludge to raise the pH of the sewage sludge to 12 after 2 hours of contact.

[1]Chapter 9 provides a detailed description of these technologies.

5.4 Sewage Sludge Alternative 3: Use of Processes Equivalent to PSRP [503.32(b)(4)]

The former Part 257 regulation allowed the sewage sludge to be treated by a process determined to be *equivalent to* a PSRP. Under Alternative 3, sewage sludge treated by any process determined to be equivalent to a PSRP is considered to be a Class B sewage sludge.

Table 5-2 provides a partial list of processes that were recommended as equivalent to PSRP under 40 CFR Part 257. Because these processes are already recommended as equivalent, the sewage sludge treated by these processes is Class B with respect to pathogens.

Table 5-2. A Partial List of Processes Recommended as Equivalent to PSRP Under Part 257[1]

Operator	Process Description
Town of Telluride, Colorado	Combination oxidation ditch, aerated storage, and drying process. Sewage sludge is treated in an oxidation ditch for at least 26 days and then stored in an aerated holding tank for up to a week. Following dewatering to 18% solids, the sewage sludge is dried on a paved surface to a depth of 2 feet (0.6 m). The sewage sludge is turned over during drying. After drying to 30% solids, the sludge is stockpiled prior to land application. Together, the drying and stockpiling steps take approximately 1 year. To ensure that PSRP requirements are met, the stockpiling period must include one full summer season.
Comprehensive Materials Management, Inc. Houston, Texas	Use of cement kiln dust (instead of lime) to treat sewage sludge by raising sewage sludge pH to at least 12 after 2 hours of contact. Dewatered sewage sludge is mixed with cement kiln dust in an enclosed system.
N-Viro Energy Systems, Ltd. Toledo, Ohio	Use of cement kiln dust and lime kiln dust (instead of lime) to treat sewage sludge by raising the pH. Sufficient lime or kiln dust is added to sewage sludge to produce a pH of 12 for at least 12 hours of contact.
Public Works Department Everett, Washington	Anaerobic digestion of lagooned sewage sludge. Suspended solids had accumulated in a 30-acre (12-hectare) aerated lagoon that had been used to aerate wastewater. The lengthy detention time in the lagoon (up to 15 years) resulted in a level of treatment exceeding that provided by conventional anaerobic digestion. The percentage of fresh or relatively unstabilized sewage sludge was very small compared to the rest of the accumulation (probably much less than 1% of the whole).
Haikey Creek Wastewater Treatment Plant Tulsa, Oklahoma	Oxidation ditch treatment plus storage. Sewage sludge is processed in aeration basins followed by storage in aerated sludge holding tanks. The total sewage sludge aeration time is greater than the aerobic digestion operating conditions specified in the Part 503 regulation of 40 days at 20°C (68°F) to 60 days at 15°C (59°F). The oxidation ditch sludge is then stored in batches for at least 45 days in an unaerated condition or 30 days under aerated conditions.
Ned K. Burleson & Associates, Inc. Fort Worth, Texas	Aerobic digestion for 20 days at 30°C (86°F) or 15 days at 35°C (95°F).

[1]All processes were recommended for site-specific equivalency, except the N-Viro System, which was recommended for national equivalency (see Section 11.1 for definition of site-specific and national equivalency).

Part 503 gives the permitting authority responsibility for determining equivalency. The Pathogen Equivalency Committee is available as a resource to provide guidance and recommendations on equivalency determinations to the permitting authorities (see Chapter 11).

5.5 Site Restrictions [503.32(b)(5)]

Sewage sludge that meets the Class B requirements may contain reduced but still significant densities of pathogenic bacteria, viruses, and viable helminth ova. Thus, site restrictions are needed to further reduce pathogenic organisms if the sewage sludge is applied to land. These requirements are based on scientific data regarding how rapidly pathogens die off on the soil surface or within the soil (EPA, 1992). The site restrictions for Class B sewage sludges are summarized below. The regulatory language is given in italics.

Food Crops with Harvested Parts That Touch the Sewage Sludge/Soil Mixture

503.32(b)(5)(i): Food crops with harvested parts that touch the sewage sludge/soil mixture and are totally above the land surface shall not be harvested for 14 months after application of sewage sludge.

This time frame is sufficient to enable environmental conditions such as sunlight, temperature, and desiccation, to reduce pathogens on the land surface to below detection limits. Note that the restriction applies to harvesting. Growing of these food crops (such as melons, cucumbers, or strawberries) can begin prior to 14 months after application, as long as the crops are not ready for harvesting earlier than 14 months after sewage sludge application.

Food Crops with Harvested Parts Below the Land Surface

503.32(b)(5)(ii): Food crops with harvested parts below the surface of the land shall not be harvested for 20 months after application of sewage sludge when the sewage sludge remains on the land surface for 4 months or longer prior to incorporation into the soil.

For example, for a September 1994 harvest, sewage sludge could be applied to the soil surface up to the end of December 1992, plowed or disked into the soil in April 1993, and the crop could be harvested in September 1994. Examples of crops with harvested parts below the land surface are potatoes, radishes, and carrots.

503.32(b)(5)(iii): Food crops with harvested parts below the surface of the land shall not be harvested for 38 months after application of sewage sludge when the sewage sludge remains on the land surface for less than 4 months prior to incorporation into the soil.

Contamination of the surface of root crops with viable helminth ova is a principal concern under these circumstances. Four months is considered the minimum time for environmental conditions to reduce viable helminth ova in sewage sludge on the land surface to negligible levels. Sewage sludge incorporated into the soil surface less than 4 months after application may contain significant numbers of viable helminth ova. Once incorporated into the soil, die-off of these organisms proceeds much more slowly; therefore, a substantially longer waiting period is required to protect public health.

Food Crops, Feed Crops, and Fiber Crops

503.32(b)(5)(iv): Food crops, feed crops, and fiber crops shall not be harvested for 30 days after application of sewage sludge.

This restriction covers food crops that are not covered by 503.32(b)(i-iii) (i.e., it covers food crops that do not have harvested parts that touch the sewage sludge/soil mixture or that are below the land surface). The restriction also applies to all feed and fiber crops. These crops may become contaminated when sewage sludge is applied to the land. Harvesting of these crops could result in the transport of sewage sludge pathogens from the growing site to the outside environment. After 30 days, however, any pathogens in sewage sludge that may have adhered to the crop during application will likely have been reduced to negligible levels. Hay is an example of a crop covered by this restriction.

Animal Grazing

503.32(b)(5)(v): Animals shall not be allowed to graze on the land for 30 days after application of sewage sludge.

Sewage sludge can adhere to animals that walk on sewage-sludge-amended land and thereby be brought into potential contact with humans who come in contact with the animals (for example, riding horses and milking cows allowed to graze on a sewage-sludge-amended pasture). Thirty days is sufficient to substantially reduce the pathogens in surface-applied sewage sludge, thereby significantly reducing the risk of human and animal contamination.

Turf Growing

503.32(b)(5)(vi): Turf grown on land where sewage sludge is applied shall not be harvested for 1 year after application of the sewage sludge when the harvested turf is placed on either land with a high potential for public exposure or a lawn, unless otherwise specified by the permitting authority.

The 1-year waiting period is designed to significantly reduce pathogens in the soil so that subsequent contact of the turf layer will not pose a risk to public health and animals. A permitting authority may reduce this time period in low-risk applications, e.g., turf applied by the commercial grower's staff to lawns that will not experience public traffic immediately after application.

Public Access

503.32(b)(5)(vii): Public access to land with a high potential for public exposure shall be restricted for 1 year after application of the sewage sludge.

As with the turf requirement above, a 1-year waiting period is necessary to protect public health and animals in a potentially high-exposure situation. A baseball diamond or a soccer field is an example of land with a high potential for public exposure. The land gets heavy use and contact with the soil is substantial (players fall on it and dust is raised which is inhaled and ingested).

503.32(b)(5)(viii): Public access to land with a low potential for public exposure shall be restricted for 30 days after application of the sewage sludge.

A farm field used to grow corn or soybeans is an example of a low potential for public exposure. Even farm family members walk about very little on such fields.

5.6 Domestic Septage [503.32(c)]

Under Part 503.32(c), pathogen reduction in domestic septage applied to agricultural land, a forest, or a reclamation site[2] may be reduced in one of two ways:

- *Either* all the Class B site restrictions under 503.32(b)(5)—see Section 5.5, above—must be met,
- *Or* the pH of the domestic septage must be raised to 12 or higher by alkali addition and maintained at pH 12 or higher for 30 minutes without adding more alkali, *and* the site restrictions on crop harvesting in 503.32(b)(5)(i-iv) must be met (see Section 5.5). The regulation uses the term alkali in the broad sense to mean any substance that causes an increase in pH.

The pH requirement applies to every container of domestic septage applied to the land, which means that the pH of each container must be monitored.

The first alternative reduces exposure to pathogens in land-applied domestic septage while environmental factors attenuate pathogens. The second alternative relies on alkali treatment to reduce pathogens and contains the added safeguard of restricting crop harvesting, which prevents exposure to crops grown on domestic septage-amended soils.

[2]Class A or B sewage sludge requirements apply to domestic septage applied to all other types of land. No pathogen-related requirements apply to domestic septage placed on a surface disposal site.

Chapter 6
Requirements for Reducing Vector Attraction

6.1 Introduction

The pathogens in sewage sludge pose a disease risk only if there are routes by which the pathogens are brought into contact with humans or animals. A principal route for transport of pathogens is vector transmission. Vectors are any living organisms capable of transmitting a pathogen from one organism to another either mechanically (by simply transporting the pathogen) or biologically by playing a specific role in the life cycle of the pathogen. Vectors for sewage sludge pathogens would most likely include insects, rodents, and birds.

The Part 503 regulation contains 12 options, described below and summarized in Table 6-1, for demonstrating reduced vector attraction of sewage sludge. These requirements are designed to either reduce the attractiveness of sewage sludge to vectors (Options 1 through 8 and 12) or prevent the vectors from coming in contact with the sewage sludge (Options 9 through 11). Guidance on when and where to sample sewage sludge to meet these requirements is provided in Chapter 7.

As mentioned in Chapter 3, compliance with the vector attraction reduction requirements must be demonstrated separately from compliance with the pathogen reduction requirements. Therefore, demonstration of adequate vector attraction reduction (e.g., through reduction of volatile solids by 38% as described below) does not demonstrate achievement of adequate pathogen reduction.

6.2 Option 1: Reduction in Volatile Solids Content [503.33(b)(1)]

Under this option, reduction of vector attraction is achieved if the mass of volatile solids in the sewage sludge is reduced by at least 38% during sludge treatment. (This is the amount of volatile solids reduction that can be attained at the "good practice" recommended conditions for anaerobic digestion of 15 days residence time at 35°C [95°F] in a completely mixed high-rate digester.) The volatile solids reduction can include any additional volatile solids reduction that occurs before the sewage sludge leaves the treatment works, such as might occur when the sewage sludge is processed on drying beds or in lagoons, or when sewage sludge is composted. Volatile solids reduction is calculated by a volatile solids balance around the digester or by the Van Kleek formula (Fisher, 1984). Guidance on methods of calculation is provided in Appendix C.

Volatile solids reduction is typically achieved by anaerobic or aerobic digestion. These processes degrade most of the biodegradable material to lower activity forms. Any biodegradable material that remains characteristically degrades slowly—so slowly that the vectors that would be attracted to unprocessed sewage sludge are not drawn to it.

6.3 Option 2: Additional Digestion of Anaerobically Digested Sewage Sludge [503.33(b)(2)]

Under this option, an anaerobically digested sewage sludge is considered to have achieved satisfactory vector attraction reduction if it loses less than 17% additional volatile solids when it is anaerobically batch-digested in the laboratory in a bench-scale unit at 30°C to 37°C (86°F to 99°F) for an additional 40 days. Procedures for this test are presented in Appendix D.

Frequently, sewage sludges have been recycled through the biological wastewater treatment section of a treatment works or have resided for long periods of time in the wastewater collection system. During this time they undergo substantial biological degradation. If they are subsequently treated by anaerobic digestion for a period to time, they are adequately reduced in vector attraction, but because they entered the digester already partially stabilized, the volatile solids reduction after treatment is frequently less than 38%. The additional digestion test is used to demonstrate that these sewage sludges are indeed satisfactorily reduced in vector attraction.

6.4 Option 3: Additional Digestion of Aerobically Digested Sewage Sludge [503.33(b)(3)]

Under this option, an aerobically digested sewage sludge with 2% or less solids is considered to have achieved satisfactory vector attraction reduction if it loses less than 15% additional volatile solids when it is aerobically batch-digested in the laboratory in a bench-scale unit at 20°C (68°F) higher for an additional 30 days. Procedures for this test are presented in Appendix D. The test can be run on sewage sludges up to 2% solids and does not require a temperature correction for sewage sludges not initially digested at 20°C (68°F). Sewage sludges with greater than 2% solids can be diluted to 2% solids with effluent, and the test can then be run on the diluted sludge.

This option is appropriate for aerobically digested sewage sludges that cannot meet the 38% volatile solids reduction re-

Table 6-1. Summary of Requirements for Vector Attraction Reduction Under Part 503

Requirement	What Is Required?	Most Appropriate for:
Option 1 **503.33(b)(1)**	At least 38% reduction in volatile solids during sewage sludge treatment	Sewage sludge processed by: • Anaerobic biological treatment • Aerobic biological treatment • Chemical oxidation
Option 2 **503.33(b)(2)**	Less than 17% additional volatile solids loss during bench-scale anaerobic batch digestion of the sewage sludge for 40 additional days at 30°C to 37°C (86°F to 99°F)	Only for anaerobically digested sewage sludge that cannot meet the requirements of Option 1
Option 3 **503.33(b)(3)**	Less than 15% additional volatile solids reduction during bench-scale aerobic batch digestion for 30 additional days at 20°C (68°F)	Only for aerobically digested sewage sludge with 2% or less solids that cannot meet the requirements of Option 1—e.g., sewage sludges treated in extended aeration plants
Option 4 **503.33(b)(4)**	SOUR at 20°C (68°F) is ≤1.5 mg oxygen/hr/g total sewage sludge solids	Sewage sludges from aerobic processes (should not be used for composted sludges)
Option 5 **503.33(b)(5)**	Aerobic treatment of the sewage sludge for at least 14 days at over 40°C (104°F) with an average temperature of over 45°C (113°F)	Composted sewage sludge (Options 3 and 4 are likely to be easier to meet for sludges from other aerobic processes)
Option 6 **503.33(b)(6)**	Addition of sufficient alkali to raise the pH to at least 12 at 25°C (77°F) and maintain a pH ≥12 for 2 hours and a pH ≥11.5 for 22 more hours	Alkali-treated sewage sludge (alkalies include lime, fly ash, kiln dust, and wood ash)
Option 7 **503.33(b)(7)**	Percent solids ≥75% prior to mixing with other materials	Sewage sludges treated by an aerobic or anaerobic process (i.e., sewage sludges that do not contain unstabilized solids generated in primary wastewater treatment)
Option 8 **503.33(b)(8)**	Percent solids ≥90% prior to mixing with other materials	Sewage sludges that contain unstabilized solids generated in primary wastewater treatment (e.g., any heat-dried sewage sludges)
Option 9 **503.33(b)(9)**	Sewage sludge is injected into soil so that no significant amount of sewage sludge is present on the land surface 1 hour after injection, except Class A sewage sludge which must be injected within 8 hours after the pathogen reduction process.	Sewage sludge applied to the land or placed on a surface disposal site. Domestic septage applied to agricultural land, a forest, or a reclamation site, or placed on a surface disposal site
Option 10 **503.33(b)(10)**	Sewage sludge is incorporated into the soil within 6 hours after application to land or placement on a surface disposal site, except Class A sewage sludge which must be applied to or placed on the land surface within 8 hours after the pathogen reduction process.	Sewage sludge applied to the land or placed on a surface disposal site. Domestic septage applied to agricultural land, forest, or a reclamation site, or placed on a surface disposal site
Option 11 **503.33(b)(11)**	Sewage sludge placed on a surface disposal site must be covered with soil or other material at the end of each operating day.	Sewage sludge or domestic septage placed on a surface disposal site
Option 12 **503.33(b)(12)**	pH of domestic septage must be raised to ≥12 at 25°C (77°F) by alkali addition and maintained at ≥12 for 30 minutes without adding more alkali.	Domestic septage applied to agricultural land, a forest, or a reclamation site or placed on a surface disposal site

quired by Option 1. These include sewage sludges from extended aeration and oxidation ditch plants, where the nominal residence time of sewage sludge leaving the wastewater treatment processes section generally exceeds 20 days. In these cases, the sewage sludge may already have been substantially reduced in biological degradability prior to aerobic digestion.

6.5 Option 4: Specific Oxygen Uptake Rate (SOUR) for Aerobically Digested Sewage Sludge [503.33(b)(4)]

For an aerobically digested sewage sludge, reduction in vector attraction can also be demonstrated if the SOUR of the sewage sludge to be used or disposed is determined to be equal to or less than 1.5 mg of oxygen per hour per gram of total sewage sludge solids (dry weight basis) at 20°C (68°F).[1] This test is based on the fact that if the sewage sludge consumes very little oxygen, its value as a food source for vectors is very low and therefore vectors are unlikely to be attracted to the sewage sludge.

Frequently aerobically digested sewage sludges are circulated through the aerobic biological wastewater treatment proc-

[1]SOUR is defined in Part 503 as the mass of oxygen consumed per unit time per unit mass of total solids (dry weight basis) in the sewage sludge. SOUR is usually based on total suspended volatile solids rather than total solids, because it is assumed that it is the volatile matter in the sewage sludge that is being oxidized. The SOUR definition in Part 503 is based on total solids primarily to reduce the number of different determinations needed. Generally the range in the ratio of volatile solids to total solids in aerobically digested sewage sludges is not large. The standard required for SOUR based on total solids will merely be slightly lower than if it had been based on volatile suspended solids to indicate the same endpoint.

ess for as long as 30 days. In these cases, the sewage sludge entering the aerobic digester is already partially digested, which makes it difficult to demonstrate the 38% reduction required by Option 1.

The oxygen uptake rate depends on the conditions of the test and, to some degree, on the nature of the original sewage sludge before aerobic treatment. It should be noted that the SOUR method may be unreliable at solids content above 2% and that it requires a poorly defined temperature correction at temperatures differing substantially from 20°C (68°F). Guidance on performing the SOUR test and on sewage sludge-dependent factors are provided in Appendix D.

6.6 Option 5: Aerobic Processes at Greater Than 40°C [503.33(b)(5)]

The sewage sludge must be aerobically treated for 14 days or longer during which time the temperature must be over 40°C (104°F) and the average temperature higher than 45°C (113°F). This option applies primarily to composted sewage sludge. These processed sewage sludges generally contain substantial amounts of partially decomposed organic bulking agents, in addition to sewage sludge. Application of the other options for aerobic sewage sludges described above to composted products is either impossible (because the percent volatile solids reduction of the sewage sludge fraction cannot be assessed) or has not been adequately investigated in terms of reliability.

The regulation does not specifically mention or limit this option to composting. This option can be applied to sewage sludge from other aerobic processes such as aerobic digestion, but other methods such as Options 3 and 4 are likely to be easier to meet for these sludges.

6.7 Option 6: Addition of Alkali [503.33(b)(6)]

Sewage sludge is considered to be adequately reduced in vector attraction if sufficient alkali is added to:

- Raise the pH to at least 12.
- Maintain a pH of at least 12 without addition of more alkali for 2 hours.
- Maintain a pH of at least 11.5 without addition of more alkali for an additional 22 hours.

As noted in Section 5.6, the term "alkali" is intended to mean a substance that causes an increase in pH. Raising sewage sludge pH through alkali addition reduces vector attraction by reducing or stopping biological activity. However, this reduction in biological activity is not permanent. If the pH drops, the surviving bacterial spores become biologically active and the sewage sludge will again putrefy and potentially attract vectors. (The more soluble the alkali, the faster this is likely to happen.) The conditions required under this option are designed to ensure that the sewage sludge can be stored for at least several days at the treatment works, transported, and applied to soil without the pH falling to the point where biological activity results in vector attraction.

6.8 Option 7: Moisture Reduction of Sewage Sludge Containing No Unstabilized Solids [503.33(b)(7)]

Under this option, vector attraction is considered to be reduced if the sewage sludge does not contain unstabilized solids generated during primary wastewater treatment and if the solids content of the sewage sludge is at least 75% before the sewage sludge is mixed with other materials. Thus, the reduction must be achieved by removing water, not by adding inert materials.[2]

It is important that the sewage sludge not contain unstabilized solids because the partially degraded food scraps likely to be present in such a sewage sludge would attract birds, some mammals, and possibly insects, even if the solids content of the sewage sludge exceeded 75%.

The way dried sewage sludge is handled or stored before use or disposal can create or prevent vector attraction. If dried sewage sludge is exposed to high humidity, the outer surface of the sludge could equilibrate to a lower solids content and attract vectors. Steps should be taken to prevent this from happening.

6.9 Option 8: Moisture Reduction of Sewage Sludge Containing Unstabilized Solids [503.33(b)(8)]

Vector attraction of any sewage sludge is considered to be adequately reduced if the solids content of the sewage sludge is increased to 90% or greater. This extreme desiccation deters vectors in all but the most unusual situations. As noted in the footnote for Option 7, the solids increase should be achieved by removal of water and not by dilution with inert solids. Drying to this extent severely limits biological activity and strips off or decomposes the volatile compounds that attract vectors.

6.10 Option 9: Injection [503.33(b)(9)]

Vector attraction reduction can be demonstrated by injecting the sewage sludge below the ground. Under this option, no significant amount of the sewage sludge can be present on the land surface within 1 hour after injection, and, if the sewage sludge is Class A with respect to pathogens, it must be injected within 8 hours after discharge from the pathogen-reduction process.

Injection of sewage sludge beneath the soil places a barrier of earth between the sewage sludge and vectors. The soil quickly removes water from the sludge, which reduces the mo-

[2]The moisture reduction may be achieved by adding active materials that remove water by reaction [e.g., CaO reacting with water to form $Ca(OH)_2$], by adsorption, or as water of crystallization (e.g., formation of $CaSO_4 \cdot 2H_2O$). The best way to determine whether the material added is active or inert is to subject the sewage sludge/solid mixture to a drying determination at mild conditions since the objective is to determine available water. Method 2540B in "Standard Methods" (APHA, 1992) is appropriate. The sewage sludge or mixture is dried at 103°C to 105°C (217°F to 221°F). Drying time should be more than 1 but less than 2 hours.

bility and odor of the sewage sludge. Odor is usually present at the site during the injection process, but it quickly dissipates when injection is complete.

Special restrictions apply to Class A sludges because these sewage sludges are a medium for regrowth (see Section 4.3). During the first 8 hours of regrowth, levels of pathogenic bacteria should still be quite low; however, after this point, the regrowth of pathogenic bacteria may rapidly increase.

6.11 Option 10: Incorporation of Sewage Sludge into the Soil [503.33(b)(10)]

Under this option, sewage sludge applied to the land surface or placed on a surface disposal site must be incorporated into the soil within 6 hours after application to or placement on the land. If the sewage sludge is Class A with respect to pathogens, the time between processing and incorporation after application or placement must not exceed 8 hours—the same as for injection under Option 9.

When applied at agronomic rates, the loading of sewage sludge solids typically is about 1/200th of the mass of soil in the plow layer. If mixing is reasonably good, the dilution of sewage sludge in the soil surface is equivalent to that achieved with soil injection. Odor will be present and vectors will be attracted temporarily, as the sewage sludge dewaters on the soil surface. This attraction diminishes and is virtually eliminated when the sewage sludge is mixed with the soil. The mixing method applies to liquid sewage sludges, dewatered sewage sludge cake, and even to dry sewage sludges.

The 6 hours allowed to complete the mixing of sewage sludge into the soil should be adequate to allow for proper incorporation. As a practical matter, it may be wise to complete the incorporation in a much shorter time. Clay soils tend to become unmanageably slippery and muddy if the liquid sewage sludge is allowed to soak into the first inch or two of topsoil.

6.12 Option 11: Covering Sewage Sludge [503.33(b)(11)]

Under this option, sewage sludge placed on a surface disposal site must be covered with soil or other material at the end of each operating day. Daily covering reduces vector attraction by creating a physical barrier between the sewage sludge and vectors, while environmental factors work to reduce pathogens.

6.13 Option 12: Raising the pH of Domestic Septage [503.33(b)(12)]

This option applies only to domestic septage. Vector attraction is reduced if the pH is raised to at least 12 through alkali addition and maintained at 12 or higher for 30 minutes without adding more alkali. (These conditions also accomplish pathogen reduction for domestic septage—see Section 5.6.) When this option is used, every container must be monitored to demonstrate that it meets the requirement. As noted in Section 5.6, "alkali" refers to a substance that causes an effect similar to an alkali; that is, it causes an increase in pH.

This vector attraction reduction requirement is slightly less stringent than the alkali addition method for sewage sludge. The method is geared to the practicalities of the use or disposal of domestic septage, which is typically treated by lime addition to the domestic septage hauling truck. The treated septage is typically applied to the land shortly after lime addition. During the very short time interval, the pH is unlikely to fall to a level at which vector attraction could occur.

Chapter 7
Meeting the Quantitative Requirements of the Regulation

7.1 Introduction

The Part 503 regulation contains operational standards for pathogen and vector attraction reduction. This chapter suggests ways to satisfy these requirements.

The regulation provides only minimal guidance on the amount of information that must be obtained during a monitoring event to prove that a standard has been met or to demonstrate that process conditions have been maintained. The final decision is up to the permitting authority. Sufficient information should be provided to the permitting authority to enable a qualified reviewer to determine if the requirements have been met. This chapter *suggests* how much information will satisfy this need. For purposes of this discussion:

- A *sampling event* is defined as the period during which the samples needed for monitoring are collected.
- A *monitoring event* includes the sampling period *and* the period to analyze the samples and provide the results needed to determine compliance.

7.2 Process Conditions

Sufficient information must be collected about sludge processing conditions so that a regulator can be reasonably certain that the process is being operated as claimed. How this information is collected and how much information is needed depend on the process. The following example illustrates the kind of information and the level of detail that are appropriate. Consider the case of a treatment works that meets the pathogen reduction requirement for a Class B sludge by using anaerobic digestion conducted at the PSRP conditions of 35°C (95°F) with a 15-day residence time. To meet the pathogen reduction requirement, the monitoring results must demonstrate that the 35°C (95°F) temperature and 15-day residence time are maintained whenever the process is being used to produce sewage sludge that will meet the Class B requirement. The following are suggested as reasonable monitoring requirements for this situation:

> The treatment works operator should measure the temperature of the sludge in the digester at least every shift. Preferably, temperature should be continuously recorded. Information should be available on typical uniformity of temperature within the digester. The temperature measuring device must be calibrated. To calculate the residence time, the volume of the digester, depth of liquid in the digester, and flow rate into or out of the digester must be known. Mixing equipment should be capable of mixing the entire contents of the digester. Information should be available on the true working volume of the digester. Since poorly maintained digesters are sometimes as much as one quarter filled with stagnant grit and scum, there should be evidence that the digester is cleaned on a reasonable schedule. The flow rate can be determined by means of a flow meter, or by observing rise or fall of liquid level during charging of feed or withdrawal of the treated sewage sludge and the number of feedings or withdrawals per day. If a flow meter is used, there should be records showing that the meter is calibrated periodically and its readings are correct.
>
> Residence time in the digester is determined from the volume of sludge in the digester and the daily average flow rate. The temperature-time requirements of the PSRP process of anaerobic digestion must be equaled or exceeded, because the regulation requires that all sewage sludge that is used or disposed meet the requirements. The 35°C (95°F) temperature requirement is sometimes difficult to meet. Lower temperatures and longer residence times may be used, as determined by a linear interpolation between 35°C (95°F) and 15 days and 20°C (68°F) and 60 days. It is suggested that a running average temperature and a running average residence time be computed, and that the average temperature and time must meet or exceed the requirements of the linear time-temperature relationship. The period of the running average must be approximately equal to the average residence time. Whenever the time-temperature relationship is not met, the sludge does not meet Class B requirements and must be diverted from agricultural use.

Other processes will have different requirements. For example, some treatment works collect liquid sewage sludge in batches, treat it with lime and either dewater it or apply it to the land in the liquid state. They often import sewage sludges or domestic septage and treat them in the same fashion. If feedstock changes substantially in character from batch to batch, records of performance will have to be kept on every batch. If sludge is consistent in quality and records demonstrate this to be true, it may be necessary to check only every third batch to determine if pH at 2 hours and at 24 hours meets the requirements of the regulation for pathogen and vector attraction reduction (see Sections 5.3 and 6.7 for requirements). If records show that the treated sewage sludge is virtually never off specification, the measurement frequency could be changed to one randomly selected batch a day. (This determination would have to be made through a permit, or at least with the agreement of the regulator.) For other processes, such as static pile composting, one of several piles constructed in a day could be monitored, probably with several thermocouples at different

elevations in the pile, to demonstrate conformance for the whole day's production.

Frequently, processes do not conform to process conditions. In such cases, the operator should keep records showing that the treated sludge produced was either recycled to be processed again or diverted in some manner for use or disposal consistent with the sludge quality (e.g., disposal in a landfill with daily cover or, if the sludge meets the Class B requirements, application as a Class B [rather than a Class A] sludge).

7.3 Monitoring Events: Needs and Duration

Monitoring events are intended to reflect the average performance of the treatment works. Conditions should be as stable as possible before the monitoring event. Day-to-day variations in feed rate and quality are inevitable in sewage sludge treatment, and the processes are designed to perform satisfactorily despite these variations. However, major process changes should be avoided before monitoring events, because long periods of time—as much as 3 months if anaerobic digestion is part of the process train—are required before steady state operation is established.

Monitoring for Microbiological Quality

To meet the Part 503 pathogen reduction requirements, sewage sludges may have to be monitored to determine densities of fecal coliforms, *Salmonella* sp., enteric viruses, and viable helminth ova. Monitoring for these microorganisms presents special problems, primarily caused by the length of time it takes to be sure that the treated sludge meets the Class A and Class B microbiological density requirements. Variations in the microbiological quality of the treated sludge and intrinsic variation in the analytical methods are generally large enough that a single measurement of a microbiological parameter is inadequate for deciding whether a process meets or fails to meet a requirement.

The Pathogen Equivalency Committee recommends that the monitoring event include several samples taken over a period of approximately 2 weeks (see Section 7.7). In addition, the microbiological tests themselves take time to complete. The MPN test for fecal coliform takes about 4 days to complete. The MPN test for salmonellae takes about 5 days, but the difficulty of conducting the test may require the use of an off-site laboratory, so it could take 7 days to get results. Enteric virus determinations take over 2 weeks and, in almost all cases, must be done by an outside laboratory. The situation is similar for viable helminth ova analysis, except the test takes about 4 weeks to conduct. Thus, the time required for a monitoring event could range from 3 to 7 weeks. During this time, the quality of the treated sewage sludge is unknown. This means that the sludge processed during the monitoring event should either be stored until it is demonstrated that the processed sludge meets the quality requirements for use as a Class A or B sludge, or—if the sludge is being monitored for Class A requirements—used or disposed as a Class B sludge (provided it meets the Class B requirements). Another option is to complete the sampling, which might take 2 weeks, and store all the sewage sludge produced during sampling (or, if possible, divert it to a Class B use), and then shut down until all the analytical results have been reported back to the treatment works operator. This latter course is only practicable for processes that produce on-specification sludge shortly after start-up.

Monitoring for Vector Attraction Reduction

Not all the vector attraction reduction options listed in the regulation (see Chapter 6) require special monitoring. Four of the methods (treatment of sewage sludge in an aerobic process for 14 days or longer, injection below the surface of the land, incorporation of sludge into the land, and placement of sludge on a surface disposal site and covering it at the end of each day) are technology descriptions. These technologies have to be maintained throughout the year in the manner described in the regulation. Examples of the kind of information needed to demonstrate adequate performance are provided in Section 7.2.

The remaining vector attraction reduction options all have performance requirements that are monitoring goals. All have some technology element. For example, the oxygen uptake rate test is only appropriate for a sludge from an aerobic digestion or wastewater treatment process. Even the 38% volatile solids reduction requirement (see Section 6.2) has a technology element (though none is mentioned in the regulation), since whatever was done to reduce the volatile solids content by at least 38% must be continued. The technology aspects of these options must be documented in the manner described in Section 7.2. The measures of performance are a monitoring requirement and must be evaluated according to the required monitoring schedule (see Table 3-4 in Chapter 3).

Monitoring for vector attraction reduction presents similar problems to monitoring for microbiological quality. Some of the tests can be conducted within a few hours while others can take more than a month. For the tests that can be conducted within a few hours, the sampling event must be more than a few hours to account for the variability in the feedstock (the incoming sludge) and the performance of the vector attraction reduction process as affected by the changes in feedstock. Just as for the microbiological tests, these vector attraction reduction tests should be conducted over approximately 2 weeks to minimize the expected effect of these variations. The 2-week period can be the same 2-week period during which the microbiological parameters are being determined.

Some vector attraction reduction tests—such as the additional digestion tests—take more than a month to complete. Unless the treatment works has several sets of duplicate testing equipment, it will be impossible to run these tests on enough samples during a 2-week sampling period to assess the variability in the performance of the treatment process. Storing samples taken during this period until the equipment becomes available is not an option, because samples cannot be stored for more than a limited time period (see Section 8.6). In such circumstances, if treatment works operation is fairly steady, the monitoring event should be started as much as a month early so that enough information can be provided to adequately assess treatment works performance (see also discussion under "Additional Digestion Tests" in Section 7.7).

7.4 Correspondence of Samples

The enteric virus and viable helminth ova requirements under Class A Alternatives 3 and 4 and some of the vector attraction reduction methods (e.g., percent volatile solids reduction) involve taking input and output samples that correspond (i.e., they are "before processing" and "after processing" samples of the *same* batch of sludge). Obtaining samples that correspond can be difficult for sewage sludge treatment processes, such as anaerobic digestion, that characteristically treat sludge in fully mixed reactors with long residence times. As mentioned in Section 7.3, it can take up to 3 months to demonstrate that an anaerobic digester has reached steady state after some substantive change in feed sludge or process conditions. Samples will correspond only when a process has reached steady state.

Almost all the treatment processes that might be used to reduce pathogens under Class A Alternatives 3 and 4 are either batch processes or plug flow continuous processes. In theory it is relatively simple to obtain correspondence—it is only necessary to calculate the time for the input material to pass through the system and sample the downstream sludge at that time. Achieving accurate correspondence in practice, however, is seldom easy. Consider, for example, the difficulty of obtaining good correspondence of feed and treated sludge for a composting operation in which the feed sewage sludge is to be compared to composted sludge that has been stored 3 months. Appropriate compositing of the samples of feed and treated sludge averages out the composition of these sewage sludges and reduces the correspondence problem. As indicated in Section 7.6, limitations on the periods of time over which microbiological samples can be collected limit the utility of compositing.

7.5 Adjusting for Diluents

Sewage sludge processing often introduces other substances into the sludge. For example, polymers, lime and ferric chloride, paper pulp, and recycled sludge ash are frequently added to aid in dewatering. Lime is sometimes added to increase the temperature of the sewage sludge cake to disinfecting temperature, and wood chips are added to absorb moisture and provide air channels in the sewage sludge cake being composted. These materials reduce the microbial densities by dilution and increase solids content, although the change is not as much as might be thought. For example, an increase in mass of 20% would result in a reduction in the log density of a microbiological parameter of only 0.079.

Because risk is directly related to mass of treated sludge, it is appropriate not to be concerned whether the sludge has been diluted or not by treatment, but to be concerned about the numbers of pathogenic organisms in the treated sewage sludge per unit mass of *that treated sludge*. This is the approach taken by the Part 503 regulation, which requires that the *treated* sludge meet the standards for Class A or Class B sludge. The treated sludge includes any additives, so no correction is needed for dilution effects.

For some sludges, particularly those treated by composting (these usually will be Class A sludges), the amount of additive can be considerable. Nevertheless, the regulation requires that the treated sludge meet the standard, which means that no correction need be made for dilution.

In many composting installations, wood chips are used as the bulking agent. Sometimes the compost is sold or given away without screening out the wood chips. Although the regulation requires that the treated sludge must meet the standard, it is appropriate to remove the wood chips when the microbial analyses are carried out. The primary reason is to improve the accuracy of the microbial measurements. The wood chips are so big (typically 4 cm x 4 cm x 1 cm) that a very large sample would have to be taken and blended to get a representative subsample. Sample reliability is reduced when the sample consists of a mix of sludge solids and fibrous wood-chip residue from blending. Another reason for removing the wood chips prior to microbial analysis is that the exposure of users to the compost is related to the fine particle content and not to large physically distinct wood chips. For example, a user who handles the compost gets his or her hands covered with compost particles that do not include wood chips. Similarly, the user might breathe in a dust of compost particles that would not include the wood chips. In these cases, what matters is the number of pathogenic organisms present in the material that the user is exposed to.

7.6 Representative Samples

Except when the investigator is searching for the maximum range of a parameter, a sample, even a grab sample, should be chosen to be representative of the sewage sludge being sampled. Specific procedures for sampling are discussed in Chapter 8.

Random Variability

Virtually all sewage sludge treatment processes will experience a certain amount of short-term random or cyclic variation in the feed sludge and in process performance. Evaluation of average performance over a 2-week time period is suggested as a reasonable approach to account for these variations. It permits selection of sampling days to avoid a day-to-day effect and at least allows a comparison between adjacent weeks.

Seasonal Variability

For some sewage sludge treatment processes, performance is poorer during certain parts of the year due to seasonal variations in such factors as temperature, sunshine, and precipitation. For example, aerobic digestion and some composting operations can be adversely affected by low ambient temperature. In such cases, it is critical that process performance be evaluated during the time of year when poorest performance is expected. If a treatment works is evaluated four or more times a year, there is no problem, because all seasons of the year will be covered. For small treatment works that are evaluated only once or twice a year, it is important to monitor in the time of year where performance is expected to be poorest, to avoid approving a process that is not performing adequately for much of the year. Process criteria of PSRPs and PFRPs must be

evaluated and recorded continuously, with the kind of records outlined in Section 7.2.

Composite Sampling

Composite sampling is frequently practiced in wastewater treatment. A small stream of wastewater or sludge is drawn off at rate proportional to the flow of the main stream being sampled and collected as a single sample. Typically, times of collection are for one shift (8 hours) or one day (24 hours). In this case, the accumulated sample represents a volume-average sample over the period of time the sample is drawn. The sample is chilled during the period it is being collected to prevent chemical change until it can be brought back to the laboratory for analysis. This is an excellent sampling procedure, but it must be modified when microbial analyses are intended. A composite time-average sample can be obtained by combining a series of small samples taken, for example, once every 5 minutes for a period of an hour. A composite sample for bacterial and viral testing could be taken over an hour or less under most circumstances without compromising the results. Composite sampling over 24 hours, or even longer if special precautions are taken, is possible for viable helminth ova provided the ova in the sample are not exposed to thermal or chemical stress (e.g., temperatures above 40°C [104°F] or the addition of certain chemicals such as ammonia, hydroxides, and oxidants).

Composite sampling may be possible for samples to be used in some of the procedures to determine whether vector attraction reduction is adequate. It may not be appropriate for those procedures that depend on bacterial respiration (i.e., aerobic or anaerobic digestion). This subject is discussed in Appendix D which presents procedures for three methods to demonstrate reduced vector attraction.

7.7 Regulatory Objectives and Number of Samples That Should Be Tested

The Part 503 regulation requires that stated goals for pathogen reduction and vector attraction reduction be met. Sufficient information should be collected in a monitoring event to demonstrate that the requirements have been met, but excessive efforts to collect information should be avoided. Unfortunately, the daily, weekly, and seasonal fluctuations that occur in influent to treatment works make it difficult to minimize the information-gathering task. Based on judgment, 2 weeks is suggested as a reasonable period for assessing the performance of a sewage sludge treatment process.

If the impact of the treated sludge (i.e., the disease risk it poses and the potential it creates for heavy metal uptake by crops) could be based on an average performance or composition *and* a composite collected over 2 weeks would remain stable, it might be possible to simply measure a parameter once to adequately assess performance. This is possible for certain chemical analyses. Unfortunately, with a few exceptions (see below), composites for periods exceeding an hour cannot be made with microbiological and vector attraction reduction parameters.

For the most part, several measurements must be made and averaged to determine the average performance over a 2-week period. Part 503 requires the geometric mean of seven samples to demonstrate Class B pathogen reduction, but does not specify the number of samples required to demonstrate achievement of the other pathogen reduction requirements or of the vector attraction reduction requirements. Based on judgment, seven samples are also suggested as adequate to demonstrate average performance for all other cases, except when compositing over several days is possible. However, if the performance of the sludge treatment process is highly variable, or if correspondence between feed and product is difficult to establish, more than seven tests must be made to determine a reliable average.

The various parameters that must be measured to meet the various pathogen reduction and vector attraction reduction requirements are considered below to assess the range of accuracy that can be expected when seven measurements are made, whether compositing can reduce the number of measurements needed, or whether variance is so large than more than seven measurements are needed. This discussion begins with the Class B pathogen reduction requirement (see Section 5.2), because this is the only case where the regulation actually defines the number of samples to be tested.

Class B: Monitoring for Fecal Coliform Densities

Part 503 requires that seven samples be taken to demonstrate compliance with the fecal coliform levels required of Class B sludges. Seven samples were judged adequate to account for the short-term fluctuations in treated sludge quality and allow determination of average performance. Variance of fecal coliform determinations is known to be high, but analysis (presented below) showed that if seven samples are averaged, the error band about the mean value is sufficiently compressed that treatment works with adequately treated sludge would not have difficulty meeting the standard. If the mean value does not meet the standard, the material is not a Class B sludge and must be disposed of otherwise until the standard is met.

The regulation requires that the geometric mean fecal coliform density of the seven samples be less than 2 million CFU or MPN per gram of total solids sewage sludge (dry weight basis). If a treatment works were producing a treated sewage sludge with a true mean density of exactly 2 million fecal coliform per gram, measured values of the fecal coliform density would cluster around 2 million per gram, but half would be below and half would be above it. Half the time, the treatment works would appear not to be meeting the requirement. The true mean density must be below 2 million per gram to be confident that the experimentally determined average will be below 2 million per gram. Just how much below depends on the standard error of the average.

Use of at least seven samples is expected to reduce the standard error to a reasonable value. In tests on extended aeration sludges, Farrell et al. (1990) obtained a standard deviation of the logarithm of the fecal coliform density (s) of 0.3 using the membrane filter method. This included the variability in the analysis as well as variability over time (approximately a year). Standard error for the average of seven measurements (S.E.=

s/n) is 0.11. Using the normal probability distribution, the true mean must be below 1.30 million if the geometric mean of seven measurements is to be below 2 million 95% of the time (see Table 7-1 for details of this calculation). If the standard deviation were higher, the true mean would have to be even lower to be reasonably confident that the geometric mean would be below 2 million per gram. Thus, efforts should be made to reduce variability. Steps that can be taken are:

- Reduce the standard error by increasing the number of measurements used to determine the geometric mean.
- Reduce process variability.
- Improve sampling and analytical techniques.

What action to take to reduce the geometric mean depends on the process. For anaerobic or aerobic digestion, some suggested steps are to increase temperature, increase residence time, use a draw-and-fill feeding procedure rather than fill-and-draw or continuous feeding, and increase the time between withdrawal and feeding. After an attempt at improvement, the evaluation should be repeated. If the process continues to fail, more substantial changes to the process may be appropriate.

Table 7-1. True Geometric Mean Needed If Standard Fecal Coliform Density of 2 Million CFU Per Gram Is to Be Rarely Exceeded

Assumptions

- The fecal coliform densities of the sewage sludge are log normally distributed. (The arithmetic mean of the logarithms of the fecal coliform densities is the mean of the distribution. The geometric mean is the antilog of the log mean.)
- The goal is to ensure that the measured mean value does not exceed the density requirement more than once in 20 monitoring events.
- The standard deviation of the log density is 0.30.

Calculation

To predict the expected frequency of a measurement using the normal probability distribution, the variable x is converted to the standard measure (u — see below) and its probability of occurrence is obtained from tabulated values of the probability distribution. In this case, the reverse is carried out. A certain probability of occurrence is desired and the value of the standard measure is read from the tables. From the normal distribution table (single-sided), u is 1.645 when P = 0.05 (one in 20),

Where: P = the proportion of the area under the curve to the right of u relative to the whole area under the curve.

and: u = the standard measure

$u = (\bar{x} - \mu)/s_{\bar{x}}$

Where: μ = true log mean

$\bar{x}$ = log mean of the several measurements

$s_{\bar{x}} = s/\sqrt{n}$

n = number of measurements that are averaged

s = standard deviation of a single measurement of log mean density

The logarithm of the fecal coliform density requirement (2 million CFU/g) is $\bar{x}$ ($\bar{x}$ = 6.301). This is the number that should not be exceeded more than once in 20 monitoring episodes. Substituting into Equation 1 and calculating μ,

$1.645 = (6.301 - \mu)/(0.3/\sqrt{7})$

$\mu = 6.114$

Antilog 6.114 = 1.3 million CFU/g.

Class A: Monitoring for Fecal Coliform Densities

Part 503 requires that, to qualify as a Class A sludge, sewage sludge must be monitored for fecal coliform (or *Salmonella* sp.—see below) and have a density of less than 1,000 MPN fecal coliform per gram of total solids sewage sludge (dry weight basis). The regulation does not specify the number of samples that have to be taken during a monitoring event or, if several samples are taken, how to determine the average fecal coliform density.

Available information indicates that the variability of the MPN test for fecal coliform that must be used for the Class A sludges is high. Yanko's (1987) quality assurance data on duplicate samples show high variability, indicating that several measurements are needed to get reasonable precision. For example, the 95% confidence interval of the geometric mean for a sample with a measured fecal coliform density of 1,000 MPN/g is 224 to 4,470 MPN/g (calculated from the standard deviation of the log fecal coliform density, s = 0.30 with 13 degrees of freedom, determined from Yanko's 1987 report). If seven measurements were taken, the confidence interval would be much smaller—580 to 1,740 MPN/g. The spread between the lower and upper limits has been reduced from a factor of 20 to a factor of 3.0 by increasing the number of measurements from one to seven. The advantage of taking several samples is obvious. Note that these confidence intervals are based on the variability that resulted when duplicates drawn from a larger sample were analyzed and do not include additional variability caused by fluctuations in feedstock or process performance.

The measured fecal coliform density provides an estimate of the likelihood of salmonellae detection and, if detected, the expected density. Yanko (1987) obtained a good correlation between fecal coliform density and salmonellae detections in his extensive investigation of composts derived from sewage sludge. Fraction detected is less than 10% when fecal coliform density is less than 1,000 MPN/g. Yanko also obtained a good correlation between fecal coliform density and salmonellae density for those samples for which salmonellae were detected. That correlation predicts that, for fecal coliform densities less than 1,000 MPN/g, salmonellae densities will be less than 1.0 MPN/g. Thus, at fecal coliform densities ≤1,000 MPN/g, salmonellae detections will be infrequent and, if detected, densities are expected to be below 1 MPN/g.

The standard deviation for the fecal coliform measurement for Class A sludges will probably be the same or somewhat higher than for Class B sludges. Thus, the number of samples taken should be the same or, in rare cases, more than for Class B sludges. It is suggested, then, that the sampling event extend for 2 weeks or more and that at least seven samples be collected and analyzed.

What action to take to reduce average density in case the fecal coliform requirement is not met depends on the process. For aerated deep-pile composting, thicker insulating layers on the pile, a longer period of temperature above 55°C (131°F),

improved efforts at eliminating cross-contamination between feed and treated sludge, and longer maturing times are suggested.

Class A: Monitoring for Salmonellae Densities

Part 503 allows *Salmonella* sp. to be monitored instead of fecal coliforms (see Section 4.3). The density of the *Salmonella* sp. must be below detection limits of 3 MPN/4 g of total sewage sludge solids (dry weight basis). The *Salmonella* sp. determination is superficially similar to the fecal coliform test, but it is much more expensive and requires a high experience level. In all likelihood, the salmonellae tests would have to be carried out by a contract laboratory.

Yanko (1987) found that the standard deviations of the MPN procedures, as determined from results on duplicates, were nearly identical for both the fecal coliform and salmonellae methods. In light of this information, one may conclude that if the seven measurements are appropriate to determine a mean fecal coliform density, the same number should be used for determining a mean density for salmonellae.

The determination of a mean density for salmonellae is complicated by the fact that most of the measurements will show densities below detection limits (3 MPN/4 g), and some way must be used to include these measurements into the average. The calculation of averages for "censored" data (a substantial portion of the data is below detection limits) is of considerable interest in analytical chemistry, and numerous approaches to constructing a proper average have been suggested (Newman and Dixon, 1990; Helsel, 1990). These methods are useful when a third or less of the measurements are nondetectable, but with treated sludge, most of the measurements will be nondetectable. Rather than over-interpret scarce data, it is suggested that an arithmetic mean be determined for the data points, considering the nondetectable measurements as 0 MPN/4 g. The arithmetic mean is chosen rather than the geometric mean because it relates better to the true number of salmonellae in a given mass of treated sludge than does the geometric mean. If the arithmetic mean density is greater than 3 MPN/4 g, the process does not reduce pathogens to the specified densities.

Class A: Monitoring for and Demonstration of Enteric Virus and Viable Helminth Ova Reduction

The accuracy of monitoring results demonstrating the absence of enteric viruses and helminth ova is influenced by the variability in the influent to the treatment works and the inherent error in the experimental method. Information on method error for both enteric viruses and helminth ova is available only on standard deviations calculated from duplicate samples. Goyal et al. (1984) report that, in their comparison of methods for determining enteroviruses, the log standard deviation for the virus determination in sewage sludge was 0.26 (47 degrees of freedom). A review of the work of Reimers et al. (1989) indicates that, in the range of 5 to 100 viable Ascaris ova per 10 grams sewage sludge solids, standard deviation was about half the number of viable ova. This is equivalent to a log density of 0.3, which is about the same as for fecal coliform. Thus, there is no unusually high variability in the basic test methods that would require an increased number of samples to minimize this effect.

Deciding how many samples to take for enteric viruses and viable helminth ova is more difficult than for fecal coliform and salmonella, because enteric viruses and viable helminth ova often are not present in untreated sludge. For this reason, the interpretation of the density determinations for these organisms in treated sludge depends on the quality of the feed sludge. If no enteric viruses or viable helminth ova are detected in the feed sludge, then the absence of these organisms in corresponding samples of treated sludge does not indicate in any way whether the process is or is not capable of reducing these organisms to below detectable limits. The ability of a process to reduce these organisms to below detectable limits is indicated when analysis shows that these organisms were present in the feed sludge and were not present in corresponding samples of treated sludge. One important questions is: What fraction of the total pairs of corresponding samples must show positive in the feed sludge and negative in the treated sludge to provide convincing evidence that the process consistently reduces enteric viruses and viable helminth ova to below detectable levels? This is a difficult question to answer.

Because viable helminth ova are relatively stable microorganisms, compositing is suggested as a way to obtain meaningful representative samples and analytical results. If precautions are taken, such as cooling the sample promptly to close to 0°C (32°F) and destroying or neutralizing any added chemicals such as strong bases that were added as part of the pathogen-reducing process, composites can be collected over a 2-week period. Corresponding composites of feed and treated sludge can be compared, with a much lower likelihood of not finding viable helminth ova in the feed sewage sludge. Because the analytical method itself has a high variance (see above), approximately four duplicates of the composite should be tested.

For enteric viruses, the same approach may be used as suggested above for viable helminth ova. Precautions are taken to cool the sample and destroy or neutralize any chemicals added in the pathogen-reducing process. Samples are collected on separate days and are promptly frozen at 0°F (-18°C), or -94°F (-70°C) if storage will be for more than 2 weeks. When the samples are to be analyzed, the individual samples are thawed and composited, and viral densities determined.

The density of both viable helminth ova and enteric viruses in processed sludge must be computed from the results of several measurements. Most of these measurements are expected to show below detectable densities. The averaging procedure suggested above for salmonellae should be followed. The arithmetic mean is determined considering the nondetectable measurements as zero per 4 grams. If the arithmetic mean is above 1 CFU (for viruses) or 1 viable helminth ovum (for helminths) per 4 grams, the process does not meet the Part 503 operational standard.

Vector Attraction Reduction Tests

Reduction in Volatile Solids

One way to demonstrate reduction in volatile solids requires measurement of volatile solids of the sewage sludge before and after sludge treatment. The sampling point for the "after treatment" measurement can be immediately leaving the processing unit or at the point of use or disposal, provided there has been no significant dilution downstream with inert solids.

Farrell and Bhide (1993) have determined the standard deviation of the percent volatile solids (%VS) determination for separate samples withdrawn from pilot-scale digesters to be 0.65% (total solids content ranged from 2% to 5%). Conventional statistical procedures (see Davies and Goldsmith, 1972) were used to calculate the standard error of the percent volatile solids reduction (%VSR), which is calculated from the %VS of the untreated and treated sludge. The standard error of the %VSR when calculated by the Van Kleeck equation (see Appendix D) is 2.0% in the range of interest (38% VSR). The 95% confidence limits of the %VSR are ±4%, which is excessive. If the %VSR is the average of seven determinations, the confidence interval is reduced to ±1.5%, which is a more acceptable value.

The most difficult problem with the %VSR determination, as discussed above in Section 7.4, is getting correspondence of the influent sludge with the effluent sludge. If there has been a significant change in an inlet concentration or flow rate, achieving correspondence can require several months of monitoring inlet and outlet volatile solids concentrations. If conditions have been steady and feed compositions have been fluctuating about an average value for a long period, data taken over a 2-week period would be adequate to establish steady state performance.[1] This implies that data have been collected beforehand that demonstrate that sewage sludge composition has been stable for a long period before the 2-week sampling period. It appears that regular collection of data for some months before the sampling period is unavoidable to demonstrate stable performance before the testing period. Fortunately, the total and volatile solids determinations are not costly, and they provide valuable operating information as well.

Total and volatile solids content of a sample do not change significantly over the course of a day, particularly if the sludge is cooled. Time composites collected over a course of a day can be used for these determinations. Seven or more determinations are recommended to reduce the error band around the mean to minimize the chance that a process that actually has a greater volatile solids reduction than 38% might show an average that is below this value.

Additional Digestion Tests

The essential measurement in the additional digestion tests for aerobic and anaerobic sludges (see Sections 6.3 and 6.4) is the percent volatile solids content (%VS) from which the percent volatile solids reduction is calculated (%VSR). Using the standard deviation of 0.65% determined by Farrell and Bhide (see above), the standard error of the %VSR when calculated by the Van Kleeck equation (see Appendix D) is 2.5% in the range of interest (15% VSR). The 95% confidence limits of the %VSR are ±5%. The tests (see Appendix D) require substantial internal replication which shrinks these confidence limits. Samples should also be taken to account for the variability in the process. The 2-week sampling period suggested for the microbiological tests may be excessively restrictive if several samples are to be evaluated. The equipment needed for the test is not expensive but the units take up substantial bench space. It is unlikely that a treatment works will want to have more than two sets of test equipment. Since the tests take 30 to 40 days, it is not possible to run more than one set of tests (two in a set) within a monitoring event. It is suggested that these tests be routinely carried out during the year and the results be considered applicable to the monitoring period. It is estimated on a best judgment basis that five tests are needed to account for variability in the feed sludge and in the treatment process itself.

Specific Oxygen Uptake Rate Test

The specific oxygen uptake rate test (SOUR, see Appendix D) can be completed in the laboratory in a few minutes, so there is no difficulty in completing the test during a monitoring event. The test requires the SOUR determination to be made on two subsamples of a given sample. Farrell and Bhide (1993) found that, in the target SOUR value of 1.5 mg O_2/hr/g, sludge solids replicates agreed within about ±0.1 mg O_2/hr/g. Since the test is easy to run, it is suggested that seven tests within the 2-week sampling event will adequately define the SOUR. Arithmetic average of the tests should be computed and compared against the Part 503 SOUR value.

Raise pH to 12

There are two options in the regulation that reduce vector attraction by pH adjustment. In the first, sludge is raised in pH by alkali addition so that pH is ≥12 for 2 hours after alkali addition and, without further alkali addition, remains at pH ≥11.5 for an additional 22 hours (see Section 6.7). The second method is for domestic septage. The pH is raised to pH ≥12 by alkali addition and, without further addition of alkali, remains at ≥12 for 30 minutes (see Section 6.13). As noted in Section 5.6, the term alkali is used in the broad sense to mean any substance that increases pH.

The pH requirement in the regulation was established using data obtained at room temperature (Counts and Shuckrow, 1975; Ronner and Cliver, 1987), which is presumed to have been 25°C (77°F). Consequently, pH should be measured at 25°C (77°F) or measured at the existing temperature and converted to 25°C (77°F) by use of a temperature-versus-pH conversion table determined experimentally for a treated sludge that meets the pH requirements. The correction is not trivial for alkaline solutions—it is about -0.03 pH unit/°C (-0.017 pH unit/°F) for aqueous calcium hydroxide with a pH of about 12—and should not be ignored. Note that temperature-compensated pH meters only adjust instrument parameters and do not compensate for the effect of temperature on the pH of the solution.

[1]Note that, unlike the plug flow case, there should be no displacement in time between comparisons of input and output for fully mixed reactors. Only when there has been a significant change is it necessary to wait a long time before the comparisons can be made.

Septage. Each container of domestic septage being treated with alkali addition must be monitored. The pH is monitored just after alkali addition and a half hour or more after alkali addition. Bonner and Cliver (1987) suggest that alkali (they used slaked lime) be added to the septic tank or to the septic tank truck while domestic septage is being pumped from a septic tank into the tank truck. If slaked lime is used, a dose of 0.35 lb per 10 gallons (4.2 g per liter) is sufficient to raise the pH to 12 for a typical domestic septage of about 1% solids content. The agitation from the high velocity incoming stream of septage distributes the lime and mixes it with the domestic septage. The pH is measured when the truck loading is complete. The truck then moves to the use or disposal site. Agitation generated by the motion of the truck helps in mixing and distributing the lime. The pH is again measured at the use or disposal site. The time should be at least a half hour after the addition of lime. The sample may be obtained through the top entry of the tank truck, using, for example, a stainless steel cup welded to a long handle to collect the sample. The pH is most conveniently measured with alkaline pH paper in the pH range of 11 to 13. The pH paper can age and become contaminated. It is best to use strips from two separate containers. If they do not agree, compare with a third batch and reject the one that disagrees with the others. Accuracy of these measurements is within ±0.1 pH unit. If the pH is below 12, either initially or after 30 minutes, more lime should be added and mixed in. After an additional waiting period of at least 30 minutes, the pH must again be measured to ensure that it is greater than 12.

Sewage Sludges. For addition of alkali to sewage sludges, the pH requirement is part of both the PSRP process description (see Section 5.3) and the requirement of a vector attraction option (see Section 6.7). Monitoring is required from 1 to 12 times a year (see Table 3-4 in Chapter 3), *and* the process must meet the processing operating conditions throughout the year. For vector attraction reduction, the pH requirement has to be met at least during the required monitoring episodes. For pathogens, the assumption is that the pH requirement is met all the time.

Alkali is sometimes added to liquid sludge and sometimes to dewatered sludge. The pH requirements as stated in the regulation apply in the same way for both liquid and dewatered sludge. For the first measurement of pH in liquid sludge 2 hours after addition of alkali, it is assumed that the alkali and the sludge have been mixed together for a sufficient time to reach equilibrium (not considering the gradual changes that occur over substantial periods of time). Consequently, the pH measurement can be made directly in the liquid sludge. The pH measurement is made preferably with a pH meter equipped with a temperature compensation adjustment and a low-sodium glass electrode for use at pH values over 10. The pH electrode is inserted directly in the sludge for the reading. The use of an alkaline range pH paper is acceptable if the paper is calibrated by use of pH standards. The second measurement is made 24 hours after addition of alkali. If the sludge is still in the liquid state, the pH measurement is made in the same fashion. However, if the process includes a dewatering step immediately following the alkali addition and the sludge is now a dewatered cake, the cake must be made into a slurry for the pH measurement. Acceptable procedures for preparing the sample and measuring pH are given by Block (1965) and by EPA (1986). The procedure requires adding 20 mL of distilled water (containing 0.01 M $CaCl_2$) to 10 g of sludge cake, mixing occasionally for half an hour, waiting for the sample to clarify if necessary, and then measuring pH. The important step is the mixing step that allows the alkali-treated dewatered sludge to come into equilibrium with the added water.

Number of Samples. The accuracy of pH meters and of pH paper is within ±0.1 pH unit. More than one sample is necessary if the domestic septage or sludge is not well mixed. If the lime has been added gradually over the period in which septage is being pumped into a tank truck, mixing is considered adequate and a single measurement taken at the top of the tank truck is sufficient. If alkali has been added to liquid sludge in a tank at a treatment plant, tests are easily run to establish how much mixing is required to produce a uniform pH in the sludge. If this adequate mixing time is used, a single sample withdrawn from the tank for pH measurement is sufficient.

If alkali is added to sludge cake, more sampling is suggested. Typically, alkali (usually lime) is added to sludge cake in a continuous process. The sludge from the dewatering process discharges continuously to a continuous mixer, from which it discharges to a pile or to a storage bin. Lime is metered into the mixer in proportion to the sludge flow rate. The flow rate and compositions of the sewage sludge can vary with time. To demonstrate compliance on a given day, several time-composite samples each covering about 5 minutes should be collected, and the pH measured. This procedure should be repeated several times during the course of a 2-week sampling event.

For sludge cake, the composites collected for pH measurement must be reduced in size for the pH measurement. Typically, the alkaline-treated sludge is discharged from the mixing devices in the form of irregular balls that can be up to 2 to 3 inches (5 to 7.6 cm) in diameter. It is important that the sludge to which the environment will be exposed has been treated to reduce pathogens and vector attraction to the desired level. If the alkali has not penetrated the entire pellet, one or both of these goals might not be met for the material on the interior of the pellet. How much risk is involved when the interior is not as well treated as the exterior cannot be directly measured or accurately estimated. To be conservative, the entire pellet should be at the proper pH. It is suggested that the composite be thoroughly mixed and that a subsample be taken for analysis from the mixed composite. An even more conservative approach is to sample only the interior of pellets, but then the test tends to become subjective (i.e., how close should one come to the skin of the pellet when taking out the inside of it?) and somewhat unrealistic. It seems more likely that the environment will be exposed to the average composition of the pellet rather than the interior alone, so the average composition should be analyzed.

Percent Solids Greater Than or Equal to 75% and 90%

The monitoring requirement for these vector attraction options (see Sections 6.8 and 6.9) is simply measurement of total solids. This measurement is described in Standard Methods (APHA [1992], Standard Method 2540 G). Standard Methods

states that duplicates should agree within ±0.5% of their average. For 75% solids, this would be ±3.8%, which appears unrealistically high. For a continuous process, a time-composite sample can be taken over the course of a day, and duplicate analyses carried out on this composite. This is possible because biological activity essentially ceases at high solids content, and further changes will not occur if the sludge is kept from further drying. Approximately seven such composites over the course of a 2-week sampling period would provide adequate sampling.

Some drying processes—such as drying sludge on sand drying beds—are batch processes. In such cases, it may be desirable to ascertain that the sludge meets the vector attraction reduction requirements *before* removing the sludge from the drying area. This can be done by taking two separate space-composites from the dried sludge, analyzing each of them in duplicate, and removing the sludge only if it meets the required solids content.

Chapter 8
Sampling Procedures and Analytical Methods

8.1 Introduction

Many of the Part 503 Subpart D pathogen and vector attraction reduction requirements call for monitoring and analysis of the sewage sludge to ensure that microbiological quality and vector attraction reduction meet specified requirements (see Chapters 4 to 6 for a description of the requirements). This chapter describes procedures that should be followed in obtaining samples and insuring their quality and integrity. It also summarizes the analytical procedures required under Part 503, and directs the reader to other sections of this document that describe some of those procedures.

Sampling personnel will also benefit from reading expanded presentations on the subject. Especially recommended are "Standard Methods" (APHA, 1992), "Principles of Environmental Sampling" (Keith, 1988), "Samplers and Sampling Procedures for Hazardous Waste Streams" (EPA, 1980), "Sludge Sampling and Analysis Guidance Document" (EPA, 1993), and ASTM Standard E 300-86, "Standard Practice for Sampling Industrial Chemicals" (ASTM, 1992a). The latter publication provides an in-depth description of available sampling devices and procedures.

When referring to other publications, it is important to note that most guidance on specific sampling techniques is directed toward chemical analysis. Procedures described may be inappropriate for microbiological sampling because they expose the samples to possible contamination, or may be appropriate only after some modification to reduce the risk of microbial contamination during sampling.

8.2 Safety Precautions

Sewage sludges that are being sampled should be presumed to contain pathogenic organisms, and should be handled appropriately. Both the sampler and the person carrying out the microbiological analysis must take appropriate precautions. Safety precautions that should be taken when sampling and when analyzing the samples are discussed in Standard Methods (APHA, 1992) in Sections 1060A and 1090C.

Individuals performing sampling (usually employees of wastewater treatment works) should receive training in the microbiological hazards of wastewater and sludge and in safety precautions to take when sampling. For example, gloves should be worn when handling or sampling untreated or treated sewage sludges. The person taking the samples should clean the sample containers, gloves, and his or her hands before delivering the samples to others. Hands should be washed frequently. Photocell-activated or foot-activated hand washing stations are desirable to reduced spreading of contamination to others. Employees should train themselves to avoid touching their lips or eyes. At a minimum, employees should be immunized against tetanus.

Personnel analyzing sludge samples should also receive training in awareness and safety concerning biohazards. Since microbiological laboratories have safety programs, this subject is not covered in depth here. Laboratory personnel should be aware that every sample container is probably contaminated on the outside with microorganisms, some of which may be pathogens. Personal hygiene and laboratory cleanliness are extremely important. Mouth-pipetting should be forbidden.

8.3 Sampling Free-Flowing Sewage Sludges

Sewage sludges below about 7% solids behave, at worst, like moderately viscous liquids such as an SAE 20 lubricating oil. They flow freely under small pressure gradients, and will flow readily into a sample bottle. They are heterogeneous, and concentration gradients develop upon standing. Generally settling is slow and is overcome by good mixing.

Liquid sludges may be flowing in pipes, undergoing processing, or stored in concrete or metal tanks, in tank cars or tank trucks, or in lagoons. This section describes procedures for sampling from these various situations, except for lagoons, which are discussed in Section 8.4.

Filling Containers

Liquid sludge samples are usually transferred into wide-mouth bottles or flexible plastic containers. Sludges can generate gases and pressure may build up in the container. Consequently, the bottle or container is generally not filled. If the sludge is to be used for the oxygen uptake test, the sample bottle should not be more than half full, to provide some oxygen for respiration of the sludge.

On the other hand, if the sludge is to be used for the additional anaerobic digestion test for vector attraction reduction, it is important that it not be exposed to oxygen more than

momentarily. Consequently, the bottles must be filled. They should be provided with a closure that can pop off, or else be collected in a flexible plastic container than can both stretch and assume a spherical shape to relieve any internal pressure that develops.

The containers used to collect the samples can be wide-mouth bottles that can be capped, or pails. If a pail is used and only part of its contents will be taken as a sample, the sample should be transferred to a bottle at the sampling location. Preferably, the transfer should be made by use of a ladle rather than by pouring, since some settling can occur in the pail, particularly with primary or mixed sludges of solids contents below about 3%.

Sampling Flowing Streams

If liquid sewage sludges are to be sampled, it is most desirable to sample them as they are being transferred from one vessel to another. Preferably this is done downstream of a pump that serves to mix the sludge thoroughly. Ideally, the sample is taken though a probe facing upstream in the center of the discharge pipe and is withdrawn at the velocity of the liquid at the center-line of the pipe. This approach generally is not possible with sludge, because fibrous deposits probably would build up on the probe and plug up the pipeline. Sampling through a side tap off the main discharge pipe is adequate only if the flow is turbulent and the sample point is over ten pipe diameters downstream from the pipe inlet (e.g., for a 3-inch [7.6-cm] pipe, 30 inches [76 cm] downstream) or the tap is downstream from a pump. For any kind of a slurry, the fluid at the wall contains fewer particles than the bulk of the fluid in the pipe. The sample should be withdrawn fast enough so that it minimizes the amount of thinned-out fluid from the outside pipe wall that enters the sample.

If the sludge discharges into the open as it is transferred from one vessel to another, it can be sampled by passing a sample container through the discharge stream. The container should be large enough to catch the whole stream during the sampling interval, rather than, for example, just sampling the center or the edge of the discharge. The sample container could be a pail or a beaker at the end of an extension arm.

Sample volume should be about three times what is needed for the analyses planned. Samples should be time-composited. For example, if a digester is being sampled during a withdrawal that takes about 15 minutes, a sample can be taken during the first, second, and third 5-minute period. The three separate samples should be brought back to the laboratory and composited into a single sample. The maximum allowable time for a composite for either bacteria, viral, or vector attraction reduction test samples is about 1 hour; a greater time might allow microbiological changes to occur in the first sample taken. Composite sampling over 24 hours is possible for viable helminth ova provided the ova in the sample are not exposed to chemical or thermal stress—for example, temperatures above 40°C (104°F) or certain chemicals such as ammonia, hydroxides, and oxidants.

Sampling Sewage Sludge in Tanks

The purpose of the sampling is to determine a mass-average property of the sludge, rather than, for example, to find out if there is a gradient in the property at various points in the tank. This requires that the tank be well-mixed, otherwise many subsamples must be taken throughout the tank and composited. If tanks are large, even well-mixed tanks containing sludge show gradients in composition. An enclosed tank such as an anaerobic digester must be sampled through pipelines entering the vessel. A minimum of three taps on a side wall of the tank is recommended. The sample tap pipe should project several feet into the tank. Preferably the sample line should be backflushed with water after the sample is withdrawn. When a sample is withdrawn, enough material must be withdrawn to thoroughly flush the line before a sample is collected. The sampling should be done when the tank is being agitated. An open tank such as an aerobic digester can be sampled by drawing a vacuum on a vacuum-filtering flask connected by a tube to the desired level in the tank. A weighted sampling bottle may also used to sample the liquid at three depths (see ASTM E300-86, Par. 21, in ASTM [1992a]).

8.4 Sampling Thick Sewage Sludges

If sewage sludges are above about 7% solids, they take on "plastic" flow properties; that is, they require a finite shear stress to cause flow. This effect increases as the solids content increases. Solids may thicken in lagoons to 15% solids. At these concentrations, they will not flow easily and require a substantial hydrostatic head before they will flow into a sample bottle.

Sampling of lagoons ranges from difficult to very difficult, depending on the objectives of the sampling and the nature of the sludge in the lagoon. The thickened sludge solids are generally nonuniformly distributed in all three dimensions. It is desirable first to map the distribution of depth with length and width to determine where the sampling should be concentrated. A length-width grid should be established that takes into account the nonuniformity of the solids deposit. ASTM E300-86, Figure 19 (ASTM, 1992a), shows a grid for sampling a uniform deposit in a railroad car.

The layer of water over the sludge complicates the use of many types of tube samplers because the overlying water should not be included in the sample. A thief sampler (ASTM, 1992a) that samples only the sludge layer may be useful. Weighted bottle samplers (ASTM, 1992a) that can be opened up at a desired depth can be used to collect samples at a desired depth. Samples at three depths could be taken and composited. Most likely the sludge will be as much as twice as high in solids content at the bottom of the sludge layer as at the top. Compositing of equal volumes of samples from top, middle, and bottom produces an excellent mass-average sample and adjusts for this difference in solids content. Generally there is no point in determining the gradient with depth in a parameter, because there is no practical way of separately removing layers of sludge from a lagoon. Determining whether there are gradients with length and width makes more sense because, if desired, sludge could be removed selectively from part of a lagoon, leaving behind the unacceptable material.

Sludges from dewatering equipment such as belt filter presses and centrifuges can reach 35% solids and even higher solids following some conditioning methods. They are easy to sample if they are on moving conveyors, but if they are stored in piles, the sampling problem becomes very difficult. Sampling devices such as augers (a deeply threaded screw) are used on high solids cakes (ASTM, 1992a). The auger is "turned into the pile and then pulled straight out. The sample is removed from the auger with a spatula or other suitable device." The pile should be sampled in proportion to its mass—that is, more samples are taken where the pile is deeper.

8.5 Sampling Dry Sewage Sludges

For purposes of this discussion, "dry" sewage sludges include sludges that may contain as much as 60% water. They include heat-dried sludges, composted sludges, and sludges from dewatering processes such as pressure filtration that produce a cake which is usually handled by breaking it up into pieces. Some centrifuge cakes are dry enough that they are comprised of small pieces that remain discrete when piled.

Dry sludges are best sampled when they are being transferred, usually on conveyors. Preferably material across the entire width of the conveyor is collected for a short period of time. Several of these across-width samples are collected and combined into a time-composite sample. If the entire width of the conveyor cannot be sampled, the sample is collected from various points across the breadth of the conveyor, and a space- and time-composited sample is collected.

When a material comprised of discrete particles is formed into a pile, classification occurs. If the particles are homogeneous, this creates no problem, but sometimes the particles are of different composition. For example, a composted sludge may be heterogeneous even when oversize bulking agents have been removed. It is important that the edges of such piles and the center be properly weighted. ASTM E300-86, Item 31.4 (ASTM, 1992a) suggests a sampling grid for a pile that prevents bias.

The heterogeneous nature of some composted sludges causes another problem in sampling. For example, most augers and sampling thiefs will be ineffective in getting a representative sample of the interior of a pile containing large wood chips and fine composted sludge. There may be no substitute for digging with a shovel to get to the desired location.

Even processed sewage sludge is not inert; in the presence of air it oxidizes slowly. Temperatures can rise to substantial levels. For example, a storage pile of compost or dried sludge may be at room temperature on the outside but could be at 60°C (140°F) at a depth of 2 feet (0.6 m) within the pile. The microbiological content of samples from the surface and from the interior of the pile may be considerably different. When there is a large temperature gradient in a storage pile, it is important to include an analysis of the sludge from the cooler section of the pile where the chance of regrowth of bacterial populations is greatest. In any case, samples from a large pile should be taken at various depths and along its length.

8.6 Control of Temperature, pH, and Oxygenation After Sample Collection

Samples for Microbial Tests

All samples for microbial analyses should be cooled to water-ice temperatures when collected or very soon thereafter. For example, enteric viral and bacterial densities are noticeably reduced by even 1 hour of exposure to temperatures of 35°C (95°F) or greater. The requirement for cooling limits the practical size of the sample collection container. A gallon sample bottle will take much longer to cool than a quart bottle. Use of bottles no bigger than a quart is recommended for most samples, particularly if the sludge being sampled is from a process operated at above ambient temperature. Granular solids and thick sludges will take a long time to cool, so a small container is advised. For rapid cooling, placing the sample container in a slurry of water and ice produces excellent results. Bagged ice or "blue ice" is effective in maintaining low temperatures but several hours can elapse before this kind of cooling reduces sample temperature to below 10°C (50°F) (Kent and Payne, 1988). The same is true if warm samples are placed in a refrigerator.

Standard Methods (APHA, 1992) states that for bacterial species, which include fecal coliform and salmonellae, if samples are not to be tested within 1 hour after collection, they should be cooled to below 10°C (50°F) during a maximum transport time of 6 hours. The authors then admit that this time can be unrealistic, particularly if material must be mailed or air-expressed to a testing laboratory. They then say that the time between collection and analysis should not exceed 24 hours. If samples are brought to 4°C (39.2°F) by *prompt* chilling, 24 hours between sampling and analysis should not adversely affect the results. Samples for bacteriological analyses should not be frozen.

The requirement for prompt chilling of samples is appropriate for viruses as well as bacteria. There are far fewer laboratories capable of carrying out virus tests than bacteria, so time between analysis and sampling could routinely exceed 24 hours. Fortunately, viruses are not harmed by freezing. Typically, virology laboratories store samples at -70°C (-94°F) before analysis. Deep freezers are not ordinarily available in a wastewater treatment works, but samples can be frozen in a -18°C (0°F) freezer and stored for up to 2 weeks without harm. They then can be packed in dry ice and shipped to the analyzing laboratory.

Viable helminth ova are only slightly affected by temperatures below 35°C (95°F), provided chemicals such as lime, chlorine, or ammonia have not been utilized in the treatment process. Nevertheless, chilling to 4°C (39.2°F) is advised. If the samples are held at this temperature, a period of a month can elapse between sampling and analysis. Freezing should be avoided because the effect of freezing on helminth ova is incompletely understood.

If the sludge is treated by a chemical such as lime, the lime may have to be removed (in this case by neutralization) immediately after sampling if the microbial tests are to be valid.

Failure to remove the lime extends the treatment time, and the high pH may interfere with the microbial test.

The presence or absence of oxygen is not a serious concern for the microbiological tests if the samples are promptly cooled.

Vector Attraction Reduction Tests

For the vector attraction reduction tests that measure oxygen uptake, or additional anaerobic or aerobic digestion (see Appendix D), the samples must be kept at the temperature at which they were collected. This sometimes can be done just by collecting a large sample in a large container. Covering the sample with an insulating blanket or placing it in an insulated box will be adequate protection against temperature change in most cases. Desired temperature can be maintained in the box by adding a "hot water bottle" or a bag of blue ice.

For any of the vector attraction reduction tests, no adjustment of pH is to be made. For those vector attraction processes that utilize lime, the only requirement is to measure pH after the time periods indicated in the vector attraction reduction option (see Section 6.7).

Lack of oxygen for aerobic sludges will interfere with the metabolic rate of the aerobic microorganisms in the sample. Similarly, presence of oxygen will seriously affect or even kill the anaerobic organisms that convert organic matter to gases in anaerobic digestion. For the oxygen uptake rate test, care must be exercised not to deprive the sample of air for more than an hour. The additional aerobic digestion test is more "forgiving" (because it is a long-term test and shocked bacteria can revive), so perhaps 4 hours of shortage of oxygen can be tolerated. For the additional anaerobic digestion test, the sample containers should be filled to exclude air. In any subsequent operations where there is a freeboard in the sample or testing vessel, that space should be filled with an inert gas such as nitrogen.

8.7 Sample Compositing and Size Reduction

The amount of sample collected will exceed the amount needed for analysis by a large margin. The sample generally must be reduced to a manageable size for the analyst to handle. Sample size reduction is more difficult for samples for microbial tests than for vector attraction reduction tests, because care must be exercised to minimize opportunity for microbial contamination.

Microbial Tests

For freely flowing liquids, samples can be adequately mixed in the sample bottles by shaking the bottles. There must be room in the bottle for adequate mixing. Compositing of smaller samples is accomplished by pouring them into a larger bottle with adequate freeboard and mixing it by shaking or stirring it thoroughly with a sterile paddle. Pouring off a small part of the contents of a large container into a smaller bottle is a poor procedure, because the top layer of any slurry always contains fewer solids than lower layers. Sampling with a pipette with a wide bore is an acceptable alternative, provided the bore of the pipette is as wide as possible. The sample should be drawn into the pipette slowly and the tip moved through the sample to minimize selective collection of liquid over solid particles.

Sample size reduction for thick sludges is difficult, because they cannot be mixed by shaking. Stirring with a mechanical mixer or a paddle is often inadequate (recall how long it takes to mix a can of paint). A satisfactory approach is to hand mix a composite of any subsamples, and then take a large number of small grabs from the large sample to form the smaller sample for the analyst.

Dry solids samples can generally be mixed adequately by shaking, but the individual particles are frequently large and must be reduced in size to get a representative sample. If the particles are large and a number of subsamples must be combined into a large composite, it may be necessary to reduce the particle size before they are composited. This can be done in a sterile covered chopper, blender, or grinder. The individual subsamples are then combined and mixed by shaking, rotating, and tumbling. A smaller composite is then prepared by combining a number of grabs from all parts of the combined sample. Some other methods used to reduce size, such as "coning and quartering" (ASTM, 1992a) cannot be used for microbiological samples because it is difficult to avoid contaminating the sample when using these procedures.

Vector Attraction Reduction Tests

The lack of a need to prevent microbial contamination makes compositing and size reduction easier for vector attraction reduction tests than for microbial tests. There is a need to keep the aerobic samples aerobic and to prevent the anaerobic samples from coming into contact with air. Subsamples for the anaerobic tests can be collected into individual bottles at the sampling location. As noted above, these sample bottles should be filled completely and capped. A brief exposure to air will not cause a problem, but any prolonged exposure, such as might occur when several subsamples are being blended together and reduced in size for a representative composite sample, must be avoided. One approach is to flush a sterile large bottle with nitrogen, then pour in the subsamples and blend them together with nitrogen still bleeding into the vessel. Alternatively, the nitrogen-filled vessel could be flushed with more nitrogen after the admission of the subsamples, capped, and then shaken thoroughly to accomplish the blending.

8.8 Requirements for Sample Containers and Sampling Tools

Materials of Construction

Sampling containers may be of glass or plastic that does not contain a plasticizer (teflon, polypropylene, and polyethylene are acceptable). Plastic bags are especially useful for thick sludges and free-flowing solids. Pre-sterilized bags are available. Stainless steel containers are acceptable, but steel or zinc-coated steel vessels are not appropriate.

Sterilization

The containers and tools used for sampling should be sterilized if the material is to meet the Class A microbiological requirements. Conservative microbiological practice also requires sterilization of containers and sampling tools to be used for collecting samples to be tested for meeting the Class B requirements. All equipment should be scrupulously clean. Sterilization is not required when collecting samples of sewage sludge to be used in vector attraction reduction tests, but all equipment must be clean.

Sterilization of the larger tools for sample collection can frequently be accomplished in large laboratory sterilizers. A shovel can be sterilized by enclosing the blade in a kraft paper bag, sealing the bag around the handle, and placing the entire shovel in the sterilizer. The handle gets contaminated when it is touched, but the bag around the blade is not removed until the shovel is used to take the sample, leaving it sterile. In other cases, a trowel could be sterilized and a clean but unsterile shovel used to get close to the desired sampling spot. The sterile trowel is used for the sample collection.

For larger devices, it is possible to clean the device very carefully, direct a jet of atmospheric pressure steam over the surface that would contact the sample for a few minutes, and then enclose the device in a sterile bag. In some cases, the device need not be covered but can simply be closed up until it is used to collect the sample. A tube or thief sampler could be sterilized by running atmospheric pressure steam through it for 10 minutes. This procedure does not give assurance of total sterilization, but may be the only possible option.

8.9 Packaging and Shipment

Proper packaging and shipment are important to ensure preservation.

Taping and Sealing

Sample containers should be securely taped to avoid contamination, and sealed (e.g., with gummed paper) so it is impossible to open the container without breaking the seal. Sealing ensures that sample integrity is preserved until the sample is opened in the laboratory. It is recommended that permanent labels be affixed to the samples. Suggested information for a sample label includes:

- Type of sewage sludge (e.g., "air-dried digested sludge" or "windrow composted sludge").
- Amount sampled.
- Type of sample (grab or composite).
- Type of analysis to be performed (e.g., *Salmonella* sp., fecal coliform bacteria, enteric virus, or viable helminth ova).
- Date and time the sample was taken.
- Sample identification code (if used) or a brief description of the sampling point and treatment process if no sample code system is used.
- Sample number (if more than one sample was collected at the same point on the same day).
- Facility name and address.
- Facility contact person.
- Name of the person collecting the sample.

Other information, such as sewage sludge pile temperature at representative depth (e.g., if above ambient temperature), may also be helpful (Yanko, 1987).

Shipment Container

A soundly constructed and insulated shipment box is essential to provide the proper environment for the preserving sample at the required temperature. It is recommended that the outside label of the shipment container include:

- The complete address of the receiving lab (including the name of a responsible person).
- A number clearly indicating how many samples are included.
- The words "Fragile" and "This End Up."

To maintain a low temperature in the shipment box, a blue-ice type of coolant in a sealed bag should be included in the box. If the blue ice has been stored in a 0°F (-18°C) freezer (e.g., a typical household freezer), the coolant should be "tempered" to warm it up to the melting point of ice (0°C [32°F]) before it is placed around the sample.

Adherence to Holding and Shipment Times

Adherence to sample preservation and holding time limits described in Section 8.6 is critical. Samples that are not processed within the specified time and under the proper conditions can yield erroneous results, especially with the less stable microorganisms (i.e., bacteria).

8.10 Documentation

Sampling Plan

It is recommended that all sampling procedures be documented in a sampling plan that identifies the sampling points, volumes to be drawn, days and times of collection, required equipment, instructions for labelling samples and ensuring chain of custody, and a list of contact persons and telephone numbers in case unexpected difficulties arise during sampling. If a formal sampling plan is not available, a field log that includes instructions and a sample collection form may be used (EPA, 1980).

Sampling Log

It is suggested that all information pertinent to a sampling be recorded in a bound log book, preferably with consecutively numbered pages. Suggested entries in the log book include, at a minimum:

- Purpose of sampling.
- Location of sampling.
- Grab or composite sample.
- Name and address of the field contact.
- Type of sewage sludge.
- Number and volume of the sample taken.
- Description of sampling point.
- Date and time of collection.

A good rule of thumb is to record sufficient information so that the sampling situation can be reconstructed without reliance on the collector's memory.

Chain of Custody

To establish the documentation necessary to trace sample possession from the time of collection, it is recommended that a chain-of-custody record be filled out and accompany every sample. This record is particularly important if the sample is to be introduced as evidence in litigation. Suggested information for the chain-of-custody record includes, at a minimum:

- Collector's name.
- Signature of collector.
- Date and time of collection.
- Place and address of collection.
- Signatures of the persons involved in the chain of possession.

8.11 Analytical Methods

Part 503.8(b) of the Part 503 regulation specifies methods that must be used when analyzing for enteric viruses, fecal coliform, salmonellae, viable helminth ova, specific oxygen uptake rate, and total, fixed, and volatile solids. Table 8-1 lists the required methods. Complete references for these methods can be found in Chapter 12. Appendix F presents sample preparation methods for fecal coliform tests and *Salmonella* sp. analysis and discusses reporting of results. Appendix G provides the required analytical method for *Salmonella* sp. Appendix H presents a detailed discussion of the required method for analysis of enteroviruses from sewage sludge, and Appendix I provides the required analytical method for viable helminth ova.

Table 8-1. Analytical Methods Required Under Part 503

Enteric Viruses	American Society for Testing and Materials Method D 4994-89 (ASTM, 1992b)[1]
Fecal Coliform	Standard Methods Part 9221 E or Part 9222 D (APHA, 1992)[2]
Salmonella sp. Bacteria	Standard Methods Part 9260D (APHA, 1992)[2] *or* Kenner and Clark (1974) (see Appendix G of this document)[2]
Viable Helminth Ova	Yanko (1987) (see Appendix I of this document)
Specific Oxygen Uptake Rate (SOUR)	Standard Methods Part 2710B (APHA, 1992)
Total, Fixed, and Volatile Solids	Standard Methods Part 2540G (APHA, 1992)
Percent Volatile Solids Reduction	Appendix C of this document

[1]Appendix H of this document presents a detailed discussion of this method.
[2]See Appendix F of this document for recommended sample preparation procedures and a discussion of the reporting of results.

Part 503 also refers to this document for the method to be used when calculating percent volatile solids reduction (see Appendix C of this document). Appendix D provides guidance on how to conduct the additional digestion tests to demonstrate reduced vector attraction in anaerobically and aerobically digested sewage sludge. Appendix D also provides guidance on adjusting the specific oxygen uptake rate (SOUR) determined at the temperature at which aerobic digestion is occurring in the treatment works to a SOUR for 20°C (68°F).

8.12 Quality Assurance

Quality assurance comprises establishing a sampling plan, quality control measures, and procedures for ensuring that the results of analytical and test measurements are correct. A complete presentation of this subject is beyond the scope of this manual. A concise treatment of quality assurance is found in Standard Methods (APHA, 1992) and is especially recommended. Parts 1000 to 1090 of Standard Methods are relevant to the entire sampling and analysis effort. Part 1020 discusses quality assurance, quality control, and quality assessment.

Standard Methods (Part 1020B) states that "a good quality control program consists of at least seven elements: certification of operator competence, recovery of known additions, analysis of externally supplied standards, analysis of reagent blanks, calibration with standards, analysis of duplicates, and maintenance of control charts." For most of the tests to be carried out to meet the pathogen and vector attraction reduction requirements of the Part 503 regulation, these elements cannot be met completely, but they should be kept in mind as a goal.

Microbial Tests

For the microbiological tests, quality assurance is needed to verify precision and accuracy. Quality assurance for microbiological methods is discussed in Part 9020 of Standard Methods. The quality control approach they suggest is recommended for the microbiological tests required because of Part 503. In Part 9020B-4, Analytical Quality Control Procedures, it is suggested that precision be initially established by running a number of duplicates, and that thereafter duplicates (5% of total samples) be run to determine whether precision is being maintained.

To estimate accuracy, spiking and recovery tests are needed. The spiking should be to density levels significant to the Part 503 regulation. For example, for viable helminth ova, samples should be spiked to density levels of under 100 per gram. Yanko (1987) did not find spiking useful for bacterial tests or viral tests, although it was effective for the helminth ova test. For viruses, instead of spiking, he recommends demonstrating the effectiveness of recovery on primary sewage sludges that typically contain viruses at low but consistent levels (for example, primary sludges from large cities).

Vector Attraction Reduction Tests

It is not possible to test for accuracy for any of the vector attraction reduction tests, because standard sludges with consistent qualities do not exist. Standard Methods gives guidance on precision and bias. However, for some of the vector attraction reduction options, this information was not available or was approximate. Section 7.7 provides guidance on the number of samples to take. The procedures for the three vector attraction options developed for the Part 503 regulation (additional anaerobic and aerobic digestion and the specific oxygen uptake rate test), which are presented in Appendix D, have internal quality control procedures that include replication. Since the tests are newly proposed, the data are insufficient to judge whether agreement between replicates is adequate. This kind of information will be communicated as experience with these options accumulates.

Chapter 9
Processes to Significantly Reduce Pathogens (PSRPs)

9.1 Introduction

Processes to Significantly Reduce Pathogens (PSRPs) are listed in Appendix B of Part 503. There are five PSRPs: aerobic and anaerobic digestion, air drying, composting, and lime stabilization. Under Part 503.32(b)(3), sewage sludge treated in these processes is considered to be Class B with respect to pathogens (see Section 5.3).

When operated under the conditions specified in Appendix B, PSRPs reduce fecal coliform densities to less than 2 million CFU or MPN per gram of total solids (dry weight basis) and reduce salmonellae and enteric virus densities in sewage sludge by approximately a factor of 10 (Farrell et al., 1985).

This level of pathogen reduction is required, as a minimum, by the Part 503 regulation if the sewage sludge is applied to agricultural land, a public contact site, a forest, or a reclamation site or placed on a surface disposal site.[1] Because Class B sludges do contain some pathogens, land application of a Class B sludge is allowed only if crop harvesting, animal grazing, and public access are limited for a period of time following application of a Class B sewage sludge so that pathogens can be further reduced by environmental factors (see Section 5.5).

The PSRPs listed in Part 503 are essentially identical to the PSRPs that were listed under the 40 CFR Part 257 regulation, except that all requirements related solely to reduction of vector attraction have been removed. Vector attraction reduction is now covered under separate requirements (see Chapter 6) that include some of the requirements that were part of the PSRP requirements under Part 257, as well as some new options for demonstrating vector attraction reduction. These new options provide greater flexibility to the regulated community in meeting the vector attraction reduction requirements.

This chapter provides detailed descriptions of the PSRPs listed in Appendix B. Since the conditions for the PSRPs, particularly aerobic and anaerobic digestion, are designed to meet pathogen reduction requirements, they are not necessarily the same conditions as those traditionally recommended by design texts and manuals.

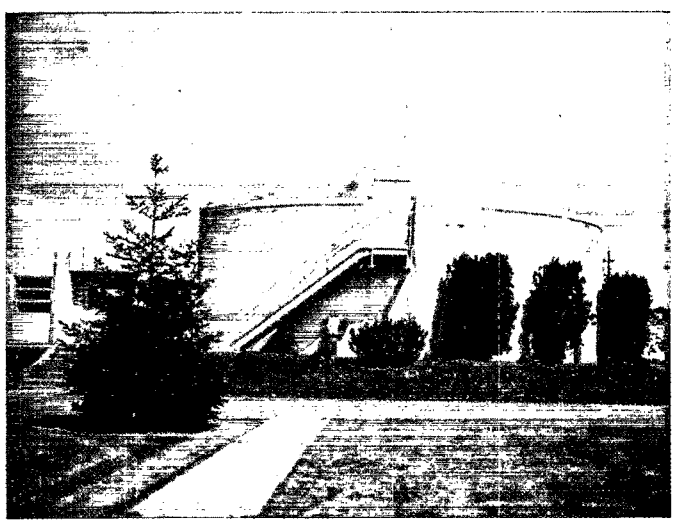
[1]Unless the active sewage sludge unit is covered at the end of each operating day, in which case no pathogen requirement applies.

9.2 Aerobic Digestion

In aerobic digestion, sewage sludge is biochemically oxidized by bacteria in an open or enclosed vessel (see photo). To supply these aerobic microorganisms with enough oxygen to carry out their task, either the sewage sludge must be agitated by a mixer or air must be forcibly injected (Figure 9-1). Under proper operating conditions, the volatile solids in sewage sludge are converted to carbon dioxide, water, and nitrate nitrogen.

Aerobic systems operate in either batch or continuous mode. In batch mode, the tank is filled with untreated sewage sludge and aerated for 2 to 3 weeks or longer, depending on the type of sewage sludge, ambient temperature, and average oxygen levels. Following aeration, the stabilized solids are allowed to settle and then separated from the clarified supernatant. The process is begun again by inoculating a new batch of untreated sewage sludge with some of the solids from the previous batch to supply the necessary biological decomposers. In continuous mode, untreated sewage sludge is fed into the digester once a day or more frequently; thickened, clarified solids are removed at the same rate.

The PSRP description in Part 503 for aerobic digestion is:



Digester in Vancouver, Washington.

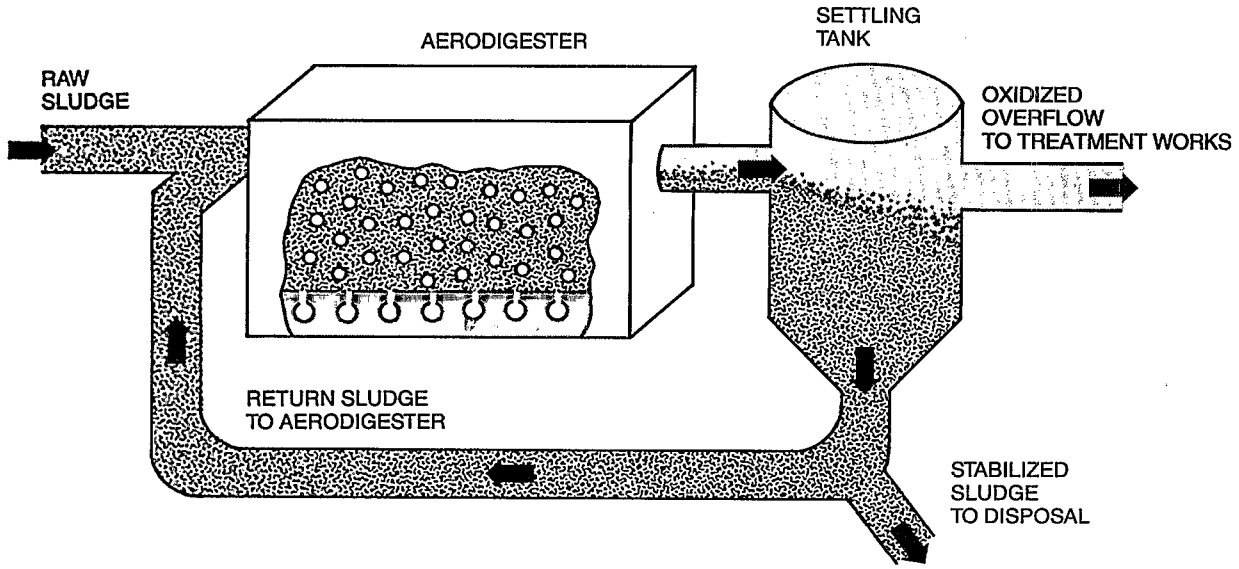


Figure 9-1. Aerobic digestion.

Sewage sludge is agitated with air or oxygen to maintain aerobic conditions for a specific mean cell residence time at a specific temperature. Values for the mean cell residence time and temperature shall be between 40 days at 20°C (68°F) and 60 days at 15°C (59°F).

The regulation does not differentiate between semi-batch and continuous operation, so either method is acceptable. The mean cell residence time is the residence time of the sewage sludge solids. The appropriate method for calculating residence time depends on the type of digester operation used:

- **Continuous-Mode, No Supernatant Removal.** For continuous-mode digesters where no supernatant is removed, nominal residence times may be calculated by dividing liquid volume in the digester by the average daily flow rate *in* or *out* of the digester.

- **Continuous-Mode, Supernatant Removal.** In systems where the is supernatant removed from the digester and recycled, the output volume of sewage sludge can be much less than the input volume of sewage sludge. For these systems, the flow rate of the sewage sludge *out of* the digester is used to calculate residence times.

- **Continuous-Mode Feeding, Batch Removal of Sewage Sludge.** For some aerobic systems, the digester is initially filled above the diffusers with treated effluent, and sewage sludge is wasted daily into the digester. Periodically, aeration is stopped to allow solids to settle and supernatant to be removed. As the supernatant is drawn off, the solids content in the digester gradually increases. The process is complete when either settling or supernatant removal is inadequate to provide space for the daily sewage sludge wasting requirement, or sufficient time for digestion has been provided. The batch of digested sewage sludge is then removed and the process begun again. If the daily mass of sewage sludge solids introduced has been constant, nominal residence time is one-half the total time from initial charge to final withdrawal of the digested sewage sludge.

- **Batch or Staged Reactor Mode.** A batch reactor or two or more well-mixed reactors in series are more effective in reducing pathogens than is a single well-mixed reactor at the same overall residence time. Reduction in residence time of 30% from the times given in the PSRP definition for aerobic digestion is recommended. The basis for this recommendation is given in Appendix E. These times are less than the PSRP conditions required for aerobic digestion in the regulation; consequently, approval by the regulatory authority is required.

- **Other.** Digesters are frequently operated in unique ways that do not fall into the categories above. Appendix E provides information that should be helpful in developing a calculation procedure for these cases.

Aerobic digestion carried out according to the Part 503 conditions typically reduces bacterial and viral pathogens by 90% (i.e., a factor of 10). Helminth ova are reduced to varying degrees, depending on the hardiness of the individual species. Aerobic digestion typically reduces the volatile solids content (the microbes' food source) of the sewage sludge by 40% to 50%, depending on the conditions maintained in the system.

Vector Attraction Reduction

Vector attraction reduction for aerobically digested sludges is demonstrated either when the percent volatile solids reduction during sludge treatment exceeds 38%, or when the specific oxygen uptake rate (SOUR) at 20°C (68°F) is less than or equal to 1.5 mg of oxygen per hour per gram of total solids, or when

additional volatile solids reduction during bench-scale aerobic batch digestion for 30 additional days at 20°C (68°F) is less than 15% (see Chapter 6).

Thermophilic aerobic systems (operating at higher temperatures) capable of producing Class A sludge are gaining in popularity as operators and researchers learn how to control and stabilize this comparatively delicate process. These systems are described more fully in Section 10.5.

9.3 Anaerobic Digestion

Anaerobic digestion is a biological process that uses bacteria that function in an oxygen-free environment to convert volatile solids into carbon dioxide, methane, and ammonia. These reactions take place in an enclosed tank (see Figure 9-2) that may or may not be heated. Because the biological activity consumes most of the volatile solids needed for further bacterial growth, the sewage sludge is stabilized. Currently, anaerobic digestion is one of the most widely used treatments for sewage sludge stabilization, especially in treatment works with average wastewater flow rates greater than 19,000 cubic meters/day (5 million gallons per day).

Most anaerobic digestion systems are classified as either standard-rate or high-rate systems. Standard-rate systems take place in a simple storage tank with sewage sludge added intermittently. The only agitation that occurs comes from the natural mixing caused by sewage sludge gases rising to the surface. Standard-rate operation can be carried out at ambient temperature, though heat is sometimes added to speed the biological activity.

High-rate systems use a combination of active mixing and carefully controlled, elevated temperature to increase the rate of sewage sludge stabilization. These systems sometimes use pre-thickened sewage sludge introduced at a uniform rate to maintain constant conditions in the reactor. Operating conditions in high-rate systems foster more efficient sewage sludge digestion.

The PSRP description in Part 503 for anaerobic digestion is:

Sewage sludge is treated in the absence of air for a specific mean cell residence time at a specified temperature. Values for the mean cell residence time and temperature shall be between 15 days at 35°C to 55°C (95°F to 131°F) and 60 days at 20°C (68°F).

Section 9.2 provides information on calculating residence times. Anaerobic digestion that meets the required residences times and temperatures typically reduces bacterial and viral pathogens by 90% or more. Helminth ova are not substantially reduced under mesophilic conditions (32°C to 38°C [90°F to 100°F]) and may not be completely reduced at temperatures between 38°C (100°F) and 50°C (122°F).

Vector Attraction Reduction

Anaerobic systems reduce volatile solids by 35% to 60%, depending on the nature of the sewage sludge and the system's operating conditions. Sewage sludges produced by systems that meet the operating conditions specified under Part 503 will typically have volatile solids reduced by at least 38%. Alternatively, vector attraction reduction can be demonstrated by Option 2 of the vector attraction reduction requirements, which requires that additional volatile solids loss during bench-scale anaerobic batch digestion of the sludge for 40 additional days at 30°C to 37°C (86°F to 99°F) be less than 17% (see Section 6.3).

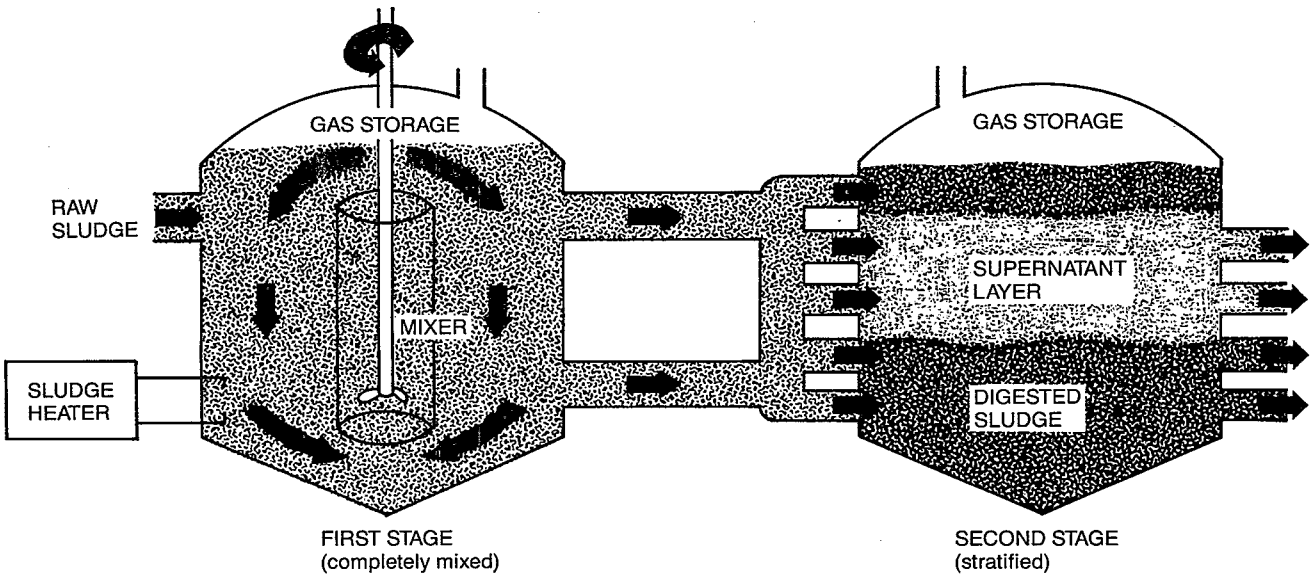
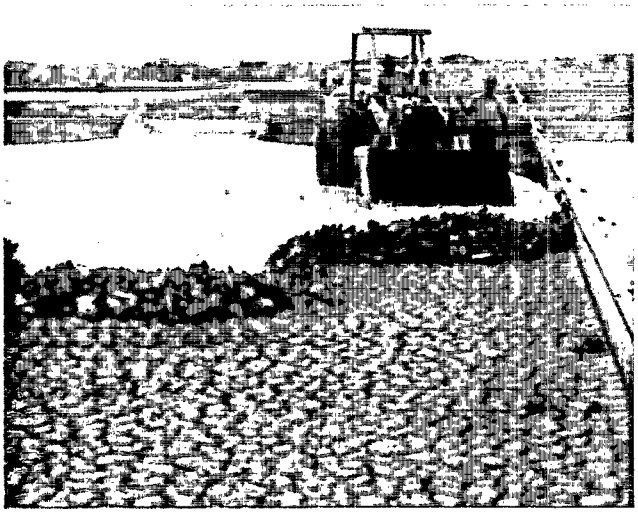


Figure 9-2. Two-stage anaerobic digestion (high rate).



Sludge drying operation. (Photo credit: East Bay Municipal Utility District)

9.4 Air Drying

Air drying allows partially digested sewage sludge to dry naturally in the open air (see photo above). Wet sewage sludge is usually applied to a depth of approximately 23 cm (9 inches) onto sand drying beds, or even deeper on paved or unpaved basins. The sewage sludge is left to drain and dry by evaporation. Sand beds have an underlying drainage system; some type of mechanical mixing or turning is frequently added to paved or unpaved basins. The effectiveness of the drying process depends very much on the local climate: drying occurs faster and more completely in warm, dry weather, and slower and less completely in cold, wet weather.

The PSRP description in Part 503 for air drying is:

Sewage sludge is dried on sand beds or on paved or unpaved basins. The sewage sludge dries for a minimum of 3 months. During 2 of the 3 months, the ambient average daily temperature is above 0°C (32°F).

In addition, it is advisable to ensure that the sewage sludge drying beds are exposed to the atmosphere (i.e., not covered with snow) during the 2 months that the daily temperature is above 0°C (32°F). Also, the sewage sludge should be at least partially digested before air drying. Under these conditions, air drying will reduce the density of pathogenic viruses and bacteria by approximately 90%. Helminth ova are reduced, except for some hardy species that remain substantially unaffected.

Vector Attraction Reduction

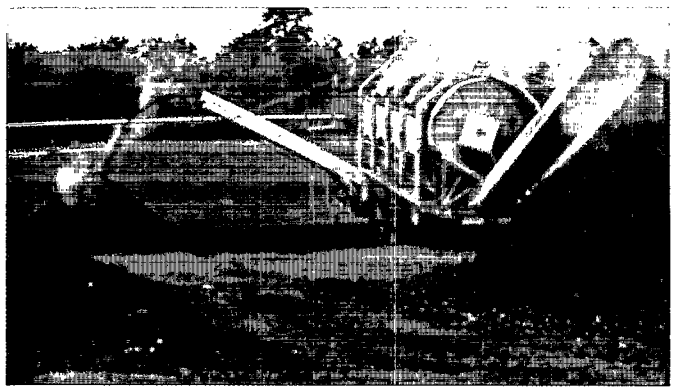
Air-dried sewage sludge typically is treated by aerobic or anaerobic digestion before it is placed on drying beds. Usually, the easiest vector attraction reduction requirement to meet is a demonstration of 38% reduction in volatile solids (Option 1—see Section 6.2), including the reduction that occurs during its residence on the drying beds.

In dry climates, vector attraction reduction can be achieved by moisture reduction (see Option 7 in Section 6.8, and Option 8 in Section 6.9).

9.5 Composting

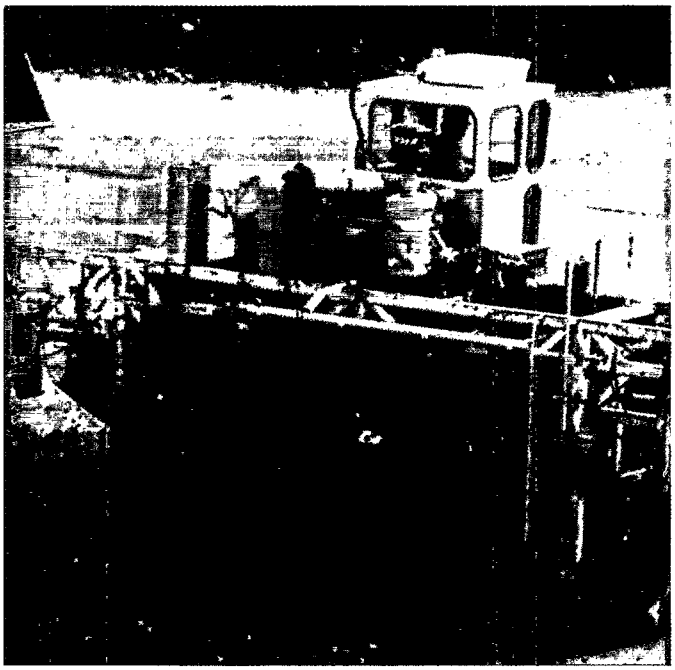
Composting involves the aerobic decomposition of organic material using controlled temperature, moisture, and oxygen levels. Composting results in a highly stabilized, humus-like material. Several different composting methods are currently in use in the United States. The three most common are windrow, aerated static pile, and within-vessel composting, all described below. Composting can yield either a Class A or Class B sludge, depending on the time and temperature variables involved in the operation.

All composting methods rely on the same basic processes. Bulking agents such as wood chips, bark, sawdust, straw, rice hulls, or even finished compost are added to the sewage sludge to absorb moisture, increase porosity, and add a source of carbon. This mixture is stored (in windrows, static piles, or enclosed tanks) for a period of intensive decomposition, during which temperatures can rise well above 55°C (131°F). Depending on ambient temperatures and the process chosen, the time required to produce a high-quality sewage sludge can range from 2 to 4 weeks. Aeration and/or frequent mixing or turning are needed to supply oxygen and remove excess heat. Following this active stage, bulking agents may or may not be screened from the completed compost for recycling (see photo below), and the composted sewage sludge is "cured" for an additional period.



Composted sludge is screened to remove the bulking agent prior to land application.

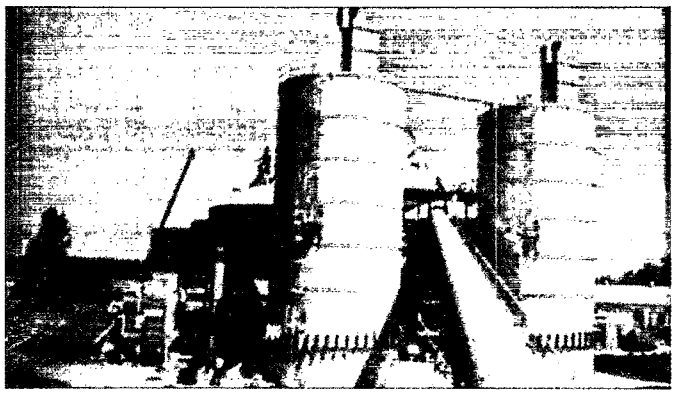
Windrow composting involves stacking the sewage sludge/bulking agent mixture into long piles, or windrows, generally 0.9 to 1.8 m high (3 to 6 feet) and 1.8 to 4.9 m wide (6 to 16 feet). These rows are regularly turned or mixed (e.g., using a front-end loader) to ensure a steady oxygen supply for the microorganisms and to reduce moisture content (see photo, next page). Active windrows are typically placed in the open air, except in areas with heavy rainfall. In colder climates, winter weather can significantly increase the amount of time needed to attain temperatures needed for pathogen control.



Compost mixing equipment turns over a windrow of compost for solar drying prior to screening. (Photo credit: East Bay Municipal Utility District)

Aerated static pile composting uses forced-air rather than mechanical mixing (see Figure 9-3) to both supply sufficient oxygen for decomposition and carry off moisture. The sewage sludge/bulking agent mixture is placed on top of either (1) a fixed underlying forced aeration system, or (2) a system of perforated piping laid on the composting pad surface and topped with a bed of bulking agent. The entire pile is covered with a layer of cured compost for insulation and noxious odor containment. Pumps are used to blow air into the compost pile or suck air through it. The latter provides greater odor control because the compost-air can be easily collected and then filtered or scrubbed.

Within-vessel composting systems vary greatly in design. They share, however, two basic techniques: the process takes place in a reactor vessel where the operating conditions can be carefully controlled (see photo below), and active aeration meets the system's high oxygen demand. Agitated bed systems (i.e., within-vessel composting) depend on continuous or periodic mixing within the vessel, followed by a curing period outside of it.



Taulman Weiss in-vessel composting facility in Portland, Oregon.

Pathogen destruction during composting depends on time and temperature variables (see photo, next page). Part 503 provides the following definition of PSRP requirement for pathogen destruction during composting:

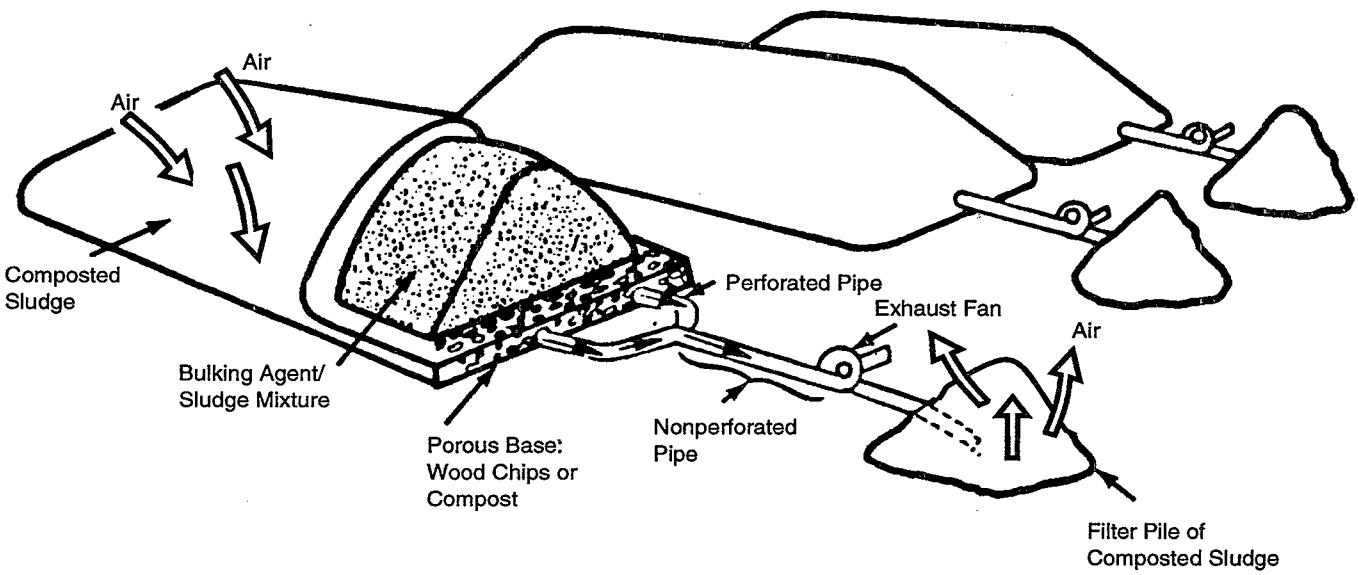


Figure 9-3. Static aerated pile composting.

Compost operator measures compost pile temperature as part of process monitoring. (Photo credit: East Bay Municipal Utility District, Oakland, California)

Using either the within-vessel, static aerated pile, or windrow composting methods, the temperature of the sewage sludge is raised to 40°C (104°F) or higher and remains at 40°C (104°F) or higher for 5 days. For 4 hours during the 5-day period, the temperature in the compost pile exceeds 55°C (131°F).

These conditions, achieved using either within-vessel, aerated static pile, or windrow methods, reduce bacterial and viral pathogens, but not helminth ova or other parasites, by more than 90% (10-fold).

A process time of only 5 days is not long enough to fully stabilize the sewage sludge solids, so the composted sewage sludge produced under these conditions will not be able to meet any of the requirements for reduced vector attraction. Complete stabilization may require 14 to 21 days for within-vessel; 21 or more days for aerated static pile; and 30 or more days for windrow composting. Many treatment works allow the finished sewage sludge to further mature or cure for at least several weeks following active composting.

Vector Attraction Reduction

Vector attraction reduction must be demonstrated for composted sewage sludge. In a few cases, this can be demonstrated by Option 1 of the vector attraction reduction requirements (38% reduction in volatile solids—see Section 6.2). However, in most cases, Option 5 is more appropriate. This option requires aerobic treatment (i.e., composting) of the sewage sludge for at least 14 days at over 40°C (104°F) with an average temperature of over 45°C (113°F).

9.6 Lime Stabilization

For 2,000 years, lime has been used to deodorize and stabilize night soil and manure. Today, lime treatment is gaining popularity as an effective option for controlling pathogens in sewage sludge. The process is relatively straightforward: lime—either hydrated lime, $Ca(OH)_2$; quicklime, CaO; or lime-containing kiln dust or fly ash—is added to sewage sludge in sufficient quantities to raise the pH above 12 after 2 hours of contact, as specified in the Part 503 PSRP description for lime stabilization:

Sufficient lime is added to the sewage sludge to raise the pH of the sewage sludge to 12 after 2 hours of contact.

Lime may be introduced to liquid sewage sludge in a mixing tank or combined with dewatered sewage sludge, providing the mixing is intimate and the sewage sludge cake is moist enough to allow aqueous contact between the sewage sludge and lime.

A variety of lime stabilization processes—some patented—are currently in use. The growing popularity of this treatment means new techniques will undoubtedly be developed in the future. The effectiveness of any lime stabilization process for controlling pathogens depends on maintaining the pH at levels that reduce microorganisms in the sewage sludge and also later inhibit bacterial growth should contamination occur after treatment. Lime stabilization can reduce bacterial and viral pathogens 99 percent or more. Such alkaline conditions have little effect on hardy species of helminth ova, however.

Lime stabilization does not reduce volatile solids. If the pH of lime-stabilized sewage sludge drops below 11, remaining pathogenic bacteria or those introduced by animal vectors may grow rapidly to substantial densities, given the rich food source. Thus long-term storage of alkali-treated sewage sludge requires either additional treatment with lime to maintain elevated pH, drying, or further treatment to reduce volatile solids (e.g., composting).

Vector Attraction Reduction

For lime-treated sludge, vector attraction reduction is demonstrated by Option 6 of the vector attraction reduction requirements. This option requires that the sludge pH remain at 12 or higher for at least 2 hours, and then at 11.5 or more for an additional 22 hours (see Section 6.7).

9.7 Equivalent Processes

Under Class B Alternative 3, sewage sludges treated in processes that are determined to be equivalent to PSRP are considered to be Class B. Table 5-2 in Chapter 5 lists some of the processes that the EPA's Pathogen Equivalency Committee has recommended as being equivalent to PSRP under Part 257. Chapter 11 discusses how the PEC makes a recommendation of equivalency.

Chapter 10
Processes to Further Reduce Pathogens (PFRPs)

10.1 Introduction

Processes to Further Reduce Pathogens (PFRPs) are listed in Appendix B of Part 503. There are seven PFRPs: composting, heat drying, heat treatment, thermophilic aerobic digestion, beta ray irradiation, gamma ray irradiation, and pasteurization. When these processes are operated under the conditions specified in Appendix B, they produce sewage sludges with pathogenic bacteria, enteric viruses, and viable helminth ova reduced to below detectable levels. Reduction of vector attraction must occur during or after PFRP treatment (see Section 4.2).

Under Part 503.32(a)(7), sewage sludge treated in these processes is considered to be Class A with respect to pathogens. Class A sludges can be used in any land application situation (including lawns and gardens) without restriction; however, Class A sludges must be monitored for fecal coliform or *Salmonella* sp. bacteria at the time of use or disposal, at the time the sewage sludge is prepared for sale or give away in a bag or other container for land application, or at the time the sewage sludge or material derived from the sewage sludge is prepared to meet the requirements in 503.10(b), 503.10(c), 503.10(e), or 503.10(f) to ensure that regrowth of bacteria has not occurred (see Section 4.3).

The PFRPs listed in Part 503 are essentially identical to the PFRPs listed under the 40 CFR Part 257 regulation, except that all requirements related solely to reduction of vector attraction have been removed. This chapter provides detailed descriptions of the seven PFRPs listed in Part 503.

10.2 Composting

As described in Chapter 9, composting reduces sewage sludge, which has generally been mixed with a bulking agent, to a humus-like material through biological degradation. There are three commonly used methods of composting: windrow, static aerated pile, and within-vessel.

To be considered a PFRP under Part 503, the composting operation must meet certain operating conditions:

Using either the within-vessel composting method or the static aerated pile composting method, the temperature of the sewage sludge is maintained at 55°C (131°F) or higher for 3 days.

Using the windrow composting method, the temperature of the sewage sludge is maintained at 55°C (131°F) or higher for 15 days or longer. During the period when the compost is maintained at 55°C (131°F) or higher, there shall be a minimum of five turnings of the windrow.

In general, within-vessel composting attains the required conditions in approximately 10 days. The static-pile and windrow processes generally require about 3 weeks. If the conditions specified by the regulation are met, all pathogenic viruses, bacteria, and parasites will be reduced to below detectable levels. However, composting under these conditions may not adequately reduce vector attraction. Longer composting periods may be necessary to fully stabilize the sewage sludge (see Section 9.5).

Under some conditions, it may be difficult to meet the Class A monitoring requirement for fecal coliforms even when *Salmonella* sp. bacteria are not present. Sewage sludge treatment involving high heat, for example, can reduce salmonellae to below detectable levels while leaving some fecal coliforms intact. If volatile solids remain in the sludge, coliforms can later regrow to significant numbers. The same thing can happen when sewage sludge is pre-treated with lime before composting. It may be necessary, therefore, to test composted sewage sludge directly for salmonellae, rather than using fecal coliforms as an indicator of pathogen control.

Vector Attraction Reduction

The options for demonstrating vector attraction reduction for both PFRP and PSRP composting are the same, and are discussed in Section 9.5.

10.3 Heat Drying

Heat drying is used to reduce both pathogens and the water content of sewage sludge. The Part 503 PFRP description of heat drying is:

Sewage sludge is dried by direct or indirect contact with hot gases to reduce the moisture content to 10% or lower. Either the temperature of the sewage sludge particles exceeds 80°C (176°F) or the wet bulb temperature of the gas in contact with the sewage sludge as it leaves the dryer exceeds 80°C (176°F).

Properly conducted heat drying will reduce pathogenic viruses, bacteria, and helminth ova to below detectable levels.

Four processes are commonly used for heat drying sewage sludge: flash dryers, spray dryers, rotary dryers, and the Carver-Greenfield process (EPA, 1979). Flash dryers used to be the most common heat drying process installed at treatment works, but current practice favors rotary dryers.

Flash Dryers

Flash dryers pulverize sewage sludge in the presence of hot gases. The process is based on exposing fine sewage sludge particles to turbulent hot gases long enough to attain at least 90% solids content.

Spray Dryers

A spray dryer typically uses centrifugal force to atomize liquid sewage sludge into a spray that is directed into a drying chamber. The drying chamber contains hot gases that rapidly dry the sewage sludge mist. Some spray drying systems use a nozzle to atomize sewage sludge.

Rotary Dryers

Rotary dryers function as horizontal cylindrical kilns. The drum rotates and may have plows or louvers that mechanically mix the sewage sludge as the drum turns. There are many different rotary kiln designs, utilizing either direct heating or indirect heating systems. Direct heating designs maintain contact between the sewage sludge and the hot gases. Indirect heating separates the two with steel shells.

Carver-Greenfield Process

The Carver-Greenfield process is a patented multiple-effect evaporative oil-immersion process in which dewatered sewage sludge is mixed with a light oil. This mixture is pumped through a series of evaporators that selectively remove the water in sewage sludge, which has a lower boiling point than the oil carrier. The oil maintains the mixture in a liquid state, even when virtually all the water has been removed. The product of this process, an oil and dry sewage sludge mixture, is put through a centrifuge to separate the dry sewage sludge solids from the oil. The recovered oil can be reused in the process.

Vector Attraction Reduction

The PFRP requirements for heat drying also meets the requirements of Option 8 for vector attraction reduction (i.e., the percent solids must be at least 90% before mixing the sludge with other materials). It exceeds the requirement of Option 7 if the sludge being dried contains no unstabilized solids.

10.4 Heat Treatment

Heat treatment processes are used both to stabilize and condition sewage sludge. The processes involve heating sewage sludge under pressure for a short period of time. The sewage sludge becomes sterilized and bacterial slime layers are solubilized, making it easier to dewater the remaining sewage sludge solids. The Part 503 PRFP description for heat treatment is:

Liquid sewage sludge is heated to a temperature of 180°C (356°F) or higher for 30 minutes.

If operated according to these requirements, the process effectively reduces pathogenic viruses, bacteria, and helminth ova to below detectable levels. Sewage sludge must be properly stored after processing because organic matter has not been reduced and, therefore, regrowth of pathogenic bacteria can occur if treated sewage sludge is reinoculated.

Two processes have been used for heat treatment: the Porteous and the Zimpro process. In the Porteous process the sewage sludge is preheated and then injected into a reactor vessel. Steam is also injected into the vessel under pressure. The sewage sludge is retained in the vessel for approximately 30 minutes after which it is discharged to a decant tank. The resulting sewage sludge can generally be concentrated and dewatered to high solids concentrations. Further dewatering may be desirable to facilitate sewage sludge handling.

The Zimpro process is similar to the Porteous process. However, air is injected into the sewage sludge before it enters the reactor and the vessel is then heated by steam to reach the required temperature. Temperatures and pressures are approximately the same for the two processes.

Vector Attraction Reduction

Heat treatment must be followed by a vector attraction reduction process. Vector attraction reduction Options 6 to 11 may be used (see Chapter 6). Options 1 to 5 are not applicable unless the sludge is subsequently digested.

10.5 Thermophilic Aerobic Digestion

Thermophilic aerobic digestion is a refinement of the conventional aerobic digestion processes discussed in Section 9.2. In this process, feed sewage sludge is generally pre-thickened and an efficient aerator is used. In some modifications, oxygen is used instead of air. Because there is less sewage sludge volume and less air to carry away heat, the heat released from biological oxidation warms the sewage sludge in the digester to as high as 60°C (140°F).

Because of the increased temperatures, this process achieves higher rates of organic solids reduction than are achieved by conventional aerobic digestion, which operates at ambient air temperature. The biodegradable volatile solids content of the sewage sludge can be reduced up to 70% in a relatively short time. The digested sewage sludge is effectively pasteurized due to the high temperatures. Pathogenic viruses, bacteria, and parasites are reduced to below detectable limits if temperatures exceed 55°C (131°F).

This process can either be accomplished using auxiliary heating of the digestion tanks or through special designs that allow the energy naturally released by the microbial digestion process to heat the sewage sludge. The Part 503 PFRP description of thermophilic aerobic digestion is:

Liquid sewage sludge is agitated with air or oxygen to maintain aerobic conditions and the mean cell residence time of the sewage sludge is 10 days at 55°C to 60°C (131°F to 140°F).

The thermophilic process requires significantly lower residence times (i.e., solids retention time) than conventional aerobic processes designed to qualify as a PSRP, which must operate 40 to 60 days at 20°C to 15°C (68°F to 59°F), respectively. Residence time is normally determined by dividing the volume of sewage sludge in the vessel by the volumetric flow rate. Operation should minimize the potential for bypassing by withdrawing treated sludge before feeding, and feeding no more than once a day.

Vector Attraction Reduction

Vector attraction reduction must be demonstrated. Although all options, except Options 3 and 12 are possible, Options 1 and 2 are the most suitable. (Option 3 is not possible because it is not yet known how to translate SOUR measurements obtained at high temperatures to 20°C [68°F].)

10.6 Beta Ray and Gamma Ray Radiation[1]

Radiation can be used to disinfect sewage sludge. Radiation destroys certain organisms by altering the colloidal nature of the cell contents (protoplasm). Gamma rays and beta rays are the two potential energy sources for use in sewage sludge disinfection. Gamma rays are high-energy photons produced by certain radioactive elements. Beta rays are electrons accelerated in velocity by electrical potentials in the vicinity of 1 millions volts. Both types of radiation destroy pathogens that they penetrate if the doses are adequate.

The Part 503 PFRP descriptions for irradiation systems are:

Beta ray irradiation—Sewage sludge is irradiated with beta rays from an accelerator at dosages of at least 1.0 megarad at room temperature (ca. 20°C [68°F]).

Gamma ray irradiation—Sewage sludge is irradiated with gamma rays from certain isotopes, such as Cobalt 60 and Cesium 137 [at dosages of at least 1.0 megarad] at room temperature (ca. 20°C [68°F]).[2]

The effectiveness of beta radiation in reducing pathogens depends on the radiation dose, which is measured in rads. A dose of 1 megarad or more will reduce pathogenic viruses, bacteria, and helminths to below detectable levels. Lower doses may successfully reduce bacteria and helminth ova but not viruses. Sewage sludge must be properly stored after processing because organic matter has not been reduced and therefore regrowth of pathogenic bacteria can occur if sewage sludge is reinoculated.

[1]Current usage by physicists reserved the term "beta rays" for high-energy electrons emitted by radioactive elements. More properly, the regulation should have used the term "high-energy electrons" instead of "beta rays."

[2]The 1.0 megarad dose was inadvertently omitted from the final Part 503 regulation.

Although the two types of radiation function similarly to inactivate pathogens, there are important differences. Gamma rays can penetrate substantial thicknesses of sewage sludge and can therefore be introduced to sewage sludge by either piping liquid sewage sludge into a vessel that surrounds the radiation source (Figure 10-1) or by carrying composted or dried sewage sludge by hopper conveyor to the radiation source. Beta rays have limited penetration ability and therefore are introduced by passing a thin layer of sewage sludge under the radiation source (Figure 10-2).

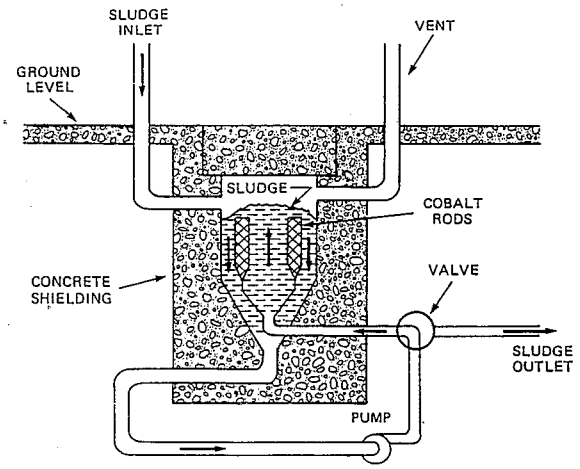


Figure 10-1. Schematic representation of cobalt-60 (gamma ray) irradiation facility at Geiselbullach, Germany. Source: EPA, 1979.

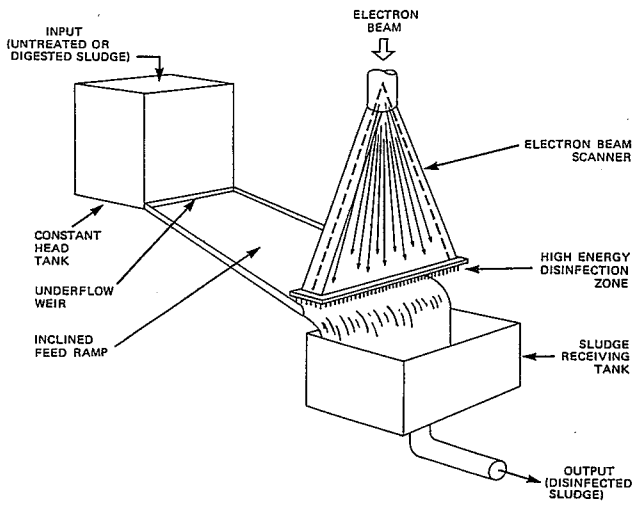


Figure 10-2. Beta ray scanner and sludge spreader. Source: EPA, 1979.

Vector Attraction Reduction

Radiation treatment must be followed by a vector attraction reduction process. The appropriate options for demonstrating vector attraction reduction are the same as for heat treatment (see Section 10.4), namely Options 6 to 11. Options 1 to 5 are not applicable unless the sludge is subsequently digested.

10.7 Pasteurization

Pasteurization involves heating sewage sludge to above a predetermined temperature for a minimum time period. For pasteurization, the Part 503 PFRP description is:

The temperature of the sewage sludge is maintained at 70°C (158°F) or higher for 30 minutes or longer.

Pasteurization reduces bacteria, enteric viruses, and helminth ova to below detectable values.

Sewage sludge can be heated by heat exchangers or by steam injection. Although sewage sludge pasteurization is uncommon in the United States, it is widely used in Europe. The steam injection method is preferred because it is more effective at maintaining even temperatures throughout the sludge batch being processed. Sewage sludge is pasteurized in batches to prevent recontamination that might occur in a continuous process. Sewage sludge must be properly stored after processing because the organic matter has not been stabilized and therefore odors and regrowth of pathogenic bacteria can occur if sewage sludge is reinoculated.

In Europe, serious problems with regrowth of *Salmonella* sp. have occurred, so pasteurization is rarely used now as a terminal treatment process. Pre-pasteurization followed by mesophilic digestion has successfully replaced the use of pasteurization after digestion in many European communities.

Vector Attraction Reduction

Pasteurization must be followed by a vector attraction reduction process. The options appropriate for demonstrating vector attraction reduction are the same as those for heat treatment (see Section 10.4), namely Options 6 to 11. Options 1 to 5 are not applicable unless the sludge is subsequently digested.

10.8 Equivalent Processes

Under Class A Alternative 6, sewage sludge treated in processes that are determined to be equivalent to PFRP are considered to be Class A with respect to pathogens (assuming the treated sewage sludges also meet the Class A regrowth requirement). Table 4-2 in Chapter 4 lists some of the processes that were found, based on the recommendation of EPA's Pathogen Equivalency Committee, to be equivalent to PFRP under Part 257. Chapter 11 discusses how the PEC makes a recommendation of equivalency.

Chapter 11
Role of EPA's Pathogen Equivalency Committee in Providing Guidance Under Part 503

11.1 Introduction

One way to meet the pathogen control requirements of Part 503 is to treat sludge in a process "equivalent to" the PFRP or PSRP processes listed in Appendix B of the regulation (see Tables 4-2 and 5-1 for a list of these processes):

- Under Class A Alternative 6, sewage sludge that is treated in a process equivalent to PFRP and meets the Class A regrowth requirement (see Section 4.3) is considered to be a Class A sludge with respect to pathogens (see Section 4.9).

- Under Class B Alternative 3, sewage sludge treated by a process equivalent to PSRP is considered to be a Class B sludge with respect to pathogens (see Section 5.4).

These alternatives provide continuity with the Part 257 regulation, which required that sewage be treated by a PSRP, PFRP, or equivalent process prior to use or disposal. There is one major difference between Part 257 and Part 503 with respect to equivalency. Under Part 257, a process had to be found equivalent in terms of both pathogen reduction and vector attraction reduction. Under Part 503, equivalency pertains only to pathogen reduction. (However, like all Class A and B sludges, sewage sludges treated by equivalent processes must also meet a separate vector attraction reduction requirement [see Chapter 6]).

What Constitutes Equivalency?

To be equivalent, a treatment process must be able to *consistently* reduce pathogens to levels comparable to the reduction achieved by the listed PSRPs or PFRPs. (These levels, described in Section 11.3, are the same levels required of all Class A and B sludges.) The process continues to be equivalent *as long as it is operated under the same conditions (e.g., time, temperature, pH) that produced the required reductions.* Equivalency is site-specific—that is, equivalency applies only to that particular operation run at that location under the specified conditions, and cannot be assumed for the same process performed at a different location, or for any modification of the process. Processes that are able to consistently produce the required pathogen reductions under the variety of conditions that may be encountered at different locations across the country may qualify for a *recommendation of national equivalency,* i.e, a recommendation that the process will likely be equivalent wherever it is operated in the United States.

Who Determines Equivalency?

The permitting authority is responsible for determining equivalency under Part 503. The permitting authority often seeks guidance from EPA's Pathogen Equivalency Committee (PEC) in making equivalency determinations. The PEC is responsible for making national equivalency recommendations.

What Are the Benefits of Equivalency?

A determination of equivalency is beneficial when it reduces the microbiological monitoring burden, i.e., when less monitoring is required to demonstrate equivalency than is required under the other Class A or B alternatives for meeting the pathogen reduction requirements of Part 503. Figure 11-1 indicates when application for equivalency may be appropriate.

PFRP Equivalency

Equivalency is not beneficial for processes already covered under Class A Alternatives 1, 2, or 5 (see Chapter 4 for a description of these alternatives). For processes not covered by Alternatives 1, 2, or 5, a determination of PFRP equivalency can reduce the enteric virus and viable helminth ova monitoring burden[1] in certain cases (see Section 11.3 for details of when PFRP equivalency may be beneficial).

PSRP Equivalency

A determination of equivalency to PSRP eliminates the fecal coliform monitoring required under Class B Alternative 1 for nonequivalent processes.

Recommendation of National Equivalency

A recommendation of national equivalency can be useful for treatment processes that will be marketed, sold, and/or used at different locations in the United States. Such a recommendation may be useful in getting PFRP or PSRP equivalency determinations from different permitting authorities across the country.

[1] A determination of PFRP equivalency will not reduce the monitoring required for salmonellae or fecal coliform since *all* Class A sludges—even sludges produced by equivalent processes—must be monitored for salmonellae or fecal coliform (see Section 4.3).

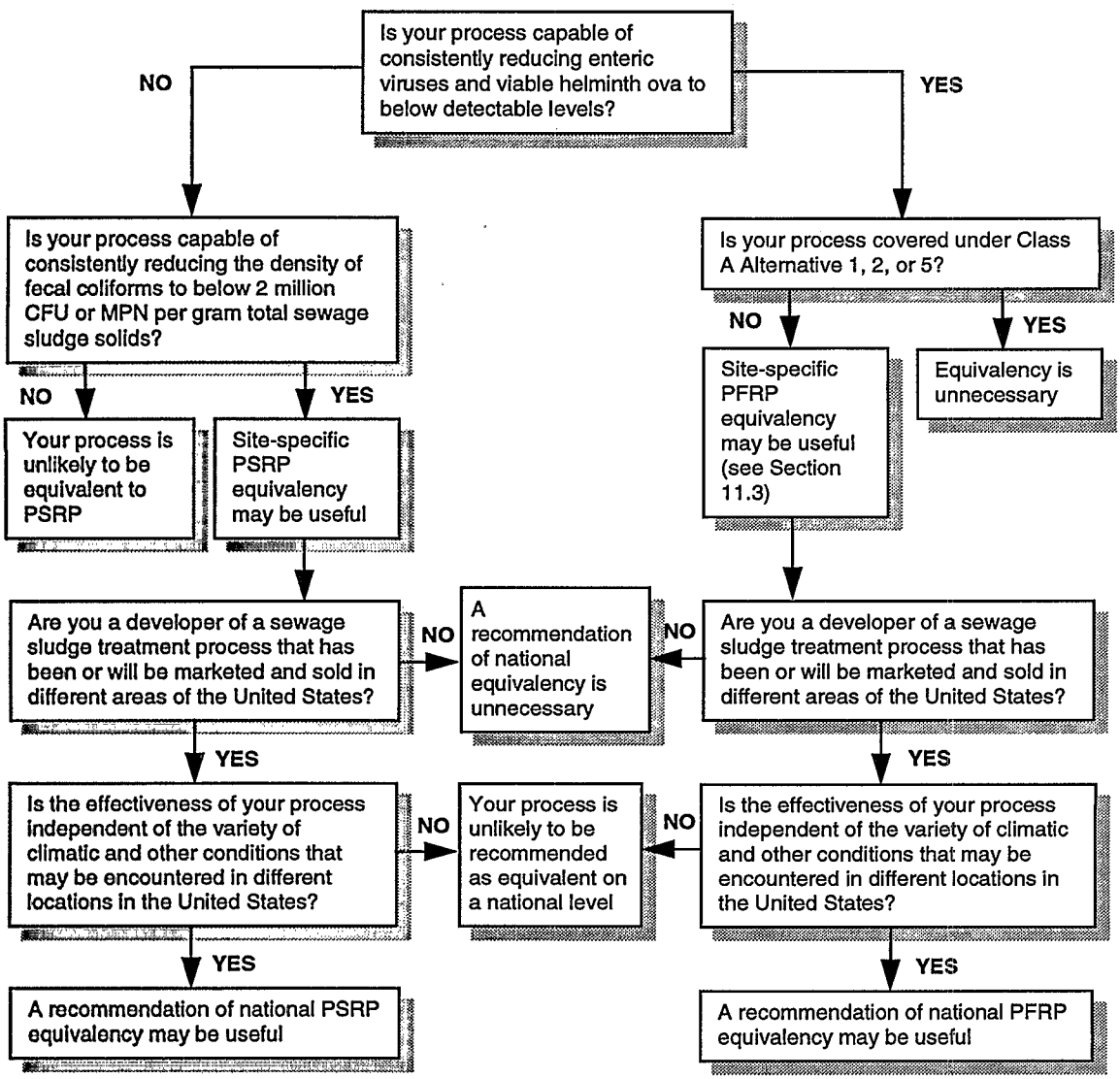


Figure 11-1. When is application for PFRP or PSRP equivalency appropriate?

Role of the Pathogen Equivalency Committee

The U.S. Environmental Protection Agency created the Pathogen Equivalency Committee (PEC) in 1985 to make *recommendations* to EPA management on applications for PSRP and PFRP equivalency under Part 257 (Whittington and Johnson, 1985). The PEC consists of approximately six members with expertise in microbiology, wastewater engineering, statistics, and sludge regulations. It includes representatives from EPA's Office of Research and Development and Office of Water.

Guidance and Technical Assistance on Equivalency Determinations

The PEC will continue to review and make recommendations to EPA management on applications for equivalency under Part 503. Its members also provide guidance to applicants on the data necessary to determine equivalency, and to permitting authorities and members of the regulated community on issues (e.g., sampling and analysis) related to meeting the Subpart D (pathogen and vector attraction reduction) requirements of Part

503. Figure 11-2 elaborates on the role of the PEC under Part 503.

National Equivalency Recommendations

The PEC can also recommend that a process be considered equivalent on a national level if the PEC finds that the process consistently produces the required pathogen reductions under the variety of conditions that may be encountered at different locations across the country.

What's in This Chapter?

This chapter explains how the PEC makes equivalency recommendations and describes how to apply for PEC guidance. The guidance in this chapter may also prove useful for permitting authorities in establishing the information they will need to make equivalency determinations.

11.2 Overview of the PEC's Equivalency Recommendation Process

The first point of contact for any equivalency determination, recommendation, or other guidance is usually the permitting authority. Appendix A provides a list of the Regional Sludge Coordinators (RSCs) and the State Sludge Coordinators (SSCs). If PEC involvement is appropriate, the permitting authority will coordinate contact with the PEC.

The PEC considers each equivalency application on a case-by-case basis. Applicants submit information on process operating parameters and/or the sewage sludge, as described in Section 11.5. The committee evaluates this information in light of current knowledge concerning sewage sludge treatment and pathogen reduction, and *recommends* one of five decisions about the process or process sequence:

- It is equivalent to PFRP.
- It is not equivalent to PFRP.
- It is equivalent to PSRP.
- It is not equivalent to PSRP.
- Additional data or other information are needed.

Most processes are recommended for site-specific equivalency. To receive a recommendation for national equivalency, the applicant must demonstrate that the process will produce the desired reductions in pathogens under the variety of conditions that may be encountered at different locations across the country. Processes affected by local climatic conditions or that use materials that may vary significantly from one part of the country to another are unlikely to be recommended as equivalent on a national basis unless specific material specifications and process procedure requirements can be identified.

If the PEC recommends, based on the information submitted, that a process is equivalent to PSRP or PFRP, the operating parameters and any other conditions critical to adequate pathogen reduction are specified. The equivalency recommendation applies only when the process is operated under the specified conditions.

If the committee finds that it cannot recommend equivalency, the committee provides an explanation for this finding. If additional data are needed, the committee describes what those data are and works with the permitting authority and/or the applicant, if necessary, to ensure that the appropriate data are gathered in an acceptable manner. The committee then reviews the revised application when the additional data are submitted.

11.3 Basis for PEC Equivalency Recommendations

As mentioned in Section 11.1, to be determined equivalent, a treatment process must consistently and reliably reduce pathogens in sludge to the same levels achievable by the listed PSRPs or PFRPs. The applicant must identify the process operating parameters (e.g., time, temperature, pH) that are necessary and sufficient for producing these reductions.

PFRP Equivalency

To be equivalent to PFRP, a treatment process must be able to consistently reduce sewage sludge pathogens to below detectable limits. For purposes of equivalency, the PEC is concerned only with the ability of a process to reduce enteric viruses and viable helminth ova to below detectable limits, because Part 503 requires ongoing monitoring of sludge produced by PFRP-equivalent processes for fecal coliform or *Salmonella* sp. (see Section 4.3) to ensure that *Salmonella* sp. are reduced to below detectable limits (i.e., to less than 3 MPN per 4 grams total solids sewage sludge [dry weight basis]). Thus, to demonstrate PFRP equivalency, the treatment process must be able to consistently reduce enteric viruses and viable helminth ova to below detectable limits, which are:

enteric viruses	less than 1 plaque-forming unit per 4 grams total solids sewage sludge (dry weight basis)
viable helminth ova	less than 1 per 4 grams total solids sewage sludge (dry weight basis)

There are two ways these reductions can be demonstrated:

- Direct monitoring of treated and untreated sewage sludge for enteric viruses and viable helminth ova.
- Comparison of the operating conditions of the process with the operating conditions of one of the listed PFRPs.

In practice, the monitoring approach to demonstrating site-specific PFRP equivalency offers no advantages as a means to fulfill Class A requirements because owners and operators can achieve the same outcome by performing the monitoring required under Class A Alternative 3 (see Section 4.6). Use of Alternative 3 may be simpler than seeking equivalency, since Alternative 3 does not require the involvement of the PEC. The monitoring approach may be of value, however, when seeking

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

JUN 15 1993

OFFICE OF WATER

MEMORANDUM

SUBJECT: The Role of the **Pathogen Equivalency Committee** Under the Part 503 *Standards for the Use or Disposal of Sewage Sludge*

FROM: Michael B. Cook, Director
Office of Wastewater Enforcement & Compliance

James A. Hanlon, Acting Director
Office of Science & Technology

TO: Water Division Directors
Regions I - X

PURPOSE

This memorandum explains the role of the **Pathogen Equivalency Committee (PEC)** in providing technical assistance and recommendations regarding pathogen reduction equivalency in implementing the Part 503 *Standards for the Use or Disposal of Sewage*. The PEC is an Agency resource available to assist your permit writers and regulated authorities. This information should be sent to your Regional Sludge Coordinators, Municipal Construction Managers, Permits and Enforcement Coordinators, and Solid Waste Offices, State Sludge Management Agencies and others concerned with sewage sludge management.

BACKGROUND

The PEC Under Part 257

The Criteria for Classification of Solid Waste Facilities and Practices (44 FR 53438, September 13, 1979), in 40 CFR Part 257 required that sewage sludge disposed on the land be treated by either a *Process to Significantly Reduce Pathogens* (PSRP) or a *Process to Further Reduce Pathogens* (PFRP). A list of PSRPs and PFRPs were included in Appendix II to Part 257.

In 1985, the PEC was formed to provide technical assistance and recommendations on whether sewage sludge treatment processes not included in Appendix II to Part 257 were equivalent to PSRP or PFRP. Under Part 257, the PEC provided technical assistance to both the permitting authority and to members of the regulated

Figure 11-2. Role of the PEC under Part 503.

community. The PEC membership has included representatives from the Office of Research & Development (ORD), Office of Wastewater Enforcement & Compliance (OWEC), and the Office of Science & Technology (OST) with extensive experience in microbiology, sludge process engineering, statistics and regulatory issues. The PEC recommendations regarding the equivalency of processes were forwarded to the Office of Science and Technology, which notified applicants about the PEC's recommendations. Final decisions on equivalency were made by the permitting authority.

The Part 503 Sewage Sludge Standards

The 40 CFR Part 503 Standards for the Use or Disposal of Sewage Sludge were published in the Federal Register on February 19, 1993 (58 FR 9248) under the authority of section 405 of the Clean Water Act, as amended. Part 503 establishes requirements for sewage sludge applied to the land, placed on a surface disposal site, or fired in a sewage sludge incinerator. Along with the 40 CFR Part 258 Municipal Solid Waste (MSW) Landfill Regulation (56 FR 50978, October 9, 1991), which established requirements for materials placed in MSW landfills, the Part 503 requirements for land application of sewage sludge and placement of sewage sludge on a surface disposal site, replaces the requirements for those practices, including the requirement to treat the sewage sludge in either a PSRP or a PFRP, in Part 257.

The Part 503 regulation addresses disease-causing organisms (i.e., pathogens) in sewage sludge by establishing requirements for sewage sludge to be classified either as Class A or Class B with respect to pathogens as an operational standard. Class A requirements are met by treating the sewage sludge to reduce pathogens to below detectable limits, while the Class B requirements rely on a combination of treatment and site restrictions to reduce pathogens. The site restrictions prevent exposure to the pathogens and rely on Natural Environmental processes to reduce the pathogens in the sewage sludge to below detectable levels. In addition to pathogen reduction, a vector attraction reduction requirement has to be met when sewage sludge is applied to the land or placed on a surface disposal site.

Vector attraction reduction requirements are imposed under Part 503 to reduce the potential for spreading of infectious disease agents by vectors (i.e., flies, rodents, and birds). A series of alternative methods for meeting the vector attraction reduction requirement are provided in the rule.

All sewage sludges that are to be sold or given away in a bag or other container for land application, or applied to lawns or home gardens must meet Class A pathogen control and vector attraction reduction requirements. All sewage sludge intended for land application must meet at least the Class B pathogen control and vector attraction reduction requirements. Surface disposal of sewage sludge requires that Class A or Class B requirements, along with one of the vector attraction reduction practices, be met unless the sewage sludge is covered with soil or other material daily.

Figure 11-2. Role of the PEC under Part 503 (continued).

A series of options are provided in the Part 503 regulation for meeting the specific requirements for the two classes of pathogen reduction. One of the Class A alternatives is to treat the sewage sludge by a process equivalent to a PFRP and one of the Class B alternatives is to treat the sewage sludge by a process equivalent to a PSRP. The permitting authority must decide whether a process is equivalent to a PFRP or a PSRP, which is the same approach used under Part 257.

THE PEC UNDER 503

Part 503 provides specific criteria and procedures for evaluating bacterial indicators (Fecal coliforms and Salmonella sp.), enteric virus and viable helminth ova as well as vector attraction reduction. The PEC will continue to support the permitting authority and members of the regulated community under the new Part 503 regulation in evaluating equivalency situations and providing technical assistance in matters such as sampling and analysis. Specifically the PEC:

- will continue to provide technical assistance to the permitting authority and regulated community, including recommendations to the permitting authority about process equivalency. The PEC also will make both site-specific and national (i.e., a process that is equivalent anywhere in the United States where it is installed and operated) recommendations on process equivalency .

- will submit recommendations on process equivalency to the Director, Health and Ecological Criteria Division, Office of Science and Technology, who will review those recommendations and then notify the applicant and appropriate permitting authorities of our recommendation.

For site-specific recommendations, requests for PEC review or assistance should be made through the appropriate Federal permitting authority (e.g., the State sludge regulatory authority for delegated programs or the EPA Regional Sludge Coordinator for non-delegated programs). For national recommendations, requests for PEC review or assistance can also be made through the Director, Health and Ecological Criteria Division (WH-586), Office of Science & Technology, U.S. EPA, 401 M St., SW, Washington, D.C. 20460 or directly to the PEC Chairman. The current PEC Chairman is: Dr. James E. Smith, Jr., U.S. EPA, CERI, (Center for Environmental Research Information) 26 W Martin Luther King Dr., Cincinnati, OH 45268 (Tele: 513/569-7355).

Additional information and guidance to supplement the pathogen reduction requirements of Part 503 and the procedures to use to reach the PEC and the assistance provided by the PEC is provided in "Control of Pathogens and Vector Attraction in Sewage

Figure 11-2. Role of the PEC under Part 503 (continued).

Sludge" (EPA 625/R-92/013), which will be updated from time to time by the PEC. This document is an update of the 1989 document "Control of Pathogens in Municipal Wastewater Sludge" (EPA/625/10-89/006), and is available from CERI.

If there are any questions about this memorandum, please contact Bob Bastian from OWEC at 202/260-7378 or Dr. Smith at CERI.

Figure 11-2. Role of the PEC under Part 503 (continued).

a *national* PFRP equivalency recommendation—something that cannot be automatically achieved through the use of Alternative 3. In this case, applicants may wish to submit microbiological monitoring data similar to that required under Alternative 3 as part of the package of information (see Section 11.5) required to demonstrate national PFRP equivalency.

The process comparison approach to demonstrating equivalency is discussed in Section 11.4.

PSRP Equivalency

For PSRP equivalency, the treatment process must consistently reduce the geometric mean of the fecal coliform density in seven samples of sewage sludge per sampling episode to less than 2 million CFU or MPN per gram of total solids (dry weight basis). Sufficient demonstrations of the required reductions are needed to ensure that the process can reliably produce the required reductions under all the different types of conditions that the process may operate. For example, for processes that may be affected by daily and seasonal variations in the weather, four or more sets of samples taken at different times of the year and during different precipitation conditions (including worst-case conditions) will be needed to make this demonstration. For national equivalency recommendations, the demonstration must show that the process can reliably produce the desired reductions under the variety of climatic and other conditions that may be encountered at different locations in the United States.

11.4 Guidance on Demonstrating Equivalency for PEC Recommendations

As described below, equivalency can be demonstrated in one of two ways:

- By comparing operating conditions to existing PFRPs or PSRPs.
- By providing performance and microbiological data.

Comparison to Operating Conditions for Existing PSRPs or PFRPs

If a process is similar to a PSRP or PFRP described in the Part 503 regulation (see Tables 4-2 and 5-1), it may be possible to demonstrate equivalency by providing performance data showing that the process consistently meets or exceeds the conditions specified in the regulation. For example, a process that consistently produces a pH of 12 after 2 hours of contact (the PSRP conditions required in Part 503 for lime stabilization) but uses a substance other than lime to raise pH could qualify as a PSRP. In such cases, microbiological data may not be necessary to demonstrate equivalency.

Process-Specific Performance Data and Microbiologic Data

In all other cases, both performance data and microbiological data (listed below) are needed to demonstrate process equivalency:

- A description of the various parameters (e.g., sludge characteristics, process operating parameters, climatic factors) that influence the microbiological characteristics of the treated sludge (see Section 11.5 for more detail on relevant parameters).
- Sampling and analytical data to demonstrate that the process has reduced microbes to the required levels (see Section 11.3 for a description of levels).
- A discussion of the ability of the treatment process to consistently operate within the parameters necessary to achieve the appropriate reductions.

Sampling and Analytical Methods

Sewage sludge should be sampled using accepted, state-of-the-art techniques for sampling and analyzed using the methods required by Part 503 (see Chapters 7 and 8).

Data Quality

The quality of the data provided is an important factor in EPA's equivalency recommendation. The following steps can help ensure data quality:

- Use of accepted, state-of-the-art sampling techniques.
- Obtaining samples that are representative of the expected variation in sludge quality.
- Developing and following quality assurance procedures for sampling.
- Using an independent, experienced laboratory to perform the analysis.

Since processes differ widely in their nature, effects, and processing sequences, the experimental plan to demonstrate that the process meets the requirements for PSRP or PFRP equivalency should be tailored to the process. Field verification and documentation by independent or third-party investigators is desirable. EPA will evaluate the study design, the accuracy of the data, and the adequacy of the results for supporting the conclusions drawn.

Can Pilot-Scale Data Be Submitted?

Operation on a full scale is desirable. However, if a pilot-scale operation truly simulates full-scale operation, testing on this reduced scale is possible. In such cases, it is important to indicate that the data were obtained from a pilot-scale operation, and to discuss why and to what extent this simulates full-scale operation. Any data available from existing full-scale operations would be useful.

The conditions of the pilot-scale operation should be at least as severe as those of full-scale operation. The arrangement of process steps, degree of mixing, nature of the flow, vessel sizing, proportion of chemicals used, etc. are all part of the requirement. Any substantial degree of departure in the process

parameters of the full-scale operation that might reduce the severity of the procedure will invalidate any PEC equivalency recommendations and permitting authority equivalency determinations and will require a retest at the new condition.

11.5 Guidance on Application for Equivalency Recommendations

The following outline and instructions are provided as *guidance* for preparing applications for equivalency recommendations by EPA's Pathogen Equivalency Committee.

Summary Fact Sheet

The application should include a brief fact sheet that summarizes key information about the process. Any important additional facts should also be included.

Introduction

The full name of the treatment works and the treatment process should be provided. The application should indicate whether it is for recommendation of:

- PSRP or PFRP equivalency.
- Site-specific or national equivalency.

Process Description

The type of sludge used in the process should be described, as well as other materials used in the process. Specifications for these materials should be provided as appropriate. Any terms used should be defined.

The process should be broken down into key steps and graphically displayed in a quantified flow diagram of the wastewater and sludge treatment processes. Details of the wastewater treatment process should be provided and the application should precisely define which steps constitute the beginning and end of sewage sludge treatment.[2] The earliest point at which sewage sludge treatment can be defined as beginning is the point at which the sludge is collected from the wastewater treatment process. Sufficient information should be provided for a mass balance calculation (i.e., actual or relative volumetric flows and solids concentration in and out of all streams, additive rates for bulking agents or other additives). A description of process parameters should be provided for each step of the process, giving typical ranges and mean values where appropriate. The specific process parameters that should be discussed will depend on the type of process and should include any of the following that affect pathogen reduction or process reliability:

[2]When defining which steps constitute the "treatment process," bear in mind that all steps included as part of a process equivalent to PSRP or PFRP must be continually operating according to the specifications and conditions that are critical to pathogen reduction. Thus, the operational and monitoring burden may be greater for a multi-step process.

Sewage Sludge Characteristics

- Total and volatile solids content of sludge before and after treatment
- Proportion and type of additives (diluents) in sludge
- Chemical characteristics (as they affect pathogen survival/destruction—e.g., pH)
- Type(s) of sludge (unstabilized vs. stabilized, primary vs. secondary, etc.)
- Wastewater treatment process performance data (as they affect sludge type, sludge age, etc.)
- Quantity of treated sludge
- Sludge age
- Sludge detention time

Process Characteristics

- Scale of the system (e.g., reactor size, flow rate)
- Sewage sludge feed process (e.g., batch vs. continuous)
- Organic loading rate (e.g., kg volatile solids/cubic meter/day)
- Operating temperature(s) (including maximum, minimum, and mean temperatures)
- Operating pressure(s) if greater than ambient
- Type of chemical additives and their loading rate
- Mixing
- Aerobic vs. anaerobic
- Duration/frequency of aeration
- Dissolved oxygen level maintained
- Residence/detention time
- Depth of sludge
- Mixing procedures
- Duration and type of storage (e.g., aerated vs. nonaerated)

Climate

- Ambient seasonal temperature range
- Precipitation
- Humidity

The application should include a description of how the process parameters are monitored, as well as process uniformity and reliability. Actual monitoring data should be provided whenever appropriate.

Description of Treated Sewage Sludge

The type of treated sludge should be described, as well as the sludge monitoring program for pathogens (if there is one). How and when are samples taken? What is analyzed for? What are the results? How long has this program been in operation?

Sampling Technique(s)

The PEC will evaluate the representativeness of the samples and the adequacy of the sampling techniques. For a recommendation of national PFRP equivalency, samples of untreated and treated sludge are usually needed (see Sections 11.3, 4.6, and 7.4). The sampling points should correspond to the beginning and end of the treatment process as defined previously under *Process Description*, above. Chapters 7 and 8 provide guidance on sampling. Samples should be representative of the sewage sludge in terms of location of collection within the sludge pile or batch. The samples taken should include samples from treatment under the least favorable operating conditions that are likely to occur (e.g., wintertime). Information should be provided on:

- Where the samples were collected from within the sewage sludge mass. (If samples were taken from a pile, include a schematic of the pile and indicate where the subsamples were taken.)
- Date and time the samples were collected. Discuss how this timing relates to important process parameters (e.g., turning over, beginning of drying).
- Sampling method used.
- How any composite samples were compiled.
- Total solids of each sample.
- Ambient temperature at time of sampling.
- Temperature of sample at time of sampling.
- Sample handling, preservation, packaging, and transportation procedures.
- The amount of time that elapsed between sampling and analysis.

Analytical Methods

Identify the analytical techniques used and the laboratory(s) performing the analysis.

Analytical Results

The analytical results should be summarized, preferably in tabular form. A discussion of the results and a summary of major conclusions should be provided. Where appropriate, the results should be graphically displayed. Copies of original data should be provided in an appendix.

Quality Assurance

The application should describe how the quality of the analytical data have been ensured. Subjects appropriate to address are: why the samples are representative; the quality assurance program; the qualifications of the in-house or contract laboratory used; and the rationale for selecting the sampling technique.

Rationale for Why Process Should Be Determined Equivalent

Finally, the application should describe why, in the applicant's opinion, the process qualifies for PSRP or PFRP equivalency. For example, it may be appropriate to describe or review particular aspects of the process that contribute to pathogen reduction, and why the process is expected to operate consistently. Complete references should be provided for any data cited. Applications for a recommendation of national equivalency should discuss why the process effectiveness is expected to be independent of the location of operation.

Appendices

A copy of the complete laboratory report(s) for any sampling and analytical data should be attached as an appendix. Any important supporting literature references should also be included as appendices.

11.6 Examples of Recommendations

Tables 4-3 and 5-2 list processes that the PEC has recommended as equivalent to PSRP or PFRP. Two of these processes are discussed below.

Raising Sewage Sludge pH Using an Alternative Chemical

The PEC evaluated a treatment process used by a Texas-based company for recommendation as equivalent to PSRP. The process was similar to lime stabilization except that cement kiln dust was used instead of lime to raise sewage sludge pH. The data provided by the applicant showed that the process reliably raised sludge pH to greater than 12 for at least 2 hours, so the PEC recommended that the process be determined equivalent to PSRP.

Use of a Chemical to Generate Heat During "Composting"

The Scarborough Sanitary District in Maine requested recommendation of its sewage sludge treatment process as a PFRP. The process was described as composting using fly ash as a

bulking agent. The applicant provided time and temperature data demonstrating that the piles reached temperatures of 60°C to 70°C (140°F to 158°F) within 24 hours and maintained this temperature range for up to 14 days. The process exceeded the PFRP requirements for static aerated pile composting. However, the PEC found that the process might not in fact be a composting process since it worked by adding an inorganic agent (fly ash) that produced high temperatures. The regulatory requirements for composting were based on the generation of heat by the biological processes that occur when an *organic* bulking agent is used. Thus, a determination of equivalency was necessary.

The applicant provided information on the location of the samples from the compost pile, so that the PEC could determine that sufficient temperatures were maintained throughout the pile to provide adequate pathogen destruction. The PEC recommended that the process be determined equivalent to PFRP because it met the time/temperature conditions that result in the reduction of pathogens in sewage sludge to below detectable limits.

Chapter 12
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Appendix A
EPA Regional and State Sludge Coordinators[1] and Map of EPA Regions

REGIONAL SLUDGE COORDINATORS

Thelma Hamilton
U.S. EPA - Region I
Municipal Facilities Branch (WMT-2111)
Water Management Division
John F. Kennedy Federal Building, Room 2103
Boston, MA 02203
617-565-3564; fax 617-565-4940

Alia Roufaeal
U.S. EPA - Region II
Water Management Division
26 Federal Plaza, Room 837
New York, NY 10278
212-264-8663; fax 212-264-9597

Ann Carkhuff
U.S. EPA - Region III
Water Management Division (3WM53)
841 Chestnut Street
Philadelphia, PA 19107
215-597-9406; fax 215-597-8541

Vince Miller
U.S. EPA - Region IV
Technology Transfer Unit
Water Management Division
345 Courtland Street
Atlanta, GA 30365
404-347-2391; fax 404-347-3269

John Colletti
U.S. EPA - Region V
Water Division
Technology Section (5WQP-16J)
77 West Jackson Boulevard
Chicago, IL 60604
312-886-6106; fax 312-886-7804

Stephanie Kordzi
U.S. EPA - Region VI
Water Management Division
Allied Bank Tower at Fountain Place
1445 Ross Avenue
Dallas, TX 75202-2733
214-655-7175; fax 214-655-6490

John Dunn
U.S. EPA - Region VII
Construction Grants Branch
Water Management Division
726 Minnesota Avenue
Kansas City, KS 66101
913-551-7594; fax 913-551-7765

Bob Brobst
U.S. EPA - Region VIII
Water Management Division/Municipal
Facilities Branch
999 18th Street, Suite 50-0
Denver, CO 80202-2413
303-293-1627; fax 303-294-1386

Lauren Fondahl
U.S. EPA - Region IX
Pretreatment Permits and Compliance
Water Management Division
75 Hawthorne Street (W-5-2)
San Francisco, CA 94105
415-744-1909; fax 415-744-1235

Laura Felten (WD-134)
U.S. EPA - Region X
Municipal Facilities Branch
1200 Sixth Avenue
Seattle, WA 98101
206-553-1647; fax 206-553-0165

[1]As of December 1992.

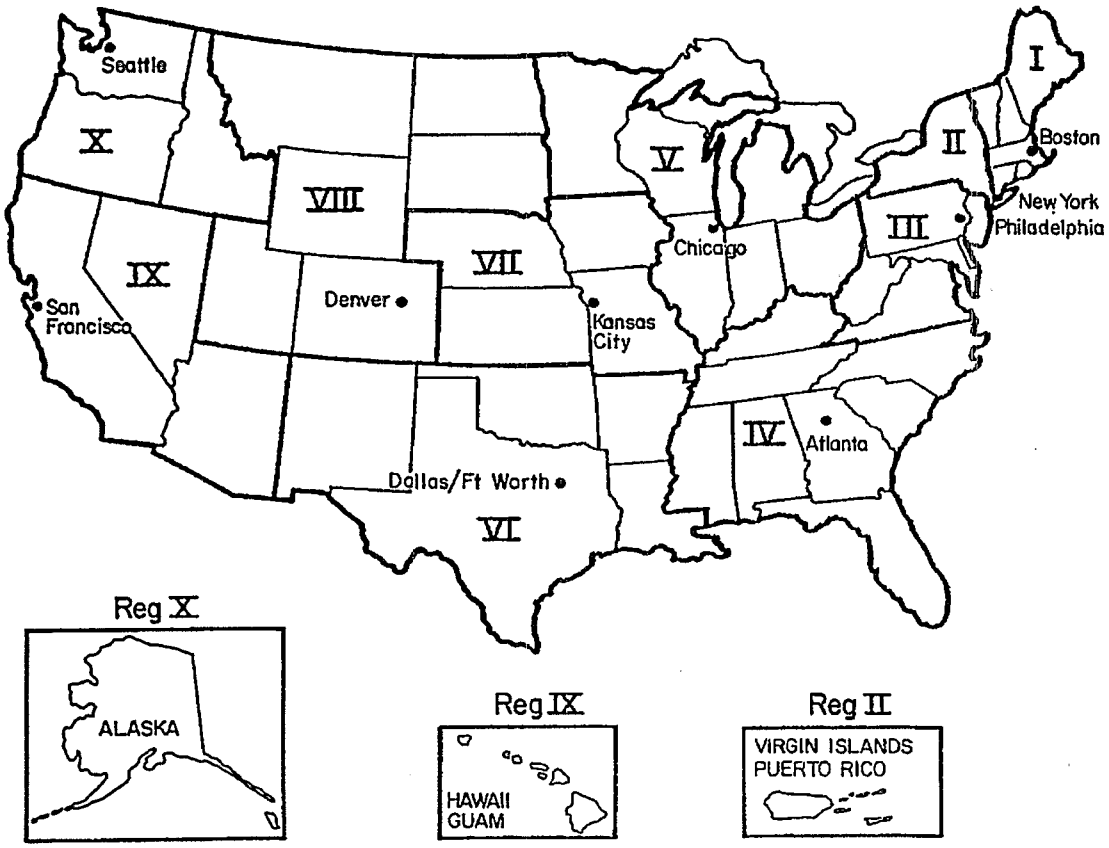

Figure A-1. EPA Regions.

STATE SLUDGE COORDINATORS[2]

Region 1

Connecticut

Warren Herzig
Department of Environmental Protection
Water Compliance Unit
122 Washington Street
Hartford, CT 06106
203-566-2154

Maine

Brian Kavannah/Steven Page
Department of Environmental Protection
Bureau of Solid Waste Management
State House, Station 17
Augusta, ME 04333
207-582-8740

Massachusetts

Dennis Dunn
Department of Environmental Protection
Division of Water Pollution Control
1 Winter Street
Boston, MA 02108
617-556-1130

New Hampshire

Selena Makofsky, Supervisor
Water Supply and Pollution Control Division
Department of Environmental Services
Sludge and Septage Management Section
P.O. Box 95
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Appendix B
Subpart D of the Part 503 Regulation

Subpart D—Pathogens and Vector Attraction Reduction

§ 503.30 Scope.

(a) This subpart contains the requirements for a sewage sludge to be classified either Class A or Class B with respect to pathogens.

(b) This subpart contains the site restrictions for land on which a Class B sewage sludge is applied.

(c) This subpart contains the pathogen requirements for domestic septage applied to agricultural land, forest, or a reclamation site.

(d) This subpart contains alternative vector attraction reduction requirements for sewage sludge that is applied to the land or placed on a surface disposal site.

§ 503.31 Special definitions.

(a) *Aerobic digestion* is the biochemical decomposition of organic matter in sewage sludge into carbon dioxide and water by microorganisms in the presence of air.

(b) *Anaerobic digestion* is the biochemical decomposition of organic matter in sewage sludge into methane gas and carbon dioxide by microorganisms in the absence of air.

(c) *Density of microorganisms* is the number of microorganisms per unit mass of total solids (dry weight) in the sewage sludge.

(d) *Land with a high potential for public exposure* is land that the public uses frequently. This includes, but is not limited to, a public contact site and a reclamation site located in a populated area (e.g, a construction site located in a city).

(e) *Land with a low potential for public exposure* is land that the public uses infrequently. This includes, but is not limited to, agricultural land, forest, and a reclamation site located in an unpopulated area (e.g., a strip mine located in a rural area).

(f) *Pathogenic organisms* are disease-causing organisms. These include, but are not limited to, certain bacteria, protozoa, viruses, and viable helminth ova.

(g) *pH* means the logarithm of the reciprocal of the hydrogen ion concentration.

(h) *Specific oxygen uptake rate (SOUR)* is the mass of oxygen consumed per unit time per unit mass of total solids (dry weight basis) in the sewage sludge.

(i) *Total solids* are the materials in sewage sludge that remain as residue when the sewage sludge is dried at 103 to 105 degrees Celsius.

(j) *Unstabilized solids* are organic materials in sewage sludge that have not been treated in either an aerobic or anaerobic treatment process.

(k) *Vector attraction* is the characteristic of sewage sludge that attracts rodents, flies, mosquitos, or other organisms capable of transporting infectious agents.

(l) *Volatile solids* is the amount of the total solids in sewage sludge lost when the sewage sludge is combusted at 550 degrees Celsius in the presence of excess air.

§ 503.32 Pathogens.

(a) *Sewage sludge—Class A.* (1) The requirement in § 503.32(a)(2) and the requirements in either § 503.32(a)(3), (a)(4), (a)(5), (a)(6), (a)(7), or (a)(8) shall be met for a sewage sludge to be classified Class A with respect to pathogens.

(2) The Class A pathogen requirements in § 503.32 (a)(3) through (a)(8) shall be met either prior to meeting or at the same time the vector attraction reduction requirements in § 503.33, except the vector attraction reduction requirements in § 503.33 (b)(6) through (b)(8), are met.

(3) *Class A—Alternative 1.* (i) Either the density of fecal coliform in the sewage sludge shall be less than 1000 Most Probable Number per gram of total solids (dry weight basis), or the density of *Salmonella* sp. bacteria in the sewage sludge shall be less than three Most Probable Number per four grams of total solids (dry weight basis) at the time the sewage sludge is used or disposed; at the time the sewage sludge is prepared for sale or give away in a bag or other container for application to the land; or at the time the sewage sludge or material derived from sewage sludge is prepared to meet the requirements in § 503.10 (b), (c), (e), or (f).

(ii) The temperature of the sewage sludge that is used or disposed shall be maintained at a specific value for a period of time.

(A) When the percent solids of the sewage sludge is seven percent or higher, the temperature of the sewage sludge shall be 50 degrees Celsius or higher; the time period shall be 20 minutes or longer; and the temperature and time period shall be determined using equation (2), except when small particles of sewage sludge are heated by either warmed gases or an immiscible liquid.

$$D = \frac{131{,}700{,}000}{10^{0.1400t}} \quad \text{Eq. (2)}$$

Where,
D=time in days.
t=temperature in degrees Celsius.

(B) When the percent solids of the sewage sludge is seven percent or higher and small particles of sewage sludge are heated by either warmed gases or an immiscible liquid, the temperature of the sewage sludge shall be 50 degrees Celsius or higher; the time period shall be 15 seconds or longer; and the temperature and time period shall be determined using equation (2).

(C) When the percent solids of the sewage sludge is less than seven percent and the time period is at least 15 seconds, but less than 30 minutes, the temperature and time period shall be determined using equation (2).

(D) When the percent solids of the sewage sludge is less than seven percent; the temperature of the sewage sludge is 50 degrees Celsius or higher; and the time period is 30 minutes or longer, the temperature and time period shall be determined using equation (3).

$$D = \frac{50{,}070{,}000}{10^{0.1400t}} \quad \text{Eq. (3)}$$

Where,
D=time in days.
t=temperature in degrees Celsius.

(4) *Class A—Alternative 2.* (i) Either the density of fecal coliform in the sewage sludge shall be less than 1000 Most Probable Number per gram of total solids (dry weight basis), or the density of *Salmonella* sp. bacteria in the sewage sludge shall be less than three Most Probable Number per four grams of total solids (dry weight basis) at the time the sewage sludge is used or disposed; at the time the sewage sludge is prepared for sale or give away in a bag or other container for application to the land; or at the time the sewage sludge or material derived from sewage sludge is prepared to meet the requirements in § 503.10 (b), (c), (e), or (f).

(ii) (A) The pH of the sewage sludge that is used or disposed shall be raised to above 12 and shall remain above 12 for 72 hours.

(B) The temperature of the sewage sludge shall be above 52 degrees Celsius for 12 hours or longer during the period that the pH of the sewage sludge is above 12.

(C) At the end of the 72 hour period during which the pH of the sewage sludge is above 12, the sewage sludge shall be air dried to achieve a percent solids in the sewage sludge greater than 50 percent.

(5) *Class A—Alternative 3.* (i) Either the density of fecal coliform in the sewage sludge shall be less than 1000 Most Probable Number per gram of total solids (dry weight basis), or the density of *Salmonella* sp. bacteria in sewage sludge shall be less than three Most Probable Number per four grams of total solids (dry weight basis) at the time the sewage sludge is used or disposed; at the time the sewage sludge is prepared for sale or give away in a bag or other container for application to the land; or at the time the sewage sludge or material derived from sewage sludge is prepared to meet the requirements in § 503.10 (b), (c), (e), or (f).

(ii) (A) The sewage sludge shall be analyzed prior to pathogen treatment to determine whether the sewage sludge contains enteric viruses.

(B) When the density of enteric viruses in the sewage sludge prior to pathogen treatment is less than one Plaque-forming Unit per four grams of total solids (dry weight basis), the sewage sludge is Class A with respect to enteric viruses until the next monitoring episode for the sewage sludge.

(C) When the density of enteric viruses in the sewage sludge prior to pathogen treatment is equal to or greater than one Plaque-forming Unit per four grams of total solids (dry weight basis), the sewage sludge is Class A with respect to enteric viruses when the density of enteric viruses in the sewage sludge after pathogen treatment is less than one Plaque-forming Unit per four grams of total solids (dry weight basis) and when the values or ranges of values for the operating parameters for the pathogen treatment process that produces the sewage sludge that meets the enteric virus density requirement are documented.

(D) After the enteric virus reduction in paragraph (a)(5)(ii)(C) of this section is demonstrated for the pathogen treatment process, the sewage sludge continues to be Class A with respect to enteric viruses when the values for the pathogen treatment process operating parameters are consistent with the values or ranges of values documented in paragraph (a)(5)(ii)(C) of this section.

(iii)(A) The sewage sludge shall be analyzed prior to pathogen treatment to determine whether the sewage sludge contains viable helminth ova.

(B) When the density of viable helminth ova in the sewage sludge prior to pathogen treatment is less than one per four grams of total solids (dry weight basis), the sewage sludge is Class A with respect to viable helminth ova until the next monitoring episode for the sewage sludge.

(C) When the density of viable helminth ova in the sewage sludge prior to pathogen treatment is equal to or greater than one per four grams of total solids (dry weight basis), the sewage sludge is Class A with respect to viable helminth ova when the density of viable helminth ova in the sewage sludge after pathogen treatment is less than one per four grams of total solids (dry weight basis) and when the values or ranges of values for the operating parameters for the pathogen treatment process that produces the sewage sludge that meets the viable helminth ova density requirement are documented.

(D) After the viable helminth ova reduction in paragraph (a)(5)(iii)(C) of this section is demonstrated for the pathogen treatment process, the sewage

sludge continues to be Class A with respect to viable helminth ova when the values for the pathogen treatment process operating parameters are consistent with the values or ranges of values documented in paragraph (a)(5)(iii)(C) of this section.

(6) *Class A—Alternative 4.* (i) Either the density of fecal coliform in the sewage sludge shall be less than 1000 Most Probable Number per gram of total solids (dry weight basis), or the density of *Salmonella* sp. bacteria in the sewage sludge shall be less than three Most Probable Number per four grams of total solids (dry weight basis) at the time the sewage sludge is used or disposed; at the time the sewage sludge is prepared for sale or give away in a bag or other container for application to the land; or at the time the sewage sludge or material derived from sewage sludge is prepared to meet the requirements in § 503.10 (b), (c), (e), or (f).

(ii) The density of enteric viruses in the sewage sludge shall be less than one Plaque-forming Unit per four grams of total solids (dry weight basis) at the time the sewage sludge is used or disposed; at the time the sewage sludge is prepared for sale or give away in a bag or other container for application to the land; or at the time the sewage sludge or material derived from sewage sludge is prepared to meet the requirements in § 503.10 (b), (c), (e), or (f), unless otherwise specified by the permitting authority.

(iii) The density of viable helminth ova in the sewage sludge shall be less than one per four grams of total solids (dry weight basis) at the time the sewage sludge is used or disposed; at the time the sewage sludge is prepared for sale or give away in a bag or other container for application to the land; or at the time the sewage sludge or material derived from sewage sludge is prepared to meet the requirements in § 503.10 (b), (c), (e), or (f), unless otherwise specified by the permitting authority.

(7) *Class A—Alternative 5.* (i) Either the density of fecal coliform in the sewage sludge shall be less than 1000 Most Probable Number per gram of total solids (dry weight basis), or the density of *Salmonella*, sp. bacteria in the sewage sludge shall be less than three Most Probable Number per four grams of total solids (dry weight basis) at the time the sewage sludge is used or disposed; at the time the sewage sludge is prepared for sale or given away in a bag or other container for application to the land; or at the time the sewage sludge or material derived from sewage sludge is prepared to meet the requirements in § 503.10(b), (c), (e), or (f).

(ii) Sewage sludge that is used or disposed shall be treated in one of the Processes to Further Reduce Pathogens described in appendix B of this part.

(8) *Class A—Alternative 6.* (i) Either the density of fecal coliform in the sewage sludge shall be less than 1000 Most Probable Number per gram of total solids (dry weight basis), or the density of *Salmonella*, sp. bacteria in the sewage sludge shall be less than three Most Probable Number per four grams of total solids (dry weight basis) at the time the sewage sludge is used or disposed; at the time the sewage sludge is prepared for sale or given away in a bag or other container for application to the land; or at the time the sewage sludge or material derived from sewage sludge is prepared to meet the requirements in § 503.10(b), (c), (e), or (f).

(ii) Sewage sludge that is used or disposed shall be treated in a process that is equivalent to a Process to Further Reduce Pathogens, as determined by the permitting authority.

(b) *Sewage sludge—Class B.* (1)(i) The requirements in either § 503.32(b)(2), (b)(3), or (b)(4) shall be met for a sewage sludge to be classified Class B with respect to pathogens.

(ii) The site restrictions in § 503.32(b)(5) shall be met when sewage sludge that meets the Class B pathogen requirements in § 503.32(b)(2), (b)(3), or (b)(4) is applied to the land.

(2) *Class B—Alternative 1.*

(i) Seven samples of the sewage sludge shall be collected at the time the sewage sludge is used or disposed.

(ii) The geometric mean of the density of fecal coliform in the samples collected in paragraph (b)(2)(i) of this section shall be less than either 2,000,000 Most Probable Number per gram of total solids (dry weight basis) or 2,000,000 Colony Forming Units per gram of total solids (dry weight basis).

(3) *Class B—Alternative 2.* Sewage sludge that is used or disposed shall be treated in one of the Processes to Significantly Reduce Pathogens described in appendix B of this part.

(4) *Class B—Alternative 3.* Sewage sludge that is used or disposed shall be treated in a process that is equivalent to a Process to Significantly Reduce Pathogens, as determined by the permitting authority.

(5) *Site Restrictions.* (i) Food crops with harvested parts that touch the sewage sludge/soil mixture and are totally above the land surface shall not be harvested for 14 months after application of sewage sludge.

(ii) Food crops with harvested parts below the surface of the land shall not be harvested for 20 months after application of sewage sludge when the sewage sludge remains on the land surface for four months or longer prior to incorporation into the soil.

(iii) Food crops with harvested parts below the surface of the land shall not be harvested for 38 months after application of sewage sludge when the sewage sludge remains on the land surface for less than four months prior to incorporation into the soil.

(iv) Food crops, feed crops, and fiber crops shall not be harvested for 30 days after application of sewage sludge.

(v) Animals shall not be allowed to graze on the land for 30 days after application of sewage sludge.

(vi) Turf grown on land where sewage sludge is applied shall not be harvested for one year after application of the sewage sludge when the harvested turf is placed on either land with a high potential for public exposure or a lawn; unless otherwise specified by the permitting authority.

(vii) Public access to land with a high potential for public exposure shall be restricted for one year after application of sewage sludge.

(viii) Public access to land with a low potential for public exposure shall be restricted for 30 days after application of sewage sludge.

(c) *Domestic septage.* (1) The site restrictions in § 503.32(b)(5) shall be met when domestic septage is applied to agricultural land, forest, or a reclamation site; or

(2) The pH of domestic septage applied to agricultural land, forest, or a reclamation site shall be raised to 12 or higher by alkali addition and, without the addition of more alkali, shall remain at 12 or higher for 30 minutes and the site restrictions in § 503.32 (b)(5)(i) through (b)(5)(iv) shall be met.

§ 503.33 Vector attraction reduction.

(a)(1) One of the vector attraction reduction requirements in § 503.33 (b)(1) through (b)(10) shall be met when bulk sewage sludge is applied to agricultural land, forest, a public contact site, or a reclamation site.

(2) One of the vector attraction reduction requirements in § 503.33 (b)(1) through (b)(8) shall be met when bulk sewage sludge is applied to a lawn or a home garden.

(3) One of the vector attraction reduction requirements in § 503.33 (b)(1) through (b)(8) shall be met when sewage sludge is sold or given away in a bag or other container for application to the land.

(4) One of the vector attraction reduction requirements in § 503.33 (b)(1) through (b)(11) shall be met when sewage sludge (other than domestic

septage) is placed on an active sewage sludge unit.

(5) One of the vector attraction reduction requirements in § 503.33 (b)(9), (b)(10), or (b)(12) shall be met when domestic septage is applied to agricultural land, forest, or a reclamation site and one of the vector attraction reduction requirements in § 503.33 (b)(9) through (b)(12) shall be met when domestic septage is placed on an active sewage sludge unit.

(b)(1) The mass of volatile solids in the sewage sludge shall be reduced by a minimum of 38 percent (see calculation procedures in "Environmental Regulations and Technology—Control of Pathogens and Vector Attraction in Sewage Sludge", EPA–625/R–92/013, 1992, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268).

(2) When the 38 percent volatile solids reduction requirement in § 503.33(b)(1) cannot be met for an anaerobically digested sewage sludge, vector attraction reduction can be demonstrated by digesting a portion of the previously digested sewage sludge anaerobically in the laboratory in a bench-scale unit for 40 additional days at a temperature between 30 and 37 degrees Celsius. When at the end of the 40 days, the volatile solids in the sewage sludge at the beginning of that period is reduced by less than 17 percent, vector attraction reduction is achieved.

(3) When the 38 percent volatile solids reduction requirement in § 503.33(b)(1) cannot be met for an aerobically digested sewage sludge, vector attraction reduction can be demonstrated by digesting a portion of the previously digested sewage sludge that has a percent solids of two percent or less aerobically in the laboratory in a bench-scale unit for 30 additional days at 20 degrees Celsius. When at the end of the 30 days, the volatile solids in the sewage sludge at the beginning of that period is reduced by less than 15 percent, vector attraction reduction is achieved.

(4) The specific oxygen uptake rate (SOUR) for sewage sludge treated in an aerobic process shall be equal to or less than 1.5 milligrams of oxygen per hour per gram of total solids (dry weight basis) at a temperature of 20 degrees Celsius.

(5) Sewage sludge shall be treated in an aerobic process for 14 days or longer. During that time, the temperature of the sewage sludge shall be higher than 40 degrees Celsius and the average temperature of the sewage sludge shall be higher than 45 degrees Celsius.

(6) The pH of sewage sludge shall be raised to 12 or higher by alkali addition and, without the addition of more alkali, shall remain at 12 or higher for two hours and then at 11.5 or higher for an additional 22 hours.

(7) The percent solids of sewage sludge that does not contain unstabilized solids generated in a primary wastewater treatment process shall be equal to or greater than 75 percent based on the moisture content and total solids prior to mixing with other materials.

(8) The percent solids of sewage sludge that contains unstabilized solids generated in a primary wastewater treatment process shall be equal to or greater than 90 percent based on the moisture content and total solids prior to mixing with other materials.

(9)(i) Sewage sludge shall be injected below the surface of the land.

(ii) No significant amount of the sewage sludge shall be present on the land surface within one hour after the sewage sludge is injected.

(iii) When the sewage sludge that is injected below the surface of the land is Class A with respect to pathogens, the sewage sludge shall be injected below the land surface within eight hours after being discharged from the pathogen treatment process.

(10)(i) Sewage sludge applied to the land surface or placed on a surface disposal site shall be incorporated into the soil within six hours after application to or placement on the land.

(ii) When sewage sludge that is incorporated into the soil is Class A with respect to pathogens, the sewage sludge shall be applied to or placed on the land within eight hours after being discharged from the pathogen treatment process.

(11) Sewage sludge placed on an active sewage sludge unit shall be covered with soil or other material at the end of each operating day.

(12) The pH of domestic septage shall be raised to 12 or higher by alkali addition and, without the addition of more alkali, shall remain at 12 or higher for 30 minutes.

Appendix C
Determination of Volatile Solids Reduction by Digestion

Introduction

Under 40 CFR Part 503, the ability of sewage sludge to attract vectors must be reduced when sewage sludge is applied to the land or placed on a surface disposal site. One way to reduce vector attraction is to reduce the volatile solids in the sewage sludge by 38% or more (see Section 6.2 of this document). Typically, volatile solids reduction is accomplished by anaerobic or aerobic digestion. Volatile solids reduction also occurs under other circumstances, such as when sewage sludge is stored in an anaerobic lagoon or is dried on sand beds. To give credit for this extra loss in volatile solids, the regulation allows the untreated sewage sludge to be compared with the treated sewage sludge that leaves the treatment works, which should account for all of the volatile solids reduction that could possibly occur. For most processing sequences, the processing steps downstream from the digester, such as short-term storage or dewatering, have no influence on volatile solids content. Consequently, the appropriate comparison is between the sewage sludge entering the digester and the sewage sludge leaving the digester. The remainder of the discussion is limited to this circumstance, except for the final section of this appendix, which compares incoming sewage sludge with the sewage sludge leaving the treatment works.

The Part 503 regulation does not specify a method for calculating volatile solids reduction. Fischer (1984) observed that the United Kingdom has a similar requirement for volatile solids reduction for digestion (40%), but also failed to prescribe a method for calculating volatile solids reduction. Fischer has provided a comprehensive discussion of the ways that volatile solids reduction may be calculated and their limitations. He presents the following equations for determining volatile solids reduction:

- Full mass balance equation
- Approximate mass balance equation
- "Constant ash" equation
- Van Kleeck equation

The full mass balance equation is the least restricted approach but requires more information than is currently collected at a wastewater treatment plant. The approximate mass balance equation assumes steady state conditions. The "constant ash" equation requires the assumption of steady state conditions as well as the assumption that the ash input rate equals the ash output rate. The Van Kleeck equation, which is the equation generally suggested in publications originating in the United States (WPCF, 1968), is equivalent to the constant ash equation. Fischer calculates volatile solids reduction using a number of examples of considerable complexity and illustrates that different methods frequently yield different results.

Fischer's paper is extremely thorough and is highly recommended for someone trying to develop a deep understanding of potential complexities in calculating volatile solids reduction. However, it was not written as a guidance document for field staff faced with the need to calculate volatile solids reduction. The nomenclature is precise but so detailed that it makes comprehension difficult. In addition, two important troublesome situations that complicate the calculation of volatile solids reduction—grit deposition in digesters and decantate removal—are not explicitly discussed. Consequently, this presentation has been prepared to present guidance that describes the major pitfalls likely to be encountered in calculating percent volatile solids reduction.

It is important to note that the calculation of volatile solids reduction is only as accurate as the measurement of volatile solids content in the sewage sludge. The principal cause of error is poor sampling. Samples should be representative, covering the entire charging and withdrawal periods. Averages should cover extended periods of time during which changes in process conditions are minimal. For some treatment, it is expected that periodic checks of volatile solids reduction will produce results so erratic that no confidence can be placed in them. In this case, adequacy of stabilization can be verified by the method described under Options 2 and 3 in Chapter 6—periodically batch digest anaerobically digested sewage sludge for 40 additional days at 30°C (86°F) to 37°C (99°F), or aerobically digested sewage sludge for 30 additional days at 20°C (68°F). If the additional VS reduction is less than 17% for the anaerobically digested sewage sludge or less than 15% for the aerobically digested sewage sludge, the sewage sludge is sufficiently stable (see Sections 6.3 and 6.4).

Equations for FVSR

The equations for fractional volatile solids reduction (FVSR) that will be discussed below are the same as those developed by Fischer (1984), except for omission of his constant ash equation. This equation gives identical results to the

Van Kleeck equation so it is not shown. Fischer's nomenclature has been avoided or replaced with simpler terms. The material balance approaches are called methods rather than equations. The material balances are drawn to fit the circumstances. There is no need to formalize the method with a rigid set of equations.

In the derivations and calculations that follow, both VS (total volatile solids content of the sewage sludge or decantate on a dry solids basis) and FVSR are expressed throughout as fractions to avoid the frequent confusion that occurs when these terms are expressed as percentages. "Decantate" is used in place of the more commonly used "supernatant" to avoid the use of "s" in subscripts. Similarly, "bottoms" is used in place of "sludge" to avoid use of "s" in subscripts.

Full Mass Balance Method

The full mass balance method must be used when steady conditions do not prevail over the time period chosen for the calculation. The chosen time period must be substantial, at least twice the nominal residence time in the digester (nominal residence time equals average volume of sludge in the digester divided by the average volumetric flow rate. Note: when there is decantate withdrawal, volume of sewage sludge withdrawn should be used to calculate the average volumetric flow rate). The reason for the long time period is to reduce the influence of short-term fluctuations in sewage sludge flow rates or compositions. If input compositions have been relatively constant for a long period of time, then the time period can be shortened.

An example where the full mass balance method would be needed is where an aerobic digester is operated as follows:

- Started with the digester 1/4 full (time zero)
- Raw sewage sludge is fed to the digester daily until the digester is full
- Supernatant is periodically decanted and raw sewage sludge is charged into the digester until settling will not occur to accommodate daily feeding (hopefully after enough days have passed for adequate digestion)
- Draw down the digester to about 1/4 full (final time), discharging the sewage sludge to sand beds

The full mass balance is written as follows:

Sum of total volatile solids inputs in feed streams during the entire digestion period = sum of volatile solids outputs in withdrawals of decantate and bottoms + loss of volatile solids + accumulation of volatile solids in the digester. (1)

Loss of volatile solids is calculated from Equation 1. FVSR is calculated by Equation 2:

$$FVSR = \frac{\text{loss in volatile solids}}{\text{sum of volatile solids inputs}} \quad (2)$$

The accumulation of volatile solids in the digester is the final volume in the digester after the drawdown times final volatile solids concentration less the initial volume at time zero times the initial volatile solids concentration.

To properly determine FVSR by the full mass balance method requires determination of all feed and withdrawal volumes, initial and final volumes in the digester, and volatile solids concentrations in all streams. In some cases, which will be presented later, simplifications are possible.

Approximate Mass Balance Method

If volumetric inputs and outputs are relatively constant on a daily basis, and there is no substantial accumulation of volatile solids in the digester over the time period of the test, an approximate mass balance (AMB) may be used. The basic relationship is stated simply:

volatile solids input rate = volatile solids output rate + rate of loss of volatile solids. (3)

The FVSR is given by Equation 2.

No Decantate, No Grit Accumulation (Problem 1)

Calculation of FVSR is illustrated for Problem 1 in Table C-1, which represents a simple situation with no decantate removal and no grit accumulation. An approximate mass balance is applied to the digester operated under constant flow conditions. Because no decantate is removed, the volumetric flow rate of sewage sludge leaving the digester equals the flow rate of sewage sludge entering the digester.

Applying Equations 3 and 2,

$$FY_f = BY_b + \text{loss} \quad (4)$$

$$\text{Loss} = 100(50-30) = 2000 \quad (5)$$

$$FVSR = \frac{\text{Loss}}{FY_f} \quad (6)$$

$$FVSR = \frac{2000}{(100)\,(50)} = 0.40 \quad (7)$$

Nomenclature is given in Table C-1. Note that the calculation did not require use of the fixed solids concentrations.

The calculation is so simple that one wonders why it is so seldom used. One possible reason is that the input and output volatile solids concentrations (Y_f and Y_b) typically will show greater coefficients of variation (standard deviation divided by arithmetic average) than the fractional volatile solids (VS is the fraction of the sewage sludge solids that is volatile—note the difference between VS and Y). If this is the case, the volatile solids reduction calculated by the approximate mass balance method from several sets of Y_f-Y_b data will show larger deviations than if it were calculated by the Van Kleeck equation using VS_f-VS_b data.

Table C-1. Quantitative Information for Example Problems[1,2,3]

Parameter	Symbol	Units	Problem Statement Number 1	2	3	4
Nominal residence time	θ	d	20	20	20	20
Time period for averages	—	d	60	60	60	60
Feed Sludge						
Volumetric flow rate	F	m^3/d	100	100	100	100
Volatile solids concentration	Y_f	kg/m^3	50	50	50	50
Fixed solids concentration	X_f	kg/m^3	17	17	17	17
Fractional volatile solids	VS_f	kg/kg	0.746	0.746	0.746	0.746
Mass flow rate of solids	M_f	kg/d	6700	6700	6700	6700
Digested Sludge (Bottoms)						
Volumetric flow rate	B	m^3/d	100	100		49.57
Volatile solids concentration	Y_b	kg/m^3	30	30	41.42	41.42
Fixed solids concentration	X_b	kg/m^3	17	15	23.50	23.50
Fractional volatile solids	VS_b	kg/kg	0.638	0.667	0.638	0.638
Mass flow rate of solids	M_b	kg/d	4700	4500		
Decantate						
Volumetric flow rate	D	m^3/d	0	0		50.43
Volatile solids concentration	Y_d	kg/m^3	—	—	12.76	12.76
Fixed solids concentration	X_d	kg/m^3	—	—	7.24	7.24
Fractional volatile solids	VS_d	kg/kg	—	—	0.638	0.638
Mass flow rate of solids	M_d	kg/d	—	—		

[1]Conditions are steady state; all daily flows are constant. Volatile solids are not accumulating in the digester, although grit may be settling out in the digester.
[2]Numerical values are given at 3 or 4 significant figures. This is unrealistic considering the expected accuracy in measuring solids concentrations and sludge volumes. The purpose of extra significant figures is to allow more understandable comparisons to be made of the different calculation methods.
[3]All volatile solids concentrations are based on total solids, not merely on suspended solids.

Grit deposition can be a serious problem in both aerobic and anaerobic digestion. The biological processes that occur in digestion dissolve or destroy the substances suspending the grit, and it tends to settle. If agitation is inadequate to keep the grit particles in suspension, they will accumulate in the digester. The approximate mass balance can be used to estimate accumulation of fixed solids.

For Problem 1, the balance yields the following:

$$FX_f = BX_b + \text{fixed solids loss} \quad (8)$$

$$(100)(17) = (100)(17) + \text{Fixed Solids Loss} \quad (9)$$

$$\text{Fixed Solids Loss} = 0 \quad (10)$$

The material balance compares fixed solids in output with input. If some fixed solids are missing, this loss term will be a positive number. Because digestion does not consume fixed solids, it is assumed that the fixed solids are accumulating in the digester. As Equation 10 shows, the fixed solids loss equals zero. Note that for this case, where input and output sewage sludge flow rates are equal, the fixed solids concentrations are equal when there is no grit accumulation.

Grit Deposition (Problem 2)

The calculation of fixed solids is repeated for Problem 2. Conditions in Problem 2 have been selected to show grit accumulation. Parameters are the same as in Problem 1 except for the fixed solids concentration (X_b) and parameters related to it. Fixed solids concentration in the sewage sludge is lower than in Problem 1. Consequently, VS is higher and the mass flow rate of solids leaving is lower than in Problem 1. A mass balance on fixed solids (input rate = output rate + rate of loss of fixed solids) is presented in Equations 11-13.

$$FX_f = BX_b + \text{Fixed Solids Loss} \quad (11)$$

$$\text{Fixed Solids Loss} = FX_f - BX_b \quad (12)$$

$$\text{Fixed Solids Loss} = (100)(7) - (100)(15) = 200\ \text{kg/d} \quad (13)$$

The material balance, which only looks at inputs and outputs, informs us that 200 kg/d of fixed solids have not appeared in the outputs as expected. Because fixed solids are not destroyed, it can be concluded that they are accumulating in the bottom of the digester. The calculation of FVSR for Problem 2 is exactly the same as for Problem 1 (see Equations 4 through

7) and yields the same result. The approximate mass balance method gives the correct answer for the FVSR despite the accumulation of solids in the digester. As will be seen later, this is not the case when the Van Kleeck equation is used.

Decantate Withdrawal, No Grit Accumulation (Problem 3)

In Problem 3, decantate is withdrawn daily. Volatile and fixed solids concentrations are known for all streams but the volumetric flow rates are not known for decantate and bottoms. It is impossible to calculate FVSR without knowing the relative volumes of these streams. However, they are determined easily by taking a total volume balance and a fixed solids balance, provided it can be assumed that loss of fixed solids (i.e., accumulation in the digester) is zero.

Selecting a basis for F of 100 m^3/d,

$$\text{Volume balance: } 100 = B + D \tag{14}$$

$$\text{Fixed solids balance: } 100\,X_f + BX_b + DX_d \tag{15}$$

Because the three Xs are known, B and D can be found. Substituting 100-D for B and the values for the Xs from Problem 3 and solving for D and B,

$$(100)(17) = (100 - D)(23.50) + (D)(7.24) \tag{16}$$

$$D = 40.0\,m^3/d,\ B = 60.0\ m^3/d \tag{17}$$

The FVSR can now be calculated by drawing a volatile solids balance:

$$FY_f + BY_b + DY_d + \text{loss} \tag{18}$$

$$FVSR = \frac{\text{loss}}{FY_f} = \frac{FY_f - By_b - DY_d}{FY_f} \tag{19}$$

$$FVSR = \frac{(100)(50) - (60)(41.42) - (40)(12.76)}{(100)(50)} = 0.40 \tag{20}$$

Unless information is available on actual volumes of decantate and sewage sludge (bottoms), it is not possible to determine whether grit is accumulating in the digester. If it is accumulating, the calculated FVSR will be in error.

When the calculations shown in Equations 18 through 20 are made, it is assumed that the volatile solids that are missing from the output streams are consumed by biological reactions that convert them to carbon dioxide and methane. Accumulation is assumed to be negligible. Volatile solids are less likely to accumulate than fixed solids, but it can happen. In poorly mixed digesters, the scum layer that collects at the surface is an accumulation of volatile solids. FVSR calculated by Equations 18 through 20 will be overestimated if the volatile solids accumulation rate is substantial.

Decantate Withdrawal and Grit Accumulation (Problem 4)

In Problem 4, there is suspected grit accumulation. The quantity of B and D can no longer be calculated by Equations 14 and 15 because Equation 15 is no longer correct. The values of B and D must be measured. All parameters in Problem 4 are the same as in Problem 3 except that measured values for B and D are introduced into Problem 4. Values of B and D calculated assuming no grit accumulation (Problem 3—see previous discussion), and measured quantities are compared below:

	Calculated	**Measured**
B	60	49.57
D	40	50.43

The differences in the values of B and D are not large but they make a substantial change in the numerical value of FVSR. The FVSR for Problem 4 is calculated below:

$$FVSR = \frac{(100)(50) - (49.57)(41.42) - (50.43)(12.76)}{(100)(50)} = 0.461 \tag{21}$$

If it had been assumed that there was no grit accumulation, FVSR would equal 0.40 (see Problem 3). It is possible to determine the amount of grit accumulation that has caused this change. A material balance on fixed solids is drawn:

$$FX_f = BX_b + DX_d + \text{Fixed Solids Loss} \tag{22}$$

The fractional fixed solids loss due to grit accumulation is found by rearranging this equation:

$$\frac{\text{Fixed Solids Loss}}{FX_f} = \frac{FX_f - BX_b - DX_d}{FX_f} \tag{23}$$

Substituting in the parameter values for Problem 4,

$$\frac{\text{Fixed Solids Loss}}{FX_f} = \frac{(100)(17) - (49.57)(23.50) - (50.43)(7.24)}{(100)(17)} = 0.100 \tag{24}$$

If this fixed solids loss of 10 percent had not been accounted for, the calculated FVSR would have been 13% lower than the correct value of 0.461. Note that if grit accumulation occurs and it is ignored, calculated FVSR will be lower than the actual value.

The Van Kleeck Equation

Van Kleeck first presented his equation without derivation in a footnote for a review paper on sewage sludge treatment processing in 1945 (Van Kleeck, 1945). The equation is easily derived from total solids and volatile solids mass balances around the digestion system. Consider a digester operated under steady state conditions with decantate and bottom sewage sludge removal. A total solids mass balance and a volatile solids mass balance are:

$$M_f = M_b + M_d + (\text{loss of total solids}) \quad (25)$$

$$M_f \cdot VS_f = M \cdot VS_b + M_d \cdot VS_d + (\text{loss of volatile solids}) \quad (26)$$

where

M_f, M_b, and M_d are the mass of solids in the feed, bottoms, and decantate streams.

The masses must be mass of *solids* rather than total mass of liquid and solid because VS is an unusual type of concentration unit—it is "mass of volatile solids per unit mass of *total solids.*"

It is now assumed that fixed solids are not destroyed and there is no grit deposition in the digester. The losses in Equations 25 and 26 then comprise only volatile solids so the losses are equal. It is also assumed that the VS of the decantate and of the bottoms are the same. This means that the bottoms may have a much higher solids content than the decantate but the proportion of volatile solids to fixed solids is the same for both streams. Assuming then that VS_b equals VS_d, and making this substitution in the defining equation for FVSR (Equation 2),

$$FVSR = \frac{\text{Loss of vol. solids}}{M_f \times VS_f} = 1 - \frac{(M_b + M_d)\, VS_b}{M_f \times VS_f} \quad (27)$$

From Equation 25, recalling that we have assumed that loss of total solids equals loss of volatile solids,

$$M_b + M_d + M_f - \text{loss of vol. solids} \quad (28)$$

Substituting for $M_b + M_d$ into Equation 27,

$$FVSR = 1 - \frac{(M_f - \text{loss of vol. solids}) \cdot VS_b}{M_f \cdot VS_f} \quad (29)$$

Simplifying further,

$$1 - (1/VS_f - FVSR) \cdot VS_b \quad (30)$$

Solving for FVSR,

$$FVSR = \frac{VS_f - VS_b}{VS_f - (VS_f \times VS_b)} \quad (31)$$

This is the form of the Van Kleeck equation found in WPCF Manual of Practice No. 16 (WPCF, 1968). Van Kleeck (1945) presented the equation in the following equivalent form:

$$FVSR = 1 - \frac{VS_b \times (1 - VS_f)}{VS_f \times (1 - VS_b)} \quad (32)$$

The Van Kleeck equation is applied below to Problems 1 through 4 in Table C-1 and compared to the approximate mass balance equation results:

	1	2	3	4
Approximate Mass Balance (AMB)	0.40	0.40	0.40	0.461
Van Kleeck (VK)	0.40	0.318	0.40	0.40

Problem 1: No decantate and no grit accumulation. Both methods give correct answer.

Problem 2: No decantate but grit accumulation. VK is invalid and incorrect.

Problem 3: Decantate but no grit accumulation. AMB method is valid. VK method is valid only if VS_b equals VS_d.

Problem 4: Decantate and grit accumulation. AMB method valid only if B and D are measured. VK method is invalid.

The Van Kleeck equation is seen to have serious shortcomings when applied to certain practical problems. The AMB method can be completely reliable, whereas the Van Kleeck method is useless under some circumstances.

Average Values

The concentrations and VS values used in the equations will all be averages. For the material balance methods, the averages should be weighted averages according to the mass of solids in the stream in question. The example below shows how to average the volatile solids concentration for four consecutive sewage sludge additions.

Addition	Volume	Total Solids Concentration	VS	
1	12 m^3	72 kg/m^3	0.75	
2	8 m^3	50 kg/m^3	0.82	
3	13 m^3	60 kg/m^3	0.80	
4	10 m^3	55 kg/m^3	0.77	(33)

For the Van Kleeck equation, the averages of VS are required. Properly they should be weighted averages based on the weight of the solids in each component of the average, although an average weighted by the volume of the component, or an arithmetic average may be sufficiently accurate if variation in VS is small. The following example demonstrates the calculation of all three averages.

Weighted by Mass

$$VS\ av = \frac{12 \times 72 \times 0.75 + 8 \times 50 \times 0.82 + 13 \times 60 \times 0.80 + 10 \times 55 \times 0.77}{12 \times 72 + 8 \times 50 + 13 \times 60 + 10 \times 55} = 0.795 \quad (34)$$

Weighted by Volume

$$VS\ av = \frac{12 \times 0.75 + 8 \times 0.82 + 13 \times 0.80 + 10 \times 0.77}{12 + 8 + 13 + 10} = 0.783 \quad (35)$$

Arithmetic Average

$$VS\ av = \frac{0.75 + 0.82 + 0.80 + 0.77}{4} = 0.785 \quad (36)$$

In this example the arithmetic average was nearly as close as the volume-weighted average to the mass-weighted average, which is the correct value.

Which Equation to Use?

Full Mass Balance Method

The full mass balance method allows calculation of volatile solids reduction for all approaches to digestion, even processes in which the final volume in the digester does not equal the initial volume and where daily flows are not steady. A serious drawback to this method is the need for volatile solids concentration and the volumes of all streams added to or withdrawn from the digester, as well as initial and final volumes and concentrations in the digester. This can be a daunting task, particularly for the small treatment works that is most likely to run digesters in other than steady flow modes. For treatment works of this kind, an "equivalent" method that shows that the sewage sludge has undergone the proper volatile solids reduction is likely to be a better approach than trying to demonstrate 38% volatile solids reduction. An aerobic sewage sludge has received treatment equivalent to a 38% volatile solids reduction if the specific oxygen uptake rate is below a specified maximum. Anaerobically digested sewage sludge has received treatment equivalent to a 38% volatile solids reduction if volatile solids reduction after batch digestion of the sewage sludge for 40 days is less than a specified maximum (EPA, 1992).

Approximate Mass Balance Method

The approximate mass balance method assumes that daily flows are steady and reasonably uniform in composition, and that digester volume and composition do not vary substantially from day to day. Results of calculations and an appreciation of underlying assumptions show that the method is accurate for all cases, including withdrawal of decantate and deposition of grit, provided that in addition to composition of all streams the quantities of decantate and bottoms (the digested sewage sludge) are known. If the quantities of decantate and bottoms are not known, the accumulation of grit cannot be determined. If accumulation of grit is substantial and FVSR is calculated assuming it to be negligible, FVSR will be lower than the true value. The result is conservative and could be used to show that minimum volatile solids reductions are being achieved.

Van Kleeck Method

The Van Kleeck equation has underlying assumptions that should be made clear wherever the equation is presented. The equation is never valid when there is grit accumulation because it assumes the fixed solids input equals fixed solids output. Fortunately, it produces a conservative result in this case. Unlike the AMB method it does not provide a convenient way to check for accumulation of grit. It can be used when decantate is withdrawn, provided VS_b equals VS_d. Just how significant the difference between these VS values can be before an appreciable error in FVSR occurs is unknown, although it could be determined by making up a series of problems with increasing differences between the VS values, calculating FVSR using the AMB method and a Van Kleeck equation, and comparing the results.

The shortcomings of the Van Kleeck equation are substantial, but the equation has one strong point: The VS of the various sewage sludge and decantate streams are likely to show much lower coefficients of variation (standard deviation divided by arithmetic average) than volatile solids and fixed solids concentration. Reviews of data are needed to determine how seriously the variation in concentrations affect the confidence interval of FVSR calculated by both methods. A hybrid approach may turn out to be advantageous. The AMB method could be used first to determine if grit accumulation is occurring. If grit is not accumulating, the Van Kleeck equation could be used. If decantate is withdrawn, the Van Kleeck equation is appropriate, particularly if the decantate is low in total solids. If not, and if VS_d differs substantially from VS_b, it could yield an incorrect answer.

Volatile Solids Loss Across All Sewage Sludge Treatment Processes

For cases when appreciable volatile solids reduction can occur downstream from the digester (for example, as would occur in air drying or lagoon storage), it is appropriate to calculate the volatile solids loss from the point at which the sewage sludge enters the digester to the point at which the sewage sludge leaves the treatment works. Under these circumstances, it is virtually never possible to use the approximate mass balance approach, because flow rates are not uniform. The full mass balance could be used in principle, but practical difficulties such as measuring the mass of the output sewage sludge (total mass, not just mass of solids) that relates to a given mass of entering sewage sludge make this also a practical impossibility. Generally then, the only option is to use the Van Kleeck equation, because only the percent volatile solids content of the entering and exiting sewage sludge is needed to make this calculation. As noted earlier, this equation will be inappropriate if there has been a selective loss of high volatility solids (e.g., bacteria) or low volatility solids (e.g., grit) in any of the sludge processing steps.

To make a good comparison, there should be good correspondence between the incoming sewage sludge and the treated sewage sludge to which it is being compared (see Section 7.4). For example, when sewage sludge is digested for 20 days, then dried on a sand bed for 3 months, and then removed, the treated sludge should be compared with the sludge fed to the digester in the preceding 3 or 4 months. If no selective loss of volatile or nonvolatile solids has occurred, the Van Kleeck equation (see Equation 31) can be used to calculate volatile solids reduction.

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Appendix D
Guidance on Three Vector Attraction Reduction Tests

This appendix provides guidance for the vector attraction reduction Options 2, 3, and 4 to demonstrate reduced vector attraction (see Chapter 6 for a description of these requirements).

1. Additional Digestion Test for Anaerobically Digested Sewage Sludge

Background

The additional digestion test for anaerobically digested sewage sludge is based on research by Jeris et al. (1985). Farrell and Bhide (1993) explain in more detail the origin of the time and volatile solids reduction requirements of the test.

Jeris et al. (1985) measured changes in many parameters including volatile solids content while carrying out additional digestion of anaerobically digested sludge from several treatment works for long periods. Samples were removed from the digesters weekly for analysis. Because substantial amount of sample was needed for all of these tests, they used continuously mixed digesters of 18 liters capacity. The equipment and procedures of Jeris et al., although not complex, appear to be more elaborate than needed for a control test. EPA staff (Farrell and Bhide, 1993) have experimented with simplified tests and the procedure recommended is based on their work.

Recommended Procedure

The essentials of the test are as follows:

- Remove, from the plant-scale digester, a representative sample of the sewage sludge to be evaluated to determine additional volatile solids destruction. Keep the sample protected from oxygen and maintain it at the temperature of the digester. Commence the test within 6 hours after taking the sample.

- Flush fifteen 100-mL volumetric flasks with nitrogen, and add approximately 50 mL of the sludge to be tested into each flask. Frequently mix the test sludge during this operation to assure that its composition remains uniform. Select five flasks at random, and determine total solids content and volatile solids content, using the entire 50 mL for the determination. Seal each of the remaining flasks with a stopper with a single glass tube through it to allow generated gases to escape.

- Connect the glass tubing from each flask through a flexible connection to a manifold. To allow generated gases to escape and prevent entry of air, connect the manifold to a water-sealed bubbler by means of a vertical glass tube. The tube should be at least 30-cm long with enough water in the bubbler so that an increase in atmospheric pressure will not cause backflow of air or water into the manifold. Maintain the flasks containing the sludge at constant temperature either by inserting them in a water bath (the sludge level in the flasks must be below the water level in the bath) or by placing the entire apparatus in a constant temperature room or box. The temperature of the additional digestion test should be the average temperature of the plant digester, which should be in the range of 30°C to 40°C (86°F to 104°F). Temperature should be controlled within ± 0.15°C (0.27°F).

- Each flask should be swirled every day to assure adequate mixing, using care not to displace sludge up into the neck of the flask. Observe the water seal for the first few days of operation. There should be evidence that gas is being produced and passing through the bubbler.

- After 20 days, withdraw five flasks at random. Determine total and volatile solids content using the entire sample for the determination. Swirl the flask vigorously before pouring out its contents to minimize the hold up of thickened sludge on the walls and to assure that any material left adhering to the flask walls will have the same average composition as the material withdrawn. Use a consistent procedure. If hold-up on walls appears excessive, a minimal amount of distilled water may be used to wash solids off the walls. Total removal is not necessary, but any solids left on the walls should be approximately of the same composition as the material removed.

- After 40 days, remove the remaining five flasks. Determine total and volatile solids content using the entire sample from each flask for the determination. Use the same precautions as in the preceding step to remove virtually all of the sludge, leaving only material with the same approximate composition as the material removed.

Total and volatile solids content are determined using the procedures of Method 2540 G of Standard Methods (APHA, 1992).

Mean values and standard deviations of the total solids content, the volatile solids content, and the percent volatile solids are calculated. Volatile solids reductions that result from the additional digestion periods of 20 and 40 days are calculated from the mean values by the Van Kleeck equation and by a material balance (refer to Appendix C for a general description of these calculations). The results obtained at 20 days give an early indication that the test is proceeding satisfactorily and will help substantiate the 40-day result.

Alternative approaches are possible. The treatment works may already have versatile bench-scale digesters available. This equipment could be used for the test, provided accuracy and reproducibility can be demonstrated. The approach described above was developed because Farrell and Bhide (1993) in their preliminary work experienced much difficulty in withdrawing representative samples from large digesters even when care was taken to stir the digesters thoroughly before sampling. If an alternative experimental setup is used, it is still advisable to carry out multiple tests for the volatile solids content in order to reduce the standard error of this measurement, because error in the volatile solids content measurement is inflated by the nature of the equation used to calculate the volatile solids reduction.

Variability in flow rates and nature of the sludge will result in variability in performance of the plant-scale digesters. It is advisable to run the additional digestion test routinely so that sufficient data are available to indicate average performance. The arithmetic mean of successive tests (a minimum of three is suggested) should show an additional volatile solids reduction of $\leq 17\%$.

Calculation Details

Appendix C, Determination of Volatile Solids Reduction by Digestion, describes calculation methods to use for digesters that are continuously fed or are fed at least once a day. Although the additional anaerobic digestion test is a batch digestion, the material balance calculations approach is the same. Masses of starting streams (input streams) are set equal to masses of ending streams (output streams).

The test requires that the fixed volatile solids reduction (FVSR) be calculated both by the Van Kleeck equation and the material balance method. The Van Kleeck equation calculations can be made in the manner described in Appendix C.

The calculation of the volatile solids reduction (and the fixed fractional solids reduction [FFSR]) by the mass balance method shown below has been refined by subtracting out the mass of gas lost from the mass of sludge at the end of the digestion step. For continuous digestion, this loss of mass usually is ignored, because the amount is small in relation to the total digesting mass, and mass before and after digestion are assumed to be the same. Considering the inherent difficulty in matching mass and composition entering to mass and composition leaving for a continuous process, this is a reasonable procedure. For batch digestion, the excellent correspondence between starting material and final digested sludge provides much greater accuracy in the mass balance calculation, so inclusion of this lost mass is worthwhile.

In the equations presented below, concentrations of fixed and volatile solids are mass fractions—mass of solids per unit mass of sludge (mass of sludge includes both the solids and the water in the sludge)—and are indicated by the symbols lowercase y and x. This is different from the usage in Appendix C where concentrations are given in mass per unit volume, and are indicated by the symbols uppercase y and x. This change has been made because masses can be determined more accurately than volumes in small-scale tests.

In the material balance calculation, it is assumed that as the sludge digests, volatile solids and fixed solids are converted to gases that escape or to volatile compounds that distill off when the sludge is dried. Any production or consumption of water by the biochemical reactions in digestion is assumed to be negligible. The data collected (volatile solids and fixed solids concentrations of feed and digested sludge) allow mass balances to be drawn on volatile solids, fixed solids, and water. As noted, it is assumed that there is no change in water mass—all water in the feed is present in the digested sludge. Fractional reductions in volatile solids and fixed solids can be calculated from these mass balances for the period of digestion. Details of the calculation of these relationships are given by Farrell and Bhide (1993). The final form of the equations for fractional volatile solids reduction (mass balance [m.b.] method) and fractional fixed solids reduction (m.b. method) are given below:

$$\mathrm{FVSR(m.b.)} = \frac{y_f\,(1-x_b) - y_b\,(1-x_f)}{y_f\,(1-x_b-y_b)} \qquad (1a)$$

$$\mathrm{FFSR(m.b.)} = \frac{x_f\,(1-y_b) - x_b\,(1-y_f)}{x_f\,(1-x_b-y_b)} \qquad (1b)$$

where:

y = mass fraction of volatile solids in the liquid sludge
x = mass fraction of fixed solids in the liquid sludge
f = indicates feed sludge at start of the test
b = indicates "bottoms" sludge at end of the test

If the fixed solids loss is zero, these two equations are reduced to Equation 2 below:

$$\mathrm{FVSR(m.b.)} = (y_f - y_b) / y_f(1-y_b) \qquad (2)$$

If the fixed solids loss is not zero but is substantially smaller than the volatile solids reduction, Equation 2 gives surprisingly accurate results. For five sludges batch-digested by Farrell and Bhide (1993), the fixed solids reductions were about one-third of the volatile solids reductions. When the FVSR(m.b.) calculated by Equation 1a averaged 15%, the FVSR(m.b.) calculated by Equation 2 averaged 14.93%, which is a trivial difference.

The disappearance of fixed solids unfortunately has a relatively large effect on the calculation of FVSR by the Van Kleeck equation. The result is lower than it should be. For five sludges

that were batch-digested by Farrell and Bhide (1993), the FVSR calculated by the Van Kleeck method averaged 15%, whereas the FVSR (m.b.) calculated by Equation 1a or 2 averaged about 20%. When the desired endpoint is an FVSR below 17%, this is a substantial discrepancy.

The additional digestion test was developed for use with the Van Kleeck equation, and the 17% requirement is based on results calculated with this equation. In the future, use of the more accurate mass balance equation may be required, with the requirement adjusted upward by an appropriate amount. This cannot be done until more data with different sludge become available.

2. Specific Oxygen Uptake Rate

Background

The specific oxygen uptake rate of a sewage sludge is an accepted method for indicating the biological activity of an activated sewage sludge mixed liquor or an aerobically digesting sludge. The procedure required by the Part 503 regulation for this test is presented in Standard Methods (APHA, 1992) as Method 2710 B, Oxygen-Consumption Rate.

The use of the specific oxygen uptake rate (SOUR) has been recommended by Eikum and Paulsrud (1977) as a reliable method for indicating sludge stability provided temperature effects are taken into consideration. For primary sewage sludges aerobically digested at 18°C (64°F), sludge was adequately stabilized (i.e., it did not putrefy and cause offensive odors) when the SOUR was less than 1.2 mg O_2/hr/g VSS (volatile suspended solids). The authors investigated several alternative methods for indicating stability of aerobically digested sludges and recommended the SOUR test as the one with the most advantages and the least disadvantages.

Ahlberg and Boyko (1972) also recommend the SOUR as an index of stability. They found that, for aerobic digesters operated at temperatures above 10°C (50°F), SOUR fell to about 2.0 mg O_2/hr/g VSS after a total sludge age of 60 days and to 1.0 mg O_2/hr/g VSS after about 120 days sludge age. These authors state that a SOUR of less than 1.0 mg O_2/hr/g VSS at temperatures above 10°C (50°F) indicates a stable sludge.

The results obtained by these authors indicate that long digestion times—more than double the residence time for most aerobic digesters in use today—are needed to eliminate odor generation from aerobically digested sludges. Since the industry is not being deluged with complaints about odor from aerobic digesters, it appears that a higher SOUR standard can be chosen than they suggest without causing problems from odor (and vector attraction).

The results of long-term batch aerobic digestion tests by Jeris et al. (1985) provide information that is helpful in setting a SOUR requirement that is reasonably attainable and still protective. Farrell and Bhide (1993) reviewed the data these authors obtained with four sewage sludges from aerobic treatment processes and concluded that a standard of 1.5 mg O_2/hr/g TS at 20°C (68°F) would discriminate between adequately stabilized and poorly stabilized sludges. The "adequately digested" sludges were not totally trouble-free, i.e., it was possible under adverse conditions to develop odorous conditions. In all cases where the sludge was deemed to be adequate, minor adjustment in plant operating conditions created an acceptable sludge.

The SOUR requirement is based on total solids rather than volatile suspended solids. This usage is preferred for consistency with the rest of the Part 503 regulation where all loadings are expressed on a total solids basis. The use of total solids concentration in the SOUR calculation is rational since the entire sludge solids and not just the volatile solids degrade and may exert some oxygen demand. Making an adjustment for the difference caused by basing the requirement on TS instead of VSS, the standard is about 1.8 times higher than Eikum and Paulsrud's recommended value and 2.1 times higher than Ahlberg and Boykos' recommendation.

Unlike anaerobic digestion, which is typically conducted at 35°C (95°F), aerobic digestion is carried out without any deliberate temperature control. The temperature of the digesting sludge will be close to ambient temperature, which can range from 5°C to 30°C (41°F to 86°F). In this temperature range, SOUR increases with increasing temperature. Consequently, if a requirement for SOUR is selected, there must be some way to convert SOUR test results to a standard temperature. Conceivably, the problem could be avoided if the sludge were simply heated or cooled to the standard temperature before running the SOUR test. Unfortunately, this is not possible, because temperature changes in digested sludge cause short-term instabilities in oxygen uptake rate (Benedict and Carlson [1973], Farrell and Bhide [1993]).

Eikum and Paulsrud (1977) recommend that the following equation be used to adjust the SOUR determined at one temperature to the SOUR for another temperature:

$$(SOUR)_{T1}/(SOUR)_{T2} = \Theta^{(T1—T2)} \qquad (3)$$

where:

$(SOUR)_{T1}$ = specific oxygen uptake rate at T_1
$(SOUR)_{T2}$ = specific oxygen uptake rate at T_2
Θ = the Streeter-Phelps temperature sensitivity coefficient

These authors calculated the temperature sensitivity coefficient using their data on the effect of temperature on the rate of reduction in volatile suspended solids with time during aerobic digestion. This is an approximate approach, because there is no certainty that there is a one-to-one relationship between oxygen uptake rate and rate of volatile solids disappearance. Another problem is that the coefficient depends on the makeup of each individual sludge. For example, Koers and Mavinic (1977) found the value of Θ to be less than 1.072 at temperatures above 15°C (59°F) for aerobic digestion of waste activated sludges, whereas Eikum and Paulsrud (1977) determined Θ to equal 1.112 for primary sludges. Grady and Lim (1980)

reviewed the data of several investigators and recommended that $\Theta = 1.05$ be used for digestion of waste-activated sludges when more specific information is not available. Based on a review of the available information and their own work, Farrell and Bhide (1993) recommend that Eikum and Paulsruds' temperature correction procedure be utilized, using a temperature sensitivity coefficient in the range of 1.05 to 1.07.

Recommended Procedure for Temperature Correction

A SOUR of 1.5 mg O_2/hr/g total solids at 20°C (68°F) was selected to indicate that an aerobically digested sludge has been adequately reduced in vector attraction.

The SOUR of the sludge is to be measured at the temperature at which the aerobic digestion is occurring in the treatment works and corrected to 20°C (68°F) by the following equation:

$$SOUR_{20} = SOUR_T \times \Theta^{(20-T)} \quad (4)$$

where
$\Theta = 1.05$ above 20°C (68°F)
1.07 below 20°C (68°F)

This correction may be applied only if the temperature of the sludge is between 10°C and 30°C (50°F and 86°F). The restriction to the indicated temperature range is required to limit the possible error in the SOUR caused by selecting an improper temperature coefficient. Farrell and Bhide's (1993) results indicate that the suggested values for Θ will give a conservative value for SOUR when translated from the actual temperature to 20°C (68°F).

The experimental equipment and procedures for the SOUR test are those described in Part 2710 B, Oxygen Consumption Rate, of Standard Methods (APHA, 1992). The method allows the use of a probe with an oxygen-sensitive electrode or a respirometer. The method advises that manufacturer's directions be followed if a respirometer is used. No further reference to respirometric methods will be made here. A timing device is needed as well as a 300-mL biological oxygen demand (BOD) bottle. A magnetic mixer with stirring bar is also required.

The procedure of Standard Method 2710 B should be followed with one exception. The total solids concentration instead of the volatile suspended solids concentration is used in the calculation of the SOUR. Total solids concentration is determined by Standard Method 2540 G. Method 2710 B cautions that if the suspended solids content of the sludge is greater than 0.5%, additional stirring besides that provided by the stirring bar be considered. Experiments by Farrell and Bhide (1993) were carried out with sludges up to 2% in solids content without difficulty if the SOUR was lower than about 3.0 mg O_2/g/h. It is possible to verify that mixing is adequate by running repeat measurements at several stirrer bar speeds. If stirring is adequate, oxygen uptake will be independent of stirrer speed.

The inert mineral solids in the wastewater in which the sludge particles are suspended do not exert an oxygen demand and properly should not be part of the total solids in the SOUR determination. Ordinarily, they are such a small part of the total solids that they can be ignored. If the ratio of inert dissolved mineral solids in the treated wastewater to the total solids in the sludge being tested is greater than 0.15, a correction should be made to the total solids concentration. Inert dissolved mineral solids in the treated wastewater effluent is determined by the method of Part 2540 B of Standard Methods (APHA, 1992). This quantity is subtracted from the total solids of the sludge to determine the total solids to be used in the SOUR calculation.

The collection of the sample and the time between sample collection and measurement of the SOUR are important. The sample should be a composite of grab samples taken within a period of a few minutes duration. The sample should be transported to the laboratory expeditiously and kept under aeration if the SOUR test cannot be run immediately. The sludge should be kept at the temperature of the digester from which it was drawn and aerated thoroughly before it is poured into the BOD bottle for the test. If the temperature differs from 20°C (68°F) by more than ±10°C (±18°F), the temperature correction may be inappropriate and the result should not be used to prove that the sewage sludge meets the SOUR requirement.

Variability in flow rates and nature of the sludge will result in variability in performance of the plant-scale digesters. It is advisable to run the SOUR test routinely so that sufficient data are available to indicate average performance. The arithmetic mean of successive tests—a minimum of seven over 2 or 3 weeks is suggested—should give a SOUR of ≤ 1.5 mg O_2/hr/g total solids.

3. Additional Digestion Test for Aerobically Digested Sewage Sludge

Background

Part 503 lists several options that can be used to demonstrate reduction of vector attraction in sewage sludge. These options include reduction of volatile solids by 38% and demonstration of the SOUR value discussed above (see also Chapter 6). These options are feasible for many, but not all, digested sludges. For example, sludges from extended aeration treatment works that are aerobically digested usually cannot meet this requirement because they already are partially reduced in volatile solids content by their exposure to long aeration times in the wastewater treatment process.

The specific oxygen uptake test can be utilized to evaluate aerobic sludges that do not meet the 38% volatile solids reduction requirement. Unfortunately, this test has a number of limitations. It cannot be applied if the sludges have been digested at temperatures lower than 10°C (50°F) or higher than 30°C (86°F). It has not been evaluated under all possible conditions of use, such as for sludges of more than 2% solids.

A straightforward approach for aerobically treated sludges that cannot meet either of the above criteria is to determine to what extent they can be digested further. If they show very little capacity for further digestion, they will have a low potential for additional biodegradation and odor generation that attracts vec-

tors. Such a test necessarily takes many days to complete, because time must be provided to get measurable biodegradation. Under most circumstances, this is not a serious drawback. If a digester must be evaluated every 4 months to see if the sewage sludge meets vector attraction reduction requirements, it will be necessary to start a regular assessment program. A record can be produced showing compliance. The sludge currently being produced cannot be evaluated quickly but it will be possible to show compliance over a period of time.

The additional digestion test for aerobically digested sludges in Part 503 is based on research by Jeris et al. (1985), and has been discussed by Farrell et al. (EPA, 1992). Farrell and Bhide (1993) explain in more detail the origin of the time and volatile solids reduction requirements of the test.

Jeris et al. (1985) demonstrated that several parameters—volatile solids reduction, COD, BOD_5, and SOUR—declined smoothly and approached asymptotic values with time as sludge was aerobically digested. Any one of these parameters potentially could be used as an index of vector attraction reduction for aerobic sludges. SOUR has been adopted (see above) for this purpose. Farrell and Bhide (1993) have shown that the additional volatile solids reduction that occurs when sludge is batch digested aerobically for 30 days correlates equally as well as SOUR with the degree of vector attraction reduction of the sludge. They recommend that a sewage sludge be accepted as suitably reduced in vector attraction when it shows less than 15% additional volatile solids reduction after 30 days additional batch digestion at 20°C (68°F). For three out of four sludges investigated by Jeris et al. (1985), the relationship between SOUR and additional volatile solids reduction showed that the SOUR was approximately equal to 1.5 mg O_2/hr/g (the Part 503 requirement for SOUR) when additional volatile solids reduction was 15%. The two requirements thus agree well with one another.

Recommended Procedure

There is considerable flexibility in selecting the size of the digesters used for the additional aerobic digestion test. Farrell and Bhide (1993) used a 20-liter fish tank. A tank of rectangular cross-section is suggested because sidewalls are easily accessible and are easily scraped clean of adhering solids. The tank should have a loose-fitting cover that allows air to escape. It is preferable to vent exhaust gas to a hood to avoid exposure to aerosols. Oil and particle-free air is supplied to the bottom of the digester through porous stones at a rate sufficient to thoroughly mix the sewage sludge. This will supply adequate oxygen to the sludge, but the oxygen level in the digesting sludge should be checked with a dissolved oxygen meter to be sure that the supply of oxygen is adequate. Oxygen level should be at least 2 mg/L. Mechanical mixers also were used to keep down foam and improve mixing.

If the total solids content of the sewage sludge is greater than 2%, the sludge must be diluted to 2% solids with secondary effluent at the start of the test. The requirement stems from the results of Reynolds (1973) and Malina (1966) which demonstrate that rate of volatile solids reduction decreases as the feed solids concentration increases. Thus, for example, a sludge with a 2% solids content that showed more than 15% volatile solids reduction when digested for 30 days might show a lower volatile solids reduction and would pass the test if it were at 4%. This dilution may cause a temporary change in rate of volatile solids reduction. However, the long duration of the test should provide adequate time for recovery and demonstration of the appropriate reduction in volatile solids content.

When sampling the sludge, care should be taken to keep the sludge aerobic and avoid unnecessary temperature shocks. The sludge is digested at 20°C (68°F) even if the digester was at some other temperature. It is expected that the bacterial population will suffer a temporary shock if there is a substantial temperature change, but the test is of sufficient duration to overcome this effect and show a normal volatile solids reduction. Even if the bacteria are shocked and do not recover completely, the test simulates what would happen to the sludge in the environment. If it passes the test, it is highly unlikely that the sludge will attract vectors when used or disposed to the environment. For example, if a sludge digested at 35°C (95°F) has not been adequately reduced in volatile solids and is shocked into biological inactivity for 30 days when its temperature is lowered to 20°C (68°F), it will be shocked in the same way if it is applied to the soil at ambient temperature. Consequently, it is unlikely to attract vectors.

The digester is charged with about 12 liters of the sewage sludge to be additionally digested, and aeration is commenced. The constant flow of air to the aerobic digestion test unit will cause a substantial loss of water from the digester. Water loss should be made up every day with distilled water. Solids that adhere to the walls above and below the water line should be scraped off and dispersed back into the sludge daily. The temperature of the digesting sludge should be approximately 20°C (68°F). If the temperature of the laboratory is maintained at about 22°C (72°F), evaporation of water from the digester will cool the sludge to about 20°C (68°F).

Sewage sludge is sampled every week for five successive weeks. Before sampling, makeup water is added (this will generally require that air is temporarily shut off to allow the water level to be established), and sludge is scraped off the walls and redistributed into the digester. The sludge in the digester is thoroughly mixed with a paddle before sampling, making sure to mix the bottom sludge with the top. The sample is comprised of several grab samples collected with a ladle while the digester is being mixed. The entire sampling procedure is duplicated to collect a second sample.

Total and volatile solids content of both samples are determined preferably by Standard Method 2540 G (APHA, 1992). Percent volatile solids is calculated from total and volatile solids content. Standard Methods (APHA, 1992) states that duplicates should agree within 5% of their average. If agreement is substantially poorer than this, the sampling and analysis should be repeated.

Calculation Details

Fraction volatile solids reduction is calculated by the Van Kleeck formula (see Appendix C) and by a mass balance

method. The mass balance (m.b.) equations become very simple, because final mass of sludge is made very nearly equal to initial mass of sludge by adjusting the volume by adding water. These equations for fractional volatile solids reduction (FVSR) and fractional fixed solids reduction (FFSR) are:

$$FVSR(m.b.) = (y_f - y_b) / y_f \quad (5a)$$

$$FFSR(m.b.) = (x_f - x_b) / x_f \quad (5b)$$

where:

y and x = mass fraction of volatile and fixed solids, respectively (see previous section on "Calculation details" for explanation of "mass fraction")

f and b = subscripts indicating initial and final sludges

This calculation assumes that initial and final sludge densities are the same. Very little error is introduced by this assumption.

The calculation of the fractional fixed solids reduction is not a requirement of the test, but it will provide useful information.

The test was developed from information based on the reduction in volatile solids content calculated by the Van Kleeck equation. As noted in the section on the additional anaerobic digestion test, for batch processes the material balance procedure for calculating volatile solids reduction is superior to the Van Kleeck approach. It is expected that the volatile solids reduction by the mass balance method will show a higher volatile solids reduction than the calculation made by using the Van Kleeck equation.

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Appendix E
Determination of Residence Time for Anaerobic and Aerobic Digestion

Introduction

The PSRP and PFRP specifications in 40 CFR 257 for anaerobic and aerobic digestion not only specify temperatures but also require minimum mean cell residence times of the sludge in the digesters. The mean cell residence time is the time that the sludge particles are retained in the digestion vessel under the conditions of the digestion. The calculation of residence time is ordinarily simple but it can become complicated under certain circumstances. This appendix describes how to make this calculation for most of the commonly encountered modes for operating digesters.

Approach

The discussion has to be divided into two parts: residence time for batch operation and for plug flow, and residence time for fully mixed digesters. For batch operation, residence time is obvious—it is the duration of the reaction. For plug flow, the liquid-solid mixture that is sludge passes through the reactor with no backward or forward mixing. The time it takes the sludge to pass through the reactor is the residence time. It is normally calculated by the following equation:

$$\theta = V/q \quad (1)$$

where
- θ = plug flow solids residence time
- V = volume of the liquid in the reactor
- q = volume of the liquid leaving the reactor

Normally the volume of liquid leaving the reactor will equal the volume entering. Conceivably, volume leaving could be smaller (e.g., because of evaporation losses) and residence time would be longer than expected if θ were based on inlet flow. Ordinarily, either inlet or outlet flow rate can be used.

For a fully mixed reactor, the individual particles of the sludge are retained for different time periods—some particles escape very soon after entry whereas others circulate in the reactor for long periods before escaping. The average time in the reactor is given by the relationship:

$$\theta_n = \frac{\Sigma (\delta s \times \theta)}{\Sigma (\delta s)} \quad (2)$$

where
- δs = an increment of sludge solids that leaves the reactor
- θ = time period this increment has been in the reactor
- θ_n = nominal average solids residence time

When the flow rates of sludge into and out of the completely mixed vessel are constant, it can be demonstrated that this equation reduces to:

$$\theta_n = \frac{VC_v}{qC_q} \quad (3)$$

where
- V = reactor volume
- q = flow rate leaving
- C_v = concentration of solids in the reactor
- C_q = concentration of solids in exiting sewage sludge

It is important to appreciate that q is the flow rate leaving the reactor. Some operators periodically shut down reactor agitation, allow a supernatant layer to form, decant the supernatant, and resume operation. Under these conditions, the flow rate entering the reactor is higher than the flow rate of sludge leaving.

Note that in Equation 3, VC_v is the mass of solids in the system and qC_q is the mass of solids leaving. Ordinarily C_v equals C_q and these terms could be canceled. They are left in the equation because they show the essential form of the residence time equation:

$$\theta_n = \frac{\text{mass of solids in the digester}}{\text{mass flow rate of solids leaving}} \quad (4)$$

Using this form, residence time for the important operating mode in which sludge leaving the digester is thickened and returned to the digester can be calculated.

In many aerobic digestion installations, digested sludge is thickened with part of the total volume returned to increase residence time and part removed as product. The calculation follows Equation 4 and is identical with the SRT (solids retention time) calculation used in activated sludge process calculations. The focus here is on the solids in the digester and the solids that ultimately leave the system. Applying Equation 4 for residence time then leads to Equation 5:

$$\theta_n = \frac{VC_v}{pC_p} \quad (5)$$

where
p = flow rate of processed sludge leaving the system
C_p = solids concentration in the processed sludge

The subscript p indicates the final product leaving the system, not the underflow from the thickener. This approach ignores any additional residence time in the thickener since this time is relatively short and not at proper digestion conditions.

Sample Calculations

In the following paragraphs, the equations and principles presented above are used to demonstrate the calculation of residence time for several commonly used digester operating modes:

Case 1

- Complete-mix reactor
- Constant feed and withdrawal at least once a day
- No substantial increase or decrease in volume in the reactor (V)
- One or more feed streams and a single product stream (q)

The residence time desired is the nominal residence time. Use Equation 3 as shown below:

$$\theta_n = \frac{VC_v}{qC_q} = \frac{V}{q}$$

The concentration terms in Equation 4 cancel out because C_v equals C_q.

Case 2a

- Complete-mix reactor
- Vessel contains a "heel" of liquid sludge (V_h) at the beginning of the digestion step
- Sludge is introduced in daily batches of volume (V_i) and solids concentration (C_i)
- When final volume (V_f) is reached, sludge is discharged until V_h remains and the process starts again

Some aerobic digesters are run in this fashion. This problem is a special case involving a batch reaction. Exactly how long each day's feeding remains in the reactor is known, but an average residence time must be calculated as shown in Equation 2:

$$\theta_n = \frac{\Sigma v_i C_i \times \text{time that batch}i \text{ remains in the reactor}}{\Sigma v_i C_i}$$

The following problem illustrates the calculation:

Let v_h = 30 m^3 (volume of "heel")
V_d = 130 m^3 (total digester volume)
v_i = each day 10 m^3 is fed to the reactor at the beginning of the day
C_i = 12 kg/m^3
V_f is reached in 10 days. Sludge is discharged at the end of Day 10.

$$\text{Then } \theta_n = \frac{(10 \cdot 12 \cdot 10 + 10 \cdot 12 \cdot 9 + \cdots + 10 \cdot 12 \cdot 1)}{(10 \cdot 12 + 10 \cdot 12 + \cdots + 10 \cdot 12)}$$

$$\theta_n = \frac{10 \cdot 12 \cdot 55}{10 \cdot 12 \cdot 10} = 5.5 \text{ days}$$

Notice that the volume of the digester or of the "heel" did not enter the calculation.

Case 2b

Same as Case 2a except:

- The solids content of the feed varies substantially from day to day
- Decantate is periodically removed so more sludge can be added to the digester

The following problem illustrates the calculation:

Let V_h = 30 m^3, and V_d = 130 m^3

Day	v_i (m^3)	Solids Content (kg/m^3)	Decantate (m^3)
1	10	10	0
2	10	15	0
3	10	20	0
4	10	15	0
5	10	15	0
6	10	10	0
7	10	20	0
8	10	25	0
9	10	15	10
10	10	10	0
11	10	15	10
12	10	20	0

$$\theta_n = (10 \cdot 10 \cdot 12 + 10 \cdot 15 \cdot 11 + 10 \cdot 20 \cdot 10 + \cdots + \cdots + 10 \cdot 10 \cdot 3 + 10 \cdot 15 \cdot 2 + 10 \cdot 20 \cdot 1)$$
$$(10 \cdot 10 + 10 \cdot 15 + 10 \cdot 20 + \cdots + 10 \cdot 10 + 10 \cdot 15 + 10 \cdot 20)$$

$$\theta_n = 11{,}950/1{,}900 + 6.29 \text{ d}$$

The volume of "heel" and sludge feedings equaled 150 m^3, exceeding the volume of the digester. This was made possible by decanting 20 m^3.

Case 3

Same as Case 2 except that after the digester is filled it is run in batch mode with no feed or withdrawals for several days.

A conservative θ_n can be calculated by simply adding the number of extra days of operation to the θ_n calculated for Case 2. The same applies to any other cases followed by batch mode operation.

Case 4

- Complete-mix reactor
- Constant feed and withdrawal at least once a day
- No substantial increase or decrease of volume in the reactor
- One or more feed streams, one decantate stream returned to the treatment works, one product stream; the decantate is removed from the digester so the sludge in the digester is higher in solids than the feed

This mode of operation is frequently used in both anaerobic and aerobic digestion in small treatment works.

Equation 3 is used to calculate the residence time:

Let V = 100 m^3
q_f = 10 m^3/d (feed stream)
C_f = 40 kg solids/m^3
q = 5 m^3/d (existing sludge stream)
C_v = 60 kg solids/m^3

$$\theta = \frac{100 \times 60}{5 \times 60} = 20 \text{ d}$$

Case 5

- Complete-mix reactor
- Constant feed and withdrawal at least once a day
- Volume in digester reasonably constant
- One or more feed streams, one product stream that is thickened, some sludge is recycled, and some is drawn off as product

This mode of operation is sometimes used in aerobic digesters. Equation 5 is used to calculate residence time.

Let V = 100 m^3
Feed flow rate = 10 m^3/d
Feed solids content = 10 kg/m^3
Flow rate from the digester = 12 m^3/d
Solids content of sludge from the digester = 13.3 kg/m^3
Flow rate of sludge from the thickener = 4 m^3/d
Solids content of sludge from the thickener = 40 kg/m^3
Flow rate of sludge returned to the digester = 2 m^3/d
Flow rate of product sludge = 2 m^3/d

$$\theta_n = \frac{100 \times 13.3}{2 \times 40} = 16.6 \text{ d}$$

The denominator is the product of the flow rate leaving the system (2 m^3/d) and the concentration of sludge leaving the thickener (40 kg/m^3). Notice that flow rate of sludge leaving the digester did not enter into the calculation.

Comments on Batch and Staged Operation

Sludge can be aerobically digested using a variety of process configurations (including continuously fed single- or multiple-stage completely mixed reactors), or it can be digested in a batch mode (batch operation may produce less volatile solids reduction for a primary sludge than the other options because there are lower numbers of aerobic microorganisms in it). Single-stage completely mixed reactors with continuous feed and withdrawal are the least effective of these options for bacterial and viral destruction, because organisms that have been exposed to the adverse condition of the digester for only a short time can leak through to the product sludge.

Probably the most practical alternative to use of a single completely mixed reactor for aerobic digestion is staged operation, such as use of two or more completely mixed digesters in series. The amount of slightly processed sludge passing from inlet to outlet would be greatly reduced compared to single-stage operation. If the kinetics of the reduction in pathogen densities are known, it is possible to estimate how much improvement can be made by staged operation.

Farrah et al. (1986) have shown that the declines in densities of enteric bacteria and viruses follow first-order kinetics. If first-order kinetics are assumed to be correct, it can be shown that a one-log reduction of organisms is achieved in half as much time in a two-stage reactor (equal volume in each stage) as in a one-stage reactor. Direct experimental verification of this prediction has not been carried out, but Lee et al. (1989) have qualitatively verified the effect.

It is reasonable to give credit for an improved operating mode. Since not all factors involved in the decay of microorganisms densities are known, some factor of safety should be introduced. It is recommended then that for staged operation using two stages of approximately equal volume, the time required be reduced to 70% of the time required for single-stage aerobic digestion in a continuously mixed reactor. This allows a 30% reduction in time instead of the 50% estimated from theoretical considerations. The same reduction is recommended for batch operation or for more than two stages in series. Thus, the time required would be reduced from 40 days at 20°C (68°F) to 28 days at 20°C (68°F), and from 60 days at 15°C (59°F) to 42 days at 15°C (59°F). These reduced times are also more than sufficient to achieve adequate vector attraction reduction.

If the plant operators desire, they may dispense with the PSRP time-temperature requirements of aerobic digestion but instead demonstrate experimentally that microbial levels in the product from their sludge digester are satisfactorily reduced. Under the current regulations, fecal coliform densities must be less than or equal to 2,000,000 CFU or MPN per gram total solids. Once this performance is demonstrated, the process would have to be operated between monitoring episodes at time-temperature conditions at least as severe as those used during their tests.

References

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Appendix F
Sample Preparation for Fecal Coliform Tests and *Salmonella* sp. Analysis

1. Sample Preparation for Fecal Coliform Tests

1.1 Class B Alternative 1

To demonstrate that a given domestic sewage sludge sample meets Class B pathogen requirements under Alternative 1, the density of fecal coliform from seven samples of treated sewage sludge must be determined and the geometric mean of the fecal coliform density must not exceed 2 million Colony Forming Units (CFU) or Most Probable Number (MPN) per gram of sewage sludge solids (dry weight basis). The solids content of treated domestic sludge can be highly variable. Therefore, an aliquot of each sample must be dried and the solids content determined in accordance with procedure 2540 G. of the 18th edition of Standard Methods for the Examination of Water and Wastewater (APHA, 1992), hereafter referred to as SM.

Sludge samples to be analyzed in accordance with SM 9221 E (Fecal Coliform MPN Procedure) and 9222 D (Fecal Coliform Membrane Filter Procedure) may require dilution prior to analysis. An ideal sample volume will yield results that accurately estimate the fecal coliform density of the sludge. Detection of fecal coliform in undiluted samples could easily exceed the detection limits of these procedures. Therefore, it is recommended that the following dilution scheme be used.

For Liquid Samples:

1. Use a sterile pipette to transfer 1.0 mL of well-mixed sample to 99 mL of sterile buffered dilution water (see SM Section 9050C) in a sterile screw cap bottle, and mix by vigorously shaking the bottle a minimum of 25 times. This is dilution "A." A volume of 1.0 mL of this mixture is 0.010 mL of the original sample.
2. Use a sterile pipette to transfer 1.0 mL of dilution "A" to a second screw cap bottle containing 99 mL of sterile buffered dilution water, and mix as before. This is dilution "B." A volume of 1.0 mL of this mixture is 0.00010 mL of the original sample. Use a sterile pipette to transfer 1.0 mL of dilution "B" to a sterile screw cap bottle containing 99 mL of sterile buffered dilution water, and mix as before. This is dilution "C." Go to Step 3 for MPN analysis or Step 5 for membrane filter (MF) analysis.
3. For MPN analysis, follow procedure 9221 E in SM. Four series of five tubes will be used for the analysis. Inoculate the first series of five tubes each with 10.0 mL of dilution "B." This is a 0.0010 dilution of the original sample. The second series of tubes should be inoculated with 1.0 mL of dilution "B" (0.00010). The third series of tubes should receive 10.0 mL of "C" (0.000010). Inoculate a fourth series of five tubes each with 1.0 mL of dilution "C" (0.0000010). Continue the procedure as described in SM.
4. Refer to Table 9221.IV in SM to estimate the MPN index/100 mL. Only three of the four series of five tubes will be used for estimating the MPN. Choose the highest dilution that gives positive results in all five tubes, and the next two higher dilutions for your estimate. Compute the MPN/g according to the following equation:

$$\text{MPN fecal coliform/g} = \frac{10 \times \text{MPN index/100 mL}}{\text{largest volume} \times \%\ \text{dry solids tested}}$$

Examples:

In the examples given below, the dilutions used to determine the MPN are underlined. The number in the numerator represents positive tubes; that in the denominator, the total number of tubes planted; the combination of positives simply represents the total number of positive tubes per dilution.

Example	0.0010 mL	0.00010 mL	0.000010 m	0.0000010 mL	Combination of Positives
a	5/5	5/5	3/5	0/5	5-3-0
b	5/5	3/5	1/5	0/5	5-3-1
c	0/5	1/5	0/5	0/5	0-1-0

For each example we will assume that the total solids content is 4.0%.

For Example a:

The MPN index/100 mL from Table 9221.4 is 80. Therefore:

$$\text{MPN/g} = \frac{10 \times 80}{0.00010 \times 4.0} = 2.0 \times 10^6$$

For Example b:

The MPN index/100 mL from Table 9221.4 is 110. Therefore:

$$\text{MPN/g} = \frac{10 \times 110}{0.0010 \times 4.0} = 2.8 \times 10^5$$

For Example c:

The MPN index/100 mL from Table 9221.4 is 2. Therefore:

$$\text{MPN/g} = \frac{10 \times 2}{0.0010 \times 4.0} = 5.0 \times 10^3$$

5. Alternately the membrane filter procedure may be used to determine fecal coliform density. Three individual filtrations should be conducted in accordance with SM 9222 D using 10.0 mL of dilution "C," and 1.0 mL and 10.0 mL of dilution "B." These represent 0.000010, 0.00010, and 0.0010 mL of the original sample. Incubate samples, and count colonies as directed. Experienced analysts are encouraged to modify this dilution scheme (e.g., half log dilutions) in order to obtain filters which yield between 20 and 60 CFU.
6. Compute the density of CFU from membrane filters that yield counts within the desired range of 20 to 60 fecal coliform colonies:

$$\text{coliform colonies/g} = \frac{\text{coliform colonies counted} \times 100}{\text{mL sample} \times \%\ \text{dry solids}}$$

For Solid Samples:

1. In a sterile dish weigh out 50.0 grams of well-mixed sample. Whenever possible, the sample tested should contain all materials which will be included in the sludge. For example, if wood chips are part of a sludge compost, some mixing or grinding means may be needed to achieve homogeneity before testing. One exception would be large pieces of wood which are not easily ground and may be discarded before blending. Transfer the sample to a sterile blender. Use 450 mL of sterile buffered dilution water to rinse any remaining sample into the blender. Cover and blend on high speed for 2 minutes. One milliliter of this sample contains O.10 g of the original sample.
2. Use a sterile pipette to transfer 11.0 mL of the blender contents to a screw cap bottle containing 99 mL of sterile buffered dilution water and shake vigorously a minimum of 25 times. One milliliter of this sample contains 0.010 g of the original sample. This is dilution "A."
3. Follow the procedures for fluid samples starting at Step 2.

Examples:

Seven samples of a treated sludge were obtained prior to land spreading. The solids concentration of each sample was determined according to SM. These were found to be:

Sample No.	Solids Concentration (%)
1	3.8
2	4.3
3	4.0
4	4.2
5	4.1
6	3.7
7	3.9

The samples were liquid with some solids. Therefore the procedure for liquid sample preparation was used. Furthermore, the membrane filter technique was used to determine if the fecal coliform concentration of the sludge would meet the criteria for Class B Alternative 1. Samples were prepared in accordance with the procedure outlined above. This yielded 21 individual membrane filters (MFs) plus controls. The results from these tests are shown in Table 1.

Table 1. Number of Fecal Coliform Colonies on MF Plates

Sample No.	0.000010 mL Filtration	0.00010 mL Filtration	0.0010 mL Filtration
1	0	1	23
2	2	18	TNTC
3	0	8	65
4	0	5	58
5	0	1	17
6	0	1	39
7	0	1	20

Whenever possible, the coliform density is calculated using only those MF plates that have between 20 and 60 blue colonies. However, there may be occasions when the total number of colonies on a plate will be above or below the ideal range. If the colonies are not discrete and appear to be growing together, results should be reported as "too numerous to count"(TNTC). If no filter has a coliform count falling in the ideal range (20 to 60), total the coliform counts on all countable filters and report as coliform colonies/g. For sample number 2 the fecal coliform density is:

$$\text{coliform colonies/g} = \frac{(2 + 18) \times 100}{(0.000010 + 0.00010) \times 4.3} = 4.2 \times 10^6$$

Sample number 3 has two filters that have colony counts outside the ideal range also. In this case, both countable plates should be used to calculate the coliform density/g. For sample number 3, the fecal coliform density is:

$$\text{coliform colonies/g} = \frac{(8 + 65) \times 100}{(0.00010 + 0.0010) \times 4.0} = 1.6 \times 10^6$$

Except for sample number 5, all the remaining samples have at least one membrane filter within the ideal range. For these samples, use the number of colonies formed on that filter to calculate the coliform density. For sample number 1, the fecal coliform density is:

$$\text{coliform colonies/g} = \frac{23 \times 100}{0.0010 \times 3.8} = 6.0 \times 10^5$$

Coliform densities of all the samples were calculated and converted to $\log_{10}$ values to compute a geometric mean. These calculated values are presented in Table 2.

Table 2. Coliform Density of Sludge Samples

Sample No.	Coliform Density	$\log_{10}$
1	6.0×10^5	5.78
2	4.2×10^6	6.62
3	1.6×10^6	6.20
4	9.0×10^5	6.14
5	4.0×10^5	5.60
6	1.0×10^6	6.02
7	5.1×10^5	5.71

The geometric mean for the seven samples is determined by averaging the $\log_{10}$ values of the coliform density and taking the antilog of that value:

$$(5.78 + 6.62 + 6.20 + 6.14 + 5.60 + 6.02 + 5.71) / 7 = 6.01$$

The antilog of $6.01 = 1.0 \times 10^6$

Therefore, the geometric mean fecal coliform density is below 2 million and the sludge meets Class B pathogen requirements under Alternative 1.

1.2 Class A Alternative 1

Part 503 requires that, to qualify as a Class A sludge, treated sewage sludge must be monitored for fecal coliform (or *Salmonella* sp.) and have a density of less than 1,000 MPN fecal coliform per gram of total solids (dry weight basis). The regulation does not specify total number of samples. However, it is suggested that a sampling event extend over 2 weeks and that at least seven samples be collected and analyzed. The membrane filter procedure may *not* be used for this determination. This is because the high concentration of solids in such sludges may plug the filter or, render the filter uncountable. The total solids content for each sample must be determined in accordance with procedure 2540 G of SM.

For Liquid Samples:

1. Follow procedure 9221 E in SM. Four series of five tubes will be used for the analysis. Use a sterile pipette to inoculate the first series of five tubes with 10.0 mL of well-mixed sample per tube (it may be convenient to use a sterile pipette with a large diameter opening capable of transferring sludge solids). The second series of tubes should receive 1.0 mL of well-mixed sample. Use a sterile pipette to transfer 1.0 mL of sample to 99 mL of sterile buffered dilution water (see SM Section 9050 C) in a sterile screw cap bottle, and mix by vigorously shaking the bottle a minimum of 25 times. This is dilution "A." Use a sterile pipette to inoculate the third series of tubes with 10.0 mL of dilution "A." The fourth series of tubes should be inoculated with 1.0 mL of dilution "A." Complete the procedure as described in SM.
2. Calculate the MPN as directed in Step 4 above.

For Solid Samples:

1. Using aseptic techniques, weigh out five portions of 10.0 grams each of well-mixed sample. For example, if wood chips are part of a sludge compost, some mixing or grinding means may be needed to achieve homogeneity before testing. One exception would be large pieces of wood that are not easily ground and may be discarded before blending. Transfer each portion to MPN tubes containing 10.0 mL of double-strength Lauryl Tryptose Broth (71.2 g/L). The second series of MPN tubes should contain 10.0 mL of single-strength Lauryl Tryptose Broth (35.6 g/L). Using aseptic technique, weigh out five portions of 1.0 g each of well-mixed sample and transfer each to individual tubes prepared for the second series of MPN tubes. Please note that some solids will not separate easily and/or may float. Since gas fermentation tubes are used for this procedure shaking is not practical. Therefore, it is recommended that a sterile loop be used to gently submerge solids in the broth. Prepare dilution "A" as described above under "Class B Alternative 1, For Solid Samples." Inoculate the third series of MPN tubes with 10.0 mL of dilution "A." The fourth series of five tubes must receive 1.0 mL of dilution "A" in each tube. Continue with the procedure in Section 9221 E of SM.
2. Calculate the MPN as directed in Step 4 above.

2. Sample Preparation for *Salmonella* Sp. Analysis

As an alternative to fecal coliform analysis, *Salmonella* sp. quantification may be used to demonstrate that a sludge meets Class A criteria. Sludges with *Salmonella* sp. densities below 3 MPN/4 g (dry weight basis) meet Class A criteria. The analytical method presented in Appendix G of this document, describes the procedure used to identify *Salmonella* sp. in a water sample. To use this MPN procedure for sewage sludges, the sample preparation step described here should be used, and the total solids content of each sample must be determined according to Method 2540 G in SM.

For Liquid Samples:

1. Follow the same procedure used for liquid sample preparation for fecal coliform analysis described under Section 1.2 above (Class A Alternative 1). However, the enrichment medium used for this analysis should be dulcitol selenite broth (DSE) as described in Appendix G, and only three series of five tubes should be used for this MPN procedure. Use a sterile open-tip pipette to transfer 10.0 mL of sample to each tube in the first series. These tubes should contain 10.0 mL of double-strength DSE broth. Each tube in the second series should contain 10.0 mL of single- strength DSE broth. These tubes should each receive 1.0 mL of sample. The final series of tubes should contain 10.0 mL

of double-strength DSE broth. These tubes should each receive 10.0 mL of dilution "A" as described above. Complete the MPN procedure as described in Appendix G.

2. Refer to Table 9221.IV in SM to estimate the MPN index/100 mL. Calculate the MPN/4 g according to the following equation:

$$\textit{Salmonella}\ \text{sp. MPN/ g} = \frac{\text{MPN index/100mL} \times 4}{\%\ \text{dry solids}}$$

Example:

If one tube in the first series was identified as being positive for *Salmonella* sp. and no other tubes were found to be positive, from Table 9221.IV one finds that a 1-0-0 combination of positives has an MPN index/100 mL of 2. If the mass of dry solids for the sample was 4.0%, then:

$$\textit{Salmonella}\ \text{sp. MPN/ g} = \frac{2 \times 4}{4.0} = 2$$

For Solid Samples:

1. Follow the procedure for solid sample preparation for fecal coliform analysis described under Section 1.2 (Class A Alternative 1) above. However, the enrichment medium used for this analysis should be dulcitol selenite broth (DSE) as described in Appendix G, and only three series of five tubes should be used for this MPN procedure. Use aseptic technique to weigh out and transfer 10.0 g of well-mixed sample to each screw cap tube in the first series, shake vigorously to mix. These tubes should contain 10.0 mL of double-strength DSE broth. Each tube in the second series should contain 10.0 mL of single-strength DSE broth. These tubes should receive 1.0 g of sample, mix as noted above. The final series of tubes should contain 10.0 mL of double-strength DSE broth. These tubes should receive 10.0 mL of dilution "A" as described above. Loosen caps before incubating the tubes. Complete the MPN procedure as described in Appendix G.

2. Refer to Table 9221.IV in SM to estimate the MPN index/100 mL. Calculate the MPN/4 g according to the following equation:

$$\textit{Salmonella}\ \text{sp. MPN/ g} = \frac{\text{MPN index/100mL} \times 4}{\%\ \text{dry solids}}$$

Appendix G
Kenner and Clark (1974) Analytical Method for *Salmonella* sp. Bacteria*

Detection and enumeration of Salmonella and Pseudomonas aeruginosa

BERNARD A. KENNER AND HAROLD P. CLARK

THE FEDERAL WATER POLLUTION CONTROL AMENDMENTS of 1972 [1-4] may well require the quantification and enumeration of pathogens such as *Salmonella* species in all classes of waters. The requirements are described by Shedroff.[5]

One of the continuing programs of the Environmental Protection Agency (EPA) is a research project concerned with the development of practical laboratory methods for the isolation, quantification, and enumeration of pathogens from polluted waters. This paper reports a monitoring method developed for the simultaneous isolation and enumeration of *Salmonella* species and *Pseudomonas aeruginosa* from potable waters, reuse waters, treatment plant effluents, receiving waters, and sludges.

The method described herein, and developed by Kenner,[6] is practical because readily available bacteriological media, chemicals, and equipment are all that are required to obtain the desired results. These results are the establishment of the absence or presence of *Salmonella* species (pathogenic hazardous bacteria) and/or *Pseudomonas aeruginosa* (potential pathogens) that affect persons who are in a debilitated condition and are very common as infectious agents in hospitals because of their resistance to antibiotic therapy.[7-9] Potable waters have also been shown to contain *Ps. aeruginosa*.[6, 10] The sources of these potential pathogens are human and animal feces and wastewaters.[11, 12]

When the monitoring method was used, it was found that 100 percent of municipal wastewaters and treatment plant sludges contained both of these potential pathogens. *Ps. aeruginosa* has been found in potable water supplies of large and small municipalities where insufficient residual chlorine is evident. Also important is the fact that these organisms may be found in the absence of fecal coliforms, whereas negative indicator tests may give a false sense of security. It is believed by the authors that these organisms may be better indicators than fecal coliforms of pollution in potable, direct reuse, bathing, and recreational waters.

MATERIALS AND METHODS

The monitoring method uses a multiple tube (MPN) procedure in which dulcitol selenite broth (DSE) [13] is used for primary enrichment medium, and is modified by the use of sodium acid selenite (BBL). The formula is proteose peptone (Bacto), 0.4 percent; yeast extract (Bacto), 0.15 percent; dulcitol, 0.4 percent; BBL, 0.5 percent; Na_2HPO_4, 0.125 percent; and KH_2PO_4, 0.125 percent in distilled water. The constituents are dissolved in a sterile flask, covered with foil, and heated to 88°C in a water bath to obtain a clear sterile medium that does not require adjustment of pH. Productivity for *Salmonella* species is enhanced by the addition of an 18-hr, 37°C culture of *Salmonella paratyphi A* (10 percent by volume) in single-strength DSE broth, killed by heating to 88°C.

Concentration of bacteria from large volumes of water is necessary when potable, direct reuse, receiving waters, and treatment effluents are being monitored.

TABLE I.—Retentive Characteristics of Several Glass Fiber Filter Papers* Compared with Membrane Filters

Filter	Total Bacteria† Filtered	Number Passing Filter	Percentage Retention
Millipore (MF) HAWG 047 HA 0.45 μ, white, grid, 47 mm, Millipore Filter Corp.	1,376	0	100
984H Ultra Glass Fiber Filter, 47 mm, Reeve Angel Corp.	1,229	25	98
GF/F Glass Paper Whatman,‡ 47 mm, Reeve Angel Corp.	2,698	6	99.8
GF/D Glass Paper Whatman,‡ 47 mm, Reeve Angel Corp.	2,622	2,166	17.4
934AH Glass Fiber Filter, 47 mm, Reeve Angel Corp.	1,049	198	81
GF/A Glass Paper Whatman, 47 mm, Reeve Angel Corp.	1,066	680	36

* The 984H Ultra Glass Fiber Filter is flexible when wet, readily allows filtration of large volumes of water, can readily be bent double with forceps, and, when placed into primary enrichment broth, disintegrates when tube is shaken and releases entrapped bacteria.

† Enteric bacteria, *E. coli*, 0.5 × 1–3 μ.

‡ A new paper filter GF/F has better retentive properties than the 984H, and has same properties (tested Oct. 1973).

Concentration is attained by filtration through glass fiber filters * in a membrane filter apparatus. After the desired volume of water is filtered through the ultra filter, the flexible filter is folded double with sterile forceps and inserted into a suitable volume of single-strength DSE medium contained in a test tube located in the first row of the multiple tube setup. The tube should then be shaken to cause the filter to disintegrate (Table I and Figure 1). To obtain MPN results per one l or per 10 l, 100 ml or 1,000 ml of sample, respectively, are filtered for each tube of DSE medium in the first row of the five-tube MPN setup. Additional dilutions are made by transferring material from tubes in the first row to tubes farther back in the setup.

Obtaining results on a per 1-gal (3.8-l) basis requires filtration of 380 ml, and on a per 10-gal (38-l) basis requires filtration of 3,800 ml for each tube in the first row. Where concentration of bacteria is not usually required, as in municipal wastewaters, sludges, or primary effluents, the regular transfer of 10 ml of sample to each tube in the first row of the setup into 10 ml of double-strength DSE is made, 1 ml of sample in 9 ml of single-strength DSE in the second row, and so on. The MPN table in "Standard Methods" [14] is used to read directly the results per volume of sample.

Incubation temperature of 40° ± 0.2°C for 1 and 2 days is critical to obtain optimum recovery of *Salmonella* sp. and *Pseudomonas aeruginosa* when DSE broth is used for primary enrichment. After primary incubation at 40°C, surface loopfuls (scum) (7 mm platinum or nichrome wire loop) are removed from each multiple-tube culture and streaked on each of two sections of a divided plate of Xylose lysine desoxycholate agar (XLD) [15] in order to isolate colonial growth. The numbered plates are inverted and incubated at 37°C for a period not to exceed 24 hr.

Commercial dehydrated XLD agars (BBL and Difco) are satisfactory if they are reconstituted in distilled water in sterile foil-covered flasks and heated to 88° or 92°C, respectively. The agar is then cooled to 55° to 60°C and distributed in sterile petri dishes. This laboratory prefers 10-ml portions in each section of a divided sterile disposable plastic dish (Figure 1).

* Reeve Angel 984H ultra glass fiber filter, 47 mm, Reeve Angel &. Co., Inc., Clifton, N. J. Mention of trade names does not constitute endorsement or recommendation by EPA.

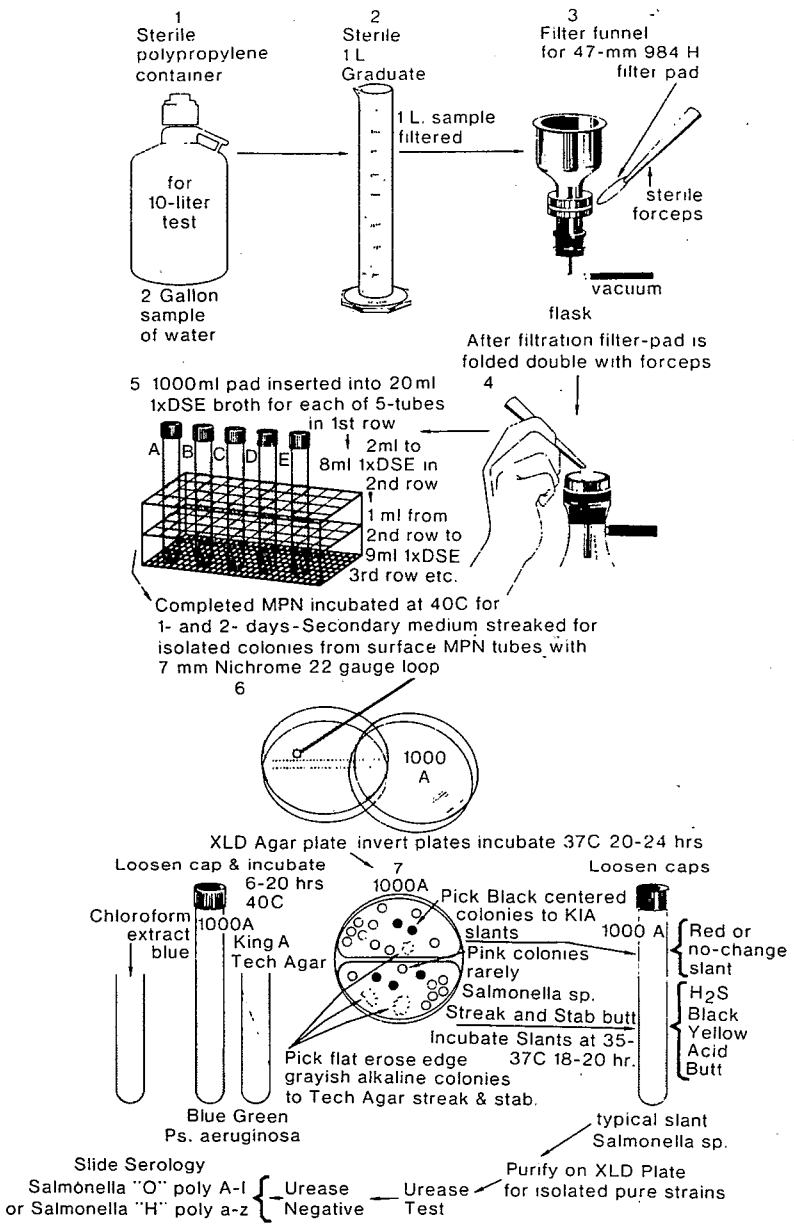

FIGURE 1.—Procedure for isolation of pathogens.

Positive incubated XLD plate cultures contain typical clear, pink-edged, black-centered *Salmonella* colonies, and flat, mucoid, grayish alkaline, pink erose-edged *Ps. aeruginosa*. The *Salmonella* colonies are picked to Kligler iron agar (KIA) or Triple sugar iron agar slants for typical appearance, purification, and identity tests. *Ps. aeruginosa* colonies are picked to King A agar slants (Tech agar BBL) for obtaining the bluegreen pyocyanin confirmation at 40°C (Figure 1).

Typically, *Salmonella* sp. slant cultures (streaked and stabbed), incubated over-

TABLE II.—Advantage of Ultra-filter 984H Use in Monitoring Suspected Waters for *Salmonella* species

Type of Sample	*Salmonella* (no./100 ml)	Serotypes Found (no./100 ml)	*Salmonella* (no./gal)	Serotypes Found (no./gal)
Stormwater runoff	4.5	*S. bareilly*[7]	210	*S. kottbus*[10] *S. bareilly*[11]
Stormwater runoff	<3.0	none	7.3	*S. java*[4] *S. muenchen*[2]
Activated sludge effluent	<3.0	none	3.6	*S.* group G[4]
Municipal wastewater	6.2	Arizona[3]	1,500	Arizona[4] *S. anatum*[2] *S. newport*[4] *S. san diego*[7] *S. worthington*[2]
Municipal wastewater	<3.0	none	110	*S. anatum*[3] *S. derby*[1] *S. newport*[3]
Activated sludge effluent	<3.0	none	28	*S. blockley*[7] *S. newport*[3]
Mississippi River water, mile 403.1	43	*S. ohio*[10]	>11,000	*S. ohio*[19] *S. derby*[2] *S. meleagridis*[6]
Municipal wastewater	3.0	*S. cholerasuis* var. kunzendorf[2]	21	*S. cholerasuis* var. kunzendorf[5] *S. newport*[6]

night at 37°C, give an unchanged or alkaline red-appearing slant; the butt is blackened by H_2S, is acid-yellow, and has gas bubbles, except for rare species. Typical-appearing slant cultures are purified by transferring them to XLD agar plates for the development of isolated colonies. The flat or umbonated-appearing colonies with large black centers and clear pink edges then are picked to KIA slants (streaked and stabbed), incubated at 37°C, and urease tested before the identification procedure (Figure 1). Urease-negative tubes are retained for presumptive serological tests and serotype identification.

Typical Tech agar slant cultures for *Ps. aeruginoca* that are incubated at 40°C overnight turn a bluegreen color from pyocyanin, a pigment produced only by this species. A reddish-blue color is caused by the additional presence of pyorubin. The blue pigment is extractable in chloroform and is light blue in color after a few hours at room temperature. No further tests are necessary. The count is read directly from the MPN table.

JUSTIFICATION FOR PROCEDURES

Choice of primary enrichment medium and secondary isolation agar. Most of the enrichment media described in contemporary literature were designed for the isolation of pathogens from clinical specimens from ill persons or from samples of suspected foods, and they work quite well for those types of samples. When they are used, however, for the isolation of pathogens from polluted waters and other types of environmental samples, such as soils, they do not prove adequate. Enrichment media that were tested and found wanting in regard to detection and selectivity were tetrathionate broth (TT), with and without brilliant green at 41.5°C; selenite cystine broth at 37°C; selenite F broth at 37°C; selenite brilliant green, with and without sulfa, at 37° and 41.5°C; and Gram-negative broth (GN) at 40° and 41.5°C.

None of the media named worked well at 37°C for the isolation of *Salmonella* sp., and isolation from wastewaters only oc-

TABLE III.—Percentage of Colony Picks from DSE-XLD Combination Positive for *Salmonella* species

Liquid Samples	No.	Total Picks from XLD	No. Positive	No. Negative	Percentage Positive	Range of *Salmonella* counts/100 ml
Municipal wastewater	15	315	250	65	79	3.0–1,500
Stockyard wastewater	1	36	36	0	100	2,100
Rivers						
Mississippi						
Ohio	8	110	84	26	76	1.5–>300
Stormwater runoffs	2	18	14	4	78	0.2–1.5
Activated sludge biological effluent	20	386	306	80	79	0.1–1,100
Trickling filter effluent	7	103	78	25	76	0.35–140
Package plant effluent	6	83	55	28	66	1.8–620
Package plant sludge	2	41	37	4	90	43–240
Chlorinated primary outfall	2	17	13	4	76	3–43
Creek 1 mile (1.6 km) below package plant outfall	2	37	16	21	43	4.5–12
Home cisterns	2	17	10	7	59	0.26–1.1
Dupont R-O						
Feed	1	20	14	6	70	4.3
Reject	1	16	8	8	50	0.91
Product-negative						
Raw primary sludge	4	80	66	14	83	13–700
Primary activated sludge	1	15	13	2	87	23
Anaerobic digester sludge	3	78	65	13	83	79–170
Anaerobic digester sludge (28 days)	1	9	3	6	33	2
Activated secondary sludge	6	189	155	34	82	11–>11,000
Total	84	1,570	1,223	347	average 78	

curred by chance and was purely qualitative. Of the above-named media used in preliminary tests, selenite brilliant green sulfa broth (SBGS) at 41.5°C gave the best isolation of *Salmonella* sp. from wastewaters (with and without the addition of *S. typhimurium* in known numbers). Of thirteen wastewater samples tested in SBGS at 41.5°C, six contained *Salmonella* or 46 percent were positive. With DSE broth at 40°C, 28 of 28, or 100 percent of wastewater samples, gave positive results.

Studies were not continued on SBGS medium when it was noted that some lots of commercially available SBGS seemed to be selective for *Salmonella* sp. while others were not. The medium was then prepared according to the original formula [16] with six different lots of brilliant green (certified), only one of which was selective. The use of brilliant green agar as a selective medium is subject to the same variability, according to Read and Reyes.[17]

The main reasons for rejection of TT, with and without brilliant green, and for selenite broth's using brilliant green agar and XLD agar as secondary media are not only fewer isolations of *Salmonella* sp., but also the poor selectivity of these combinations when they are used for monitoring polluted waters. These combinations' poor selectivity at 41.5°C is apparent in the results of Dutka and Bell,[18] where the TT broth-XLD combination yielded 26 percent confirmation of colonial picks, and selenite broth-BGA and selenite broth-XLD gave 55 and 56 percent confirmations, respectively. The authors had similar results. The GN-XLD combination was poorest for water samples at 40° and 41.5°C, yielding less than 10 percent isolations from wastewaters.

Effect of incubation temperature on isolation of *Salmonella* sp. In a study of 26 wastewater samples that was conducted with the DSE multiple tube setups at three

TABLE IV.—Serotypes of *Salmonella* Found in Polluted Waters

Serotype	No. of Strains	Rank in Water Isolations	Rank in Human Occurrence*
1 *typhimurium**	375	1	1 and 6†
2 *derby*	287	2	12
3 *cubana*	223	3	20
4 *chester*	203	4	19
5 *newport*	188	5	4
6 *kottbus*	158	6	—‡
7 *blockley*	157	7	11
8 *infantis*	141	8	9
9 *enteritidis*	128	9	5
10 *anatum*	127	10	13
11 *heidelberg*	110	11	6
12 *manhattan*	97	12	14
13 *paratyphi* B	91	13	17
14 *illinois*	77	14	—‡
15 *thompson*	63	15	3
16 *livingstone*	52	16	—‡
17 *montevideo*	47	17	16
18 *muenchen*	45	18	18
19 *oranienberg*	44	19	15
20 *san diego*	44	19	18
21 *bareilly*	42	20	23
22 *tshiongwe*	41	21	—
23 *orion*	41	21	—‡
24 *senftenberg*	39	22	25
25 *schwarzengrund*	37	23	—‡
26 *lexington*	33	24	—‡
27 *cholerasuis*	30	25	—‡
28 *binza*	29	26	24
29 *cholerasuis* var. kunzendorf	29	26	—‡
Other serotypes:			
30 *albany*	20	31	—‡
31 *benfica*	10	40	—‡
32 *braenderup*	13	38	—‡
33 *brancaster*	1		—‡
34 *bredeney*	8		23
35 *california*	2		—‡
36 *drypool*	14	37	—‡
37 *friedenau*	4		—
38 *give*	25	29	22
39 *grumpensis*	10	40	—‡
40 *haifa*	2		—‡
41 *hartford*	2		—‡
42 *havana*	16	35	—‡
43 *indiana*	10	40	—‡
44 *java*	15	36	7
45 *javiana*	13	38	8
46 *litchfield*	17	34	21
47 *lomita*	26	28	—‡
48 *meleagridis*	18	33	25
49 *mission*	14	37	—‡
50 *newington*	2		—‡
51 *newlands*	8		—‡
52 *norwich*	14	37	—‡
53 *ohio*	19	32	—‡
54 *preston*	2		—
55 *reading*	26	28	—‡
56 *rubislaw*	15	36	—‡
57 *saint paul*	21	30	10
58 *schleissheim*	12	39	—
59 *simsbury*	9		—‡
60 *taksony*	16	35	—‡
61 *tennessee*	12	39	20
62 *typhi-suis* var. voldagsen	2		—
63 *uzaramo*	28	27	—‡
64 *weil*	3		—
65 *worthington*	10	40	24
Sub total	3,417		
Arizona	151		
Incomplete serology	232		
Total	3,800		

* Rank in human occurrence Table I, Martin and Ewing.[19]

† Separation of *S. typhimurium* and var. copenhagen not done after initial identifications.

‡ Serotypes occurring in humans, 1965–1971, Center for Disease Control, Salmonella Surveillance, Annual Summary 1971, Table IX, U. S. DHEW, PHS DHEW Publ. No. (HSM) 73-8184 (Oct. 1972).

different temperatures, it was found that 100 percent of the samples contained *Salmonella* sp. and *Ps. aeruginosa* at 40°C. At 41.5°C, however, only 50 percent or 13 of the samples yielded *Salmonella* sp., and at 37°C only 8 percent or 2 of the samples yielded *Salmonella* sp.

Effect of enhancement of DSE broth with a killed culture of *S. paratyphi* A. In a study of 84 samples of activated sludge effluents, trickling filter effluents, package plant effluents, and stream waters, DSE broth enhanced with a killed culture of *S. paratyphi A* in DSE broth (10 percent by volume) yielded isolations in 64 samples or 74 percent isolated *Salmonella* sp., compared with 48 samples or 57 percent isolations when the DSE broth was used without enhancement. An improved isolation of 17 percent was achieved with enhanced DSE broth.

Ultra-filter. The advantages of ultra-filter use in testing water samples are illustrated in Table II.

RESULTS AND DISCUSSION

Of importance to those who must use bacteriological tests to obtain *Salmonella* sp. and *Ps. aeruginosa* counts from waters is the amount of work that must be done to secure accurate results. Table III presents the percentage of colony picks made with the described method that proved to be *Salmonella* sp. If there are black-centered colonies on the XLD plates, more than 75 percent of the picks will prove to be *Salmonella* sp.; thus, the method leads to less unproductive work. When other methods were used, the authors have at times had to pick 50 black-centered colonies to obtain only 5 *Salmonella* sp. strains. This type of unproductive work has given the search for pathogens in the environment an undeserved bad reputation, and it has caused some to give up.

In Table II it may readily be seen that in many cases the fault with many tests has been the testing of an insufficient volume of sample. Many people think that it involves too much work, and that only expensive fluorescent antibody techniques will work. The problem is, however, to

TABLE V.—Percentage of Various Types of Water Samples Positive for *Salmonella* species

Type of Sample	Number of Samples	Number Positive	Number Negative	Percentage Positives
Municipal wastewaters	28	28	0	100.0
Municipal primary effluents (chlorinated)	9	5	4	56.0
Activated sludge effluents (clarified)	40	29	11	72.5
Activated sludge effluents				
Before chlorination	5	4	1	80.0
After chlorination, 1.4–2.0 mg/l residual, 5 min contact	8	0	8	0.0
Trickling filter effluents	26	15	11	57.7
Package plant effluents	15	7	8	46.7
Creek 1 mile (1.6 km) below package plant	3	3	0	100.0
Ohio River above Cincinnati public landing	20	9	11	45.0
Wabash River	4	3	1	75.0
Mississippi River	4	3	1*	75.0
Streams collective	31	18	13	58.0
Stormwater runoff after heavy rain	6	3†	3‡	50.0
Farm wells	4	0	4	0.0
Home cisterns suburban	5	2	3	40.0
Septic tank sludges	6	3	3	50.0
Totals	183	114	69	

* Municipal intake.
† Positive by per-gallon technique.
‡ Negative by per-100 ml technique.

concentrate the bacteria in a 10-gal (38-l) sample or a 100-gal (380-l) sample of potable or reuse water to obtain results, and still not require even more expensive filtration or centrifugation equipment. It also seems unrealistic to test only extremely small samples of the water being examined, because they may not be representative.

Table IV contains a list of *Salmonella* serotypes isolated from polluted waters and ranked according to the frequency of serotype isolations. It will be noted that all of the serotypes except S. *typhi* were isolated from environmental samples by the monitoring method, and that only 6 of the 65 serotypes reported were not reported as occurring in humans in the U. S. over the period from 1965 to 1971.

Table V summarizes the percentage of various types of water samples positive for *Salmonella* sp. Of interest is the fact that 100 percent of the municipal wastewaters tested contain *Salmonella* sp., that 56 percent of chlorinated primary effluents tested contain the pathogens, and that 100 percent of chlorinated secondary effluents are negative for pathogens. There are more studies scheduled for testing of secondary and tertiary effluents to obtain minimal chlorine residuals. Calabro *et al.*[20] reported that more than 50 attempts at isolating *Salmonella* sp. from septic tank samples using SBGS-BGSA combinations were unsuccessful.

Table VI summarizes the isolation of *Ps. aeruginosa* from potable water supply, that is, wells, cisterns, and small municipal water supply. It should be noted that fecal coliforms were not detected in most of these samples. Fecal streptococci counts were higher than fecal coliform counts where both tests were used. *Ps. aeruginosa* were present in all but three of the tests, and *Salmonella* sp. were isolated from two different cistern samples.

It is of importance to the user of pathogen tests that the test be quantitative. In initial studies on the DSE-XLD combination, it was important to know if the enrichment broth would support the growth of a wide

TABLE VI.—Isolation of *Pseudomonas aeruginosa* from Potable Water Supply

Type of Sample	*Ps. aeruginosa* Isolation	Indicators/100 ml		
		Total Coliforms	Fecal Coliforms	Fecal Streptococci
Well 8/16/71	+	4	—	—
Well 8/25/71	+	22	—	—
Well 3/27/72	+	—	<1	—
Well 3/27/72 (chlorinated)	+	—	<1	—
Well 8/23/72	+	—	0.25	62
Well 10/ 4/72	+	—	<2	46
Suburban cisterns				
8/ 4/72	+	—	180	—
10/ 9/72*	+	—	15	156
11/ 6/72*	+	—	<2	22
11/ 6/72	+	—	<2	2
11/26/72	+	—	3	28
Municipal supplies				
Population served 54,700				
3/17/71	+	—	<1	—
6/21/71	+	—	<1	—
7/19/71	+	—	<1	—
6/19/72	+	—	0.26	—
10/ 9/72	+	—	<1	<1
5/ 8/72	0	—	<1	—
Population served 14,000				
5/ 8/72	0	—	<1	—
10/24/72	+	—	<1	18
Population served <10,000				
11/27/72	0	—	<1	<1

* *Salmonella* sp. also present in samples.

range of *Salmonella* serotypes. Laboratory cultures of *S. paratyphi A, S. typhimurium, S. bredeney, S. oranienberg, S. pullorum, S. anatum, S. give,* and *S. worthington* were tested in three enrichment broths. The time required to isolate each of the above cultures from an estimated 10 to 20 organisms/100 ml in buffer water was 48 to 72 hr for *S. paratyphi A* in TT broth, 24 hr for DSE broth, and 36 to 48 hr for SBGS broth. The rest of the cultures were isolated in estimated numbers in 14 to 24 hr in TT and DSE broths. In SBGS broth, *S. typhimurium, S. bredeney, S. anatum, S. give,* and *S. worthington* required 36 to 48 hr incubation, and *S. pullorum* and *S. oranienberg* required 48 to 72 hr incubation.

It is impossible to know if 100 percent of *Salmonella* sp. in a polluted water sample are isolated. In tests where laboratory cultures have been added in low numbers to wastewater and treatment effluent samples, all of the numbers added were detected, as well as the *Salmonella* sp. that were naturally occurring. The higher the quality of the water (for example, secondary or tertiary treatment effluent, or even potable waters), the better the possibility of isolation of all the *Salmonella* serotypes present, as well as *Ps. aeruginosa,* a potential pathogen.

SUMMARY

A practical laboratory method is presented for the simultaneous isolation and enumeration of *Salmonella* sp. and *Pseudomonas aeruginosa* from all classes of waters, including potable water supplies, with a minimum of interfering false positive isolations. The method allows for the testing

of large volumes of high quality waters, wherein the absence of indicator bacteria (that is, total coliforms, fecal coliforms, and fecal streptococci), may give a false sense of security because of the low volumes of water usually tested. Justification for each step of the procedural method is presented.

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Appendix H
Method for the Recovery and Assay of Enteroviruses from Sewage Sludge

I. INTRODUCTION

The Class A requirements of 40 CFR Part 503 can be met on the basis of several alternatives which specify the final densities of pathogenic organisms after a pathogen reduction process or in sewage sludge at the time of distribution. Where required under Part 503 (see Chapter 4), human enteric viruses (i.e., viruses that are transmitted via the fecal-oral route) must be less than 1 plaque-forming unit (PFU) per 4 g of total dry solids. The method required to demonstrate this virus density is described below.

Chapters 7 and 8 of this document describe the quantitative and sampling criteria. In some cases the collection of four or more composite samples is recommended. A composite sample should be prepared by collecting 10 representative samples of 60 mL each (600 mL total) from different locations of a batch sludge pile or on different days over a period of several weeks when testing a process sludge sample. Batch samples that cannot be assayed within 8 hours of collection must be frozen; otherwise, they should be held at 4°C until processed. Just prior to assay, frozen samples for each composite are thawed, combined, and mixed thoroughly. A 50 mL portion is removed from each sample for solids determination as described in Section II below. The remaining portion is held at 4°C while the solids determination is being performed, or frozen for later processing if the assay cannot be initiated within 8 hours.

The "enteric viruses" specified for testing in the Part 503 regulation consist of more than 100 virus types, with hepatitis A virus and Norwalk virus being the primary human viral pathogens of concern. At the present time, however, standard methods for isolating and detecting these and many other enteric viruses have not been developed. The method detailed in Section III below has been tested and approved for isolating the enterovirus group (e.g., polioviruses, coxsackieviruses, echoviruses) of enteric viruses.[1] This method and the standard procedures for virus quantitation which follow it should be rigorously followed. Enteroviruses are assayed using a BGM cell line (Section IV) and the plaque assay technique (Section V). Occasionally, components isolated from sludges along with enterovirus may show cytotoxic effects on BGM cells, leading to false negative results. Samples showing cytotoxicity should be reassayed using the method given in Section VI. Cytotoxic and other components of sludge can also produce false positive results, requiring that all potential viral isolates be confirmed as infectious virus. A method to confirm virus isolates is given in Section VII.

Aseptic techniques and sterile materials and apparatus are to be used throughout all sections of the virus procedure described below. Virus-contaminated materials must be sterilized by autoclaving at 121°C for 15 minutes before discarding.

II. DETERMINATION OF TOTAL DRY SOLIDS[2]

1. Weigh a dry weighing pan that has been held in a desiccator and is at a constant weight. Place the 50 mL sludge portion for solids determination into the pan and weigh again.

2. Place the pan and its contents into an oven maintained at 103-105°C for at least 1 hour.

3. Cool the sample to room temperature in a desiccator and weigh again.

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[1]Method D4994-89, ASTM (1992).

[2]Modified from EPA/600/4-84/013(R7), September 1989 Revision (Section 3). This and other cited EPA publications may be requested from the Virology Branch, Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268.

4. Repeat the drying (1 hour each), cooling, and weighing steps until the loss in weight is no more than 4% of the previous weight.

5. Calculate the fraction of total dry solids (T) using the formula: T = (A-C)/(B-C), where A is the weight of the sample and dish after drying, B is the weight of the sample and dish before drying, and C is the weight of the dish. Record the fraction of dry solids (T) as a decimal (e.g., 0.04).

III. ENTEROVIRUS RECOVERY FROM SLUDGES[3]

1. INTRODUCTION

Enteroviruses in sludge will primarily be associated with solids. Although the fraction of virus associated with the liquid portion will usually be small, this fraction may vary considerably with different sludge types. To correct for this variation, samples will first be treated to bind free virus to solids. Virus is then eluted from the solids and concentrated prior to assay.

The procedures in this section require dispensing the entire sample volume into a centrifuge bottle. If bottles of sufficient capacity are unavailable, the sample should be divided and then recombined after centrifugation.

2. CONDITIONING OF SUSPENDED SOLIDS

Conditioning of sludges binds unadsorbed enteroviruses present in the liquid matrix to the sludge solids.

2.1 Preparation

2.1.1 Apparatus and materials

(a) Refrigerated centrifuge capable of attaining 10,000 x *g* and screw-capped centrifuge bottles with 100 to 1,000 mL capacity.
Each bottle must be rated for the relevant centrifugal force.

(b) A pH meter with an accuracy of at least 0.1 pH unit, equipped with a combination-type electrode.

(c) Magnetic stirrer and stir bars.

2.1.2 Media and reagents

Analytical reagent or ACS grade chemicals (unless specified otherwise) and deionized, distilled water (ddH_2O) should be used to prepare all reagents. All water used must have a resistance of greater than 0.5 megohms-cm, but water with a resistance of 18 megohms-cm is preferred.

(a) Hydrochloric acid (HCl) — 1 and 5 M.
Mix 10 or 50 mL of concentrated HCl with 90 or 50 mL of ddH_2O, respectively.

(b) Aluminum chloride ($AlCl_3 \cdot 6H_2O$) — 0.05 M.
Dissolve 12.07 g of aluminum chloride in a final volume of 1,000 mL of ddH_2O. Autoclave at 121°C for 15 minutes.

(c) Sodium hydroxide (NaOH) — 1 and 5 M.
Dissolve 4 or 20 g of sodium hydroxide in a final volume of 100 mL of ddH_2O, respectively.

[3]Modified from EPA/600/4-84/013(R7), September 1989 Revision.

2.2 Conditioning procedure — Each analyzed composite sample (from the portion remaining after solids determination) must have an initial total dry solids content of at least 12 g. This amount is necessary to allow storage of one half of the sample at -70°C as a backup in case of procedural mistakes or sample cytotoxicity (see Section VI) and to use a portion of each sample for a positive control.

Figure 1 gives a flow diagram for the procedure to condition suspended solids.

Calculate the amount of sample needed from the formula: X = 12/T, where X = the milliliters of sample required to obtain 12 g, and T = the fraction of total dry solids (from Section II).[4] *Use a graduated cylinder to measure the volume. If X is not a multiple of 100 mL (100, 200, 300 mL, etc.), sterile water should be added to bring the volume to the next multiple of 100 mL. Each sample should then be aliquoted into 100 mL portions before proceeding. Samples must be mixed vigorously just before aliquoting because solids begin to settle out as soon as the mixing stops. Each aliquot should be placed into a 250 mL beaker containing a stir bar.*

CAUTION: Always avoid the formation of aerosols by slowly pouring samples down the sides of vessels.

2.2.1 The following steps must be performed on each 100 mL aliquot.

2.2.2 Place the beaker on a magnetic stirrer, cover loosely with aluminum foil, and stir at a speed sufficient to develop a vortex. Add 1 mL of 0.05 M $AlCl_3$ to the mixing aliquot.

The final concentration of $AlCl_3$ in each aliquot is approximately 0.0005 M.

2.2.3 Place a combination-type pH electrode into the mixing aliquot. Adjust the pH of the aliquot to 3.5 ± 0.1 with 5 M HCl. Continue mixing for 30 minutes.

The pH meter must be standardized at pH 7 and 4. When solids adhere to electrodes, clean electrodes by moving them up and down gently in the mixing aliquot.

The pH of the aliquot should be checked at frequent intervals. If the pH drifts up, readjust it to 3.5 ± 0.1 with 5 M HCl. If the pH drifts down, readjust it with 5 M NaOH. Use 1 M acid or base for small adjustments. Do not allow the pH to drop below 3.4.

2.2.4 Pour the conditioned aliquot into a centrifuge bottle and centrifuge at 2,500 x *g* for 15 minutes at 4°C.

To prevent the transfer of the stir bar into the centrifuge bottle when decanting the aliquot, hold another stir bar or magnet against the bottom of the beaker. Solids that adhere to the stir bar in the beaker may be removed by manipulation with a pipette. It may be necessary to pour the aliquot back and forth several times from the centrifuge bottle to the beaker to obtain all the solids in the bottle.

2.2.5 Decant the supernatant into a beaker and discard. Replace the cap onto the centrifuge bottle. Elute viruses from the solids by following the procedure described below under "Elution of Viruses from Solids."

3. ELUTION OF VIRUSES FROM SOLIDS

3.1 Apparatus and materials

In this and following sections only apparatus and materials which have not been described in previous sections are listed.

3.1.1 Membrane filter apparatus for sterilization — 47 mm diameter Swinnex filter holder and 50 mL slip-tip syringe (Millipore Corp., product no. SX00 047 00, and Becton Dickinson, product no. 1627, or equivalent for filter holder only).

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[4]This formula is based upon a reliable assumption that the density of the liquid in sludge is 1 g/mL. Only 550 mL of the original sample is available for analysis at this point. If the fraction of total dry solids is below 0.022, 550 mL will not be sufficient and the amount of initial sample collected will have to be increased to equal the volume of X + 50 mL.

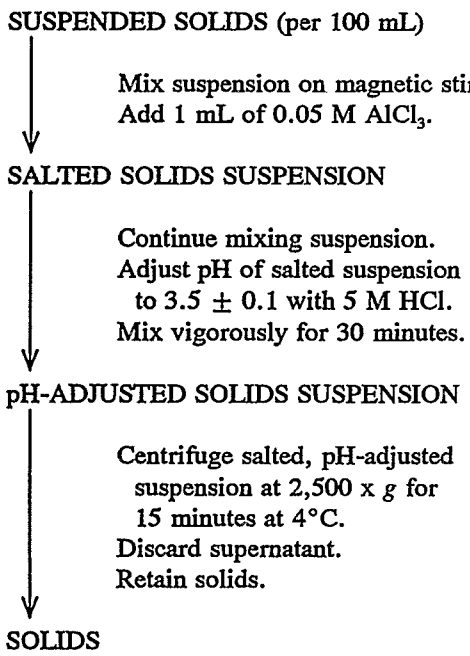

Figure 1. Flow Diagram of Method for Conditioning Suspended Solids

3.1.2 Disc filters, 47 mm diameter — 3.0, 0.45, and 0.2 μm pore size filters (Mentec America, Filterite Div., Duo-Fine series (product nos. 8025-030, 8025-034, and 8025-037, or equivalent). Filters may be cut to the proper diameter from sheet filters.

Disassemble a Swinnex filter holder. Place the filter with a 0.25 μm pore size on the support screen of the filter holder and stack the remaining filters on top in order of increasing pore size. Reassemble and tighten filter holder. Wrap filter stack in foil and sterilize by autoclaving at 121°C for 15 min.

Filters stacked in tandem as described tend to clog more slowly when turbid material is filtered through them. Prepare several filter stacks.

3.2 Media and Reagents

In this and following sections only media and reagents which have not been described in previous sections are listed.

3.2.1 Beef extract powder (BBL Microbiology Systems, product no. 12303, or equivalent).

Prepare buffered 10% beef extract by dissolving 10 g beef extract powder, 1.34 g $Na_2HPO_4 \cdot 7H_2O$ and 0.12 g citric acid in 100 mL of ddH_2O. The pH should be about 7.0. Dissolve by stirring on a magnetic stirrer. Autoclave for 15 minutes at 121°C.

Do not use paste beef extract (Difco Laboratories, product no. 0126) or beef extract V powder (BBL Microbiology Systems, product no. 97531) for virus elution. These beef extracts may elute cytotoxic materials from sludges.

3.3 Elution Procedure

A flow diagram of the virus elution procedure is given in Figure 2.

3.3.1 Place a stir bar and 100 mL of buffered 10% beef extract into the centrifuge bottle containing the solids (from Subsection 2.2.5). If more than one aliquot per sludge sample was processed, the solids should be combined at this step.

3.3.2 Place the centrifuge bottle on a magnetic stirrer, and stir at a speed sufficient to develop a vortex for 30 minutes at room temperature.

To minimize foaming (which may inactivate viruses), do not mix faster than necessary to develop a vortex.

3.3.3 Remove the stir bar from the bottle with a long forceps or a magnet retriever and centrifuge the solids-eluate mixture at 10,000 x *g* for 30 minutes at 4°C. Decant supernatant fluid (eluate) into a beaker and discard the solids.

Determine if the centrifuge bottle is appropriate for the centrifugal force that will be applied.

Centrifugation at 10,000 x g is normally required to clarify the sludge samples sufficiently to force the resulting supernatant through the filter stacks. It may be possible to use 2,500 x g for some samples.

3.3.4 Place a filter holder that contains a filter stack (from Subsection 3.1.2) onto a 250 mL Erlenmeyer receiving flask. Load a 50 mL syringe with supernatant from Step 3.3.3. Place the tip of the syringe into the filter holder and force the supernatant through the filter stack into a 250 mL receiving flask.

Take care not to break off the tip of the syringe and to minimize pressure on the receiving flask because such pressure may crack or topple the flask. If the filter stack begins to clog badly, empty the loaded syringe into the beaker containing unfiltered eluate, fill the syringe with air, and inject air into the filter stack to force residual eluate from the filters. Continue the filtration procedure with another filter holder and filter stack. Discard contaminated filter holders and filter stacks. Step 3.3.4 may be repeated as often as necessary to filter the entire volume of supernatant. Disassemble each filter holder and examine the bottom 0.25 µm filters to be certain they have not ruptured. If a bottom filter has ruptured, repeat Steps 3.3.4 with new filter holders and filter stacks.

Proceed immediately to Subsection 4.

4. CONCENTRATION OF VIRUSES FROM ELUATES BY ORGANIC FLOCCULATION

This organic flocculation concentration procedure (Katzenelson et al., 1976) is used to reduce the number of cell cultures needed for assays by concentrating enteroviruses in the eluate. Although the overall virus recovery may be diminished by this step, organic flocculation will result in considerable cost reductions in labor and materials.

Floc formation capacity of the powdered beef extract reagent must be pretested. Some powdered beef extracts may not produce sufficient floc, resulting in significantly reduced virus recoveries. Beef extract reagents may be pretested by spiking 100 mL of ddH_2O with a known amount of virus in the presence of a 47 mm nitrocellulose filter. This sample should be conditioned using Subsection 2 above to bind virus to the filter. Virus should then be eluted from the filter using the procedure in Subsection 3, and concentrated using the following procedure. Any lot of beef extract not giving a overall recovery of at least 50% should be discarded or supplemented with floc from paste beef extract or beef extract V. The procedure for the preparation of the additional floc is described in Subsection 4.1.3. The overall recovery of virus should also be at least 50% when supplementation is required.

4.1 Media and Reagents

4.1.1 Sodium phosphate, dibasic ($Na_2HPO_4 \cdot 7H_2O$) — 0.15 M.

Dissolve 40.2 g of sodium phosphate in a final volume of 1,000 mL. Autoclave at 121°C for 15 minutes.

4.1.2 Paste beef extract (Difco Laboratories, product no. 0126, or equivalent) or beef extract V (BBL Microbiology Systems, product no. 97531, or equivalent) — 3%.

Prepare a 3% paste beef extract or beef extract V stock solution by dissolving 30 g of beef extract in 1,000 mL ddH_2O. Autoclave the stock solution at 121°C for 15 minutes and use at room temperature. From this stock solution, one 330 mL aliquot is removed for each sample requiring supplementation with beef extract floc. Although the stock solution may be stored at 4°C for an extended time period, it is advisable to prepare the solutions on a weekly basis, thereby lessening the possibility of microbial contamination.

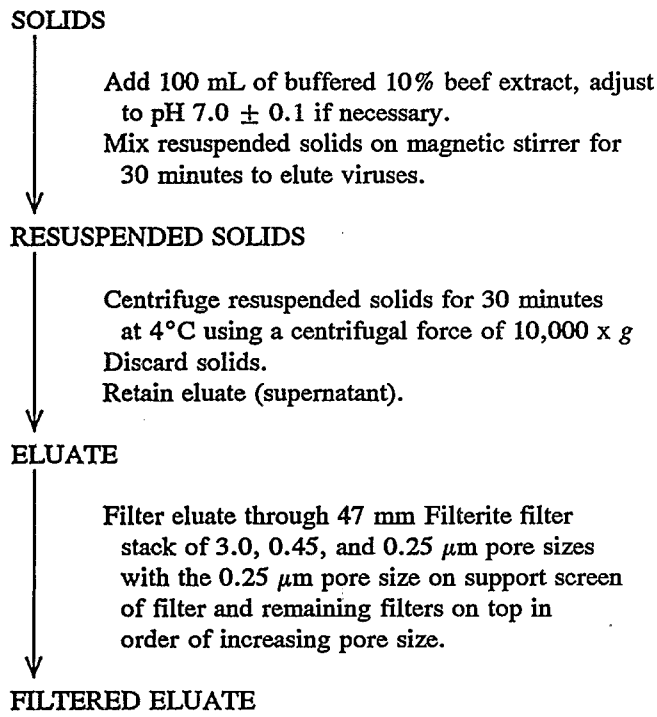

Figure 2. Flow Diagram of Method for Elution of Virus from Solids

4.1.3 Preparation of Floc from 3% Beef Extract Reagent

A flow diagram for the procedure to prepare floc is given in Figure 3.

(a) Place a stir bar and 330 mL of the 3% beef extract stock solution into a 600 mL beaker and cover loosely with aluminum foil. Place the beaker onto a magnetic stirrer, and stir at a speed sufficient to develop a vortex.

(b) Insert a combination-type pH electrode into the beef extract stock solution. Add 1 M HCl to flask slowly until the pH of beef extract reaches 3.5 ± 0.1.
The pH meter must be standardized at pH 4 and 7.
A precipitate will form.

(c) Remove the electrode and pour the contents of the beaker into a 1,000 mL centrifuge bottle. Centrifuge the precipitated beef extract suspensions at 2,500 x *g* for 15 minutes at 4°C. Pour off and discard the supernatant.
To prevent the transfer of the stir bar into the centrifuge bottle, hold another stir bar or magnet against the bottom of the beaker while decanting the contents.

(d) Retain the floc in the centrifuge bottle at 4°C for subsequent mixing with the non-flocculating buffered beef extract (Subsection 4.2.4).

4.2 Virus Concentration Procedure

A flow diagram for the virus concentration procedure is given in Figure 4.

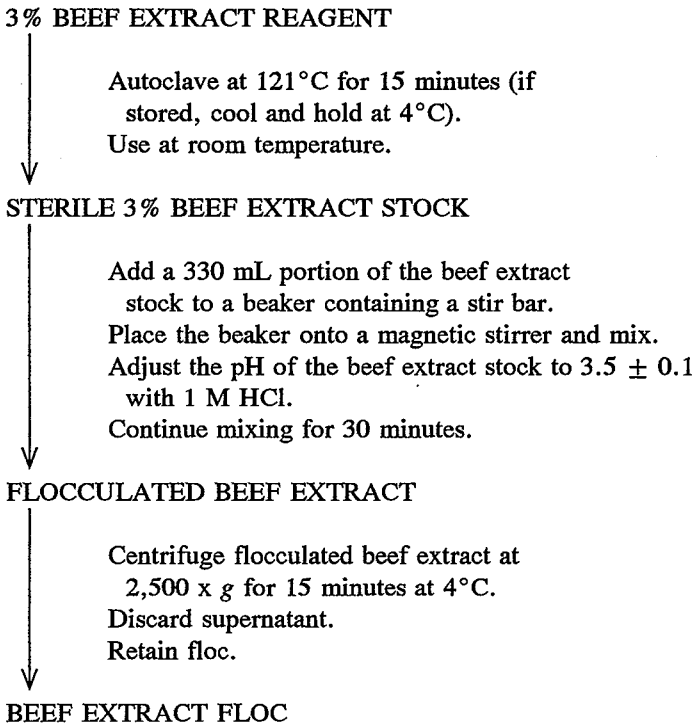

Figure 3. Flow Diagram of Method for Preparation of Floc from Beef Extract Reagent

4.2.1 Pour the filtered eluate (from Subsection 3.3.4) into a graduated cylinder, and record the volume. Transfer into a 600 mL beaker and cover loosely with aluminum foil.

4.2.2 For every 3 mL of beef extract eluate, add 7 mL of ddH_2O to the 600 mL beaker.

The concentration of beef extract is now 3%. This dilution is necessary because 10% beef extract often does not process well by the organic flocculation concentration procedure.

4.2.3 Record the total volume of the diluted eluate.

Proceed directly to Subsection 4.2.5 only if the powdered beef extract reagent used for the virus elution process (in Subsection 3) is known to form sufficient floc to efficiently concentrate virus without the additional floc. Where additional floc is required, add the diluted eluate to the floc as described in Subsection 4.2.4.

4.2.4 Pour the eluate from the beaker into the centrifuge bottle containing floc from Subsection 4.1.3(d). Disperse the floc manually using a pipette until it is dissolved in the eluate and then transfer the eluate/floc mixture into a 600 mL beaker.

4.2.5 Place a stir bar into the beaker that contains the diluted, filtered beef extract. Place the beaker on a magnetic stirrer, cover loosely with aluminum foil, and stir at a speed sufficient to develop a vortex.

To minimize foaming (which may inactivate viruses), do not mix faster than necessary to develop a vortex.

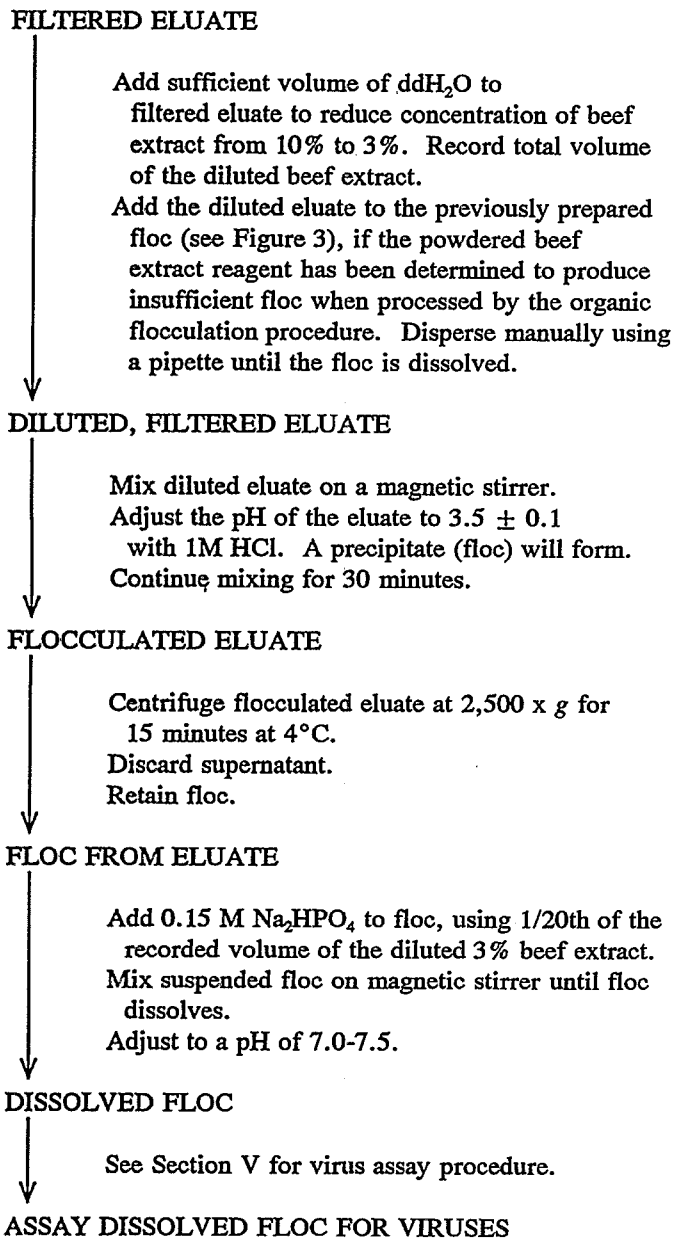

Figure 4. Flow Diagram of Method for Concentration of Viruses from Beef Extract Eluate

4.2.6 Insert a combination-type pH electrode into the diluted, filtered beef extract. Add 1 M HCl to the flask slowly until the pH of the beef extract reaches 3.5 ± 0.1. Continue to stir for 30 minutes at room temperature.

The pH meter must be standardized at pH 4 and 7.

A precipitate will form. If the pH is accidentally reduced below 3.4, add 1 M NaOH until it reaches 3.5 ± 0.1. Avoid reducing the pH below 3.4 because some inactivation of virus may occur.

4.2.7 Remove the electrode from the beaker, and pour the contents of the beaker into a 1,000 mL centrifuge bottle. Centrifuge the precipitated beef extract suspensions at 2,500 x *g* for 15 minutes at 4°C. Pour off and discard the supernatant.

To prevent the transfer of the stir bar into a centrifuge bottle, hold another stir bar or magnet against the bottom of the beaker when decanting contents.

4.2.8 Place a stir bar into the centrifuge bottle that contains the precipitate. Add a volume of 0.15 M $Na_2HPO_4 \cdot 7H_2O$ equal to exactly 1/20 of the volume recorded in Subsection 4.2.3. Place the bottle onto a magnetic stirrer, and stir slowly until the precipitate has dissolved completely.

Support the bottle as necessary to prevent toppling. Avoid foaming, which may inactivate or aerosolize viruses. The precipitate may be partially dissipated with a spatula before or during the stirring procedure.

4.2.9 Measure the pH of the dissolved precipitate.

If the pH is above or below 7.0-7.5, adjust to that range with either 1 M HCl or 1 M NaOH.

4.2.10 Freeze exactly one half of the dissolved precipitate sample at -70°C. This sample will be held as a backup to use should the sample prove to be cytotoxic (see Section VI). Record the remaining sample volume (this volume represents 6 g of total dry solids). Refrigerate the sample immediately at 4°C and maintain at that temperature until it is assayed in accordance with the instructions given in Section V below.

If the virus assay cannot be undertaken within 8 hours, store the remaining sample at -70°C.

IV. CELL CULTURE PREPARATION AND MAINTENANCE[5]

1. INTRODUCTION

This section outlines procedures and media for culturing the Buffalo green monkey (BGM) cell line and is intended for the individual who is experienced in cell culture preparation. BGM cells are a continuous cell line derived from African green monkey kidney cells. The characteristics of this line were described by Barron *et al.* (1970). Use of BGM cells for recovering viruses from environmental samples was described by Dahling *et al.* (1974). The media and methods recommended in Sections III and IV are the results of the BGM cell line optimization studies by Dahling and Wright (1986). The BGM cell line can be obtained by qualified laboratories from the Virology Branch, Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268.

BGM cells are highly susceptible to many enteric viruses (Dahling *et al.*, 1984; Dahling and Wright, 1986); however, these cells are not sensitive for detecting all enteric viruses that may be present in environmental samples. The use of several cell lines would be required to maximize the number of viruses recovered from environmental samples. Since it is difficult to specify the type of cell lines which would be best for a particular sludge sample, the use of the BGM cell line only will be sufficient to meet the requirements of the 40 CFR Part 503 regulation.

2. MEDIUM PREPARATION

2.1 Apparatus and Materials

2.1.1 Glassware, Pyrex (Corning, product no. 1395, or equivalent).

Storage vessels must be equipped with airtight closures.

2.1.2 Autoclavable inner-braided tubing with metal quick-disconnect connectors or with screw clamps for connecting tubing to equipment to be used under pressure.

Quick-disconnect connectors can be used only after equipment has been properly adapted.

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[5]Modified from EPA/600/4-84/013(R9), January 1987 Revision.

2.1.3 Positive pressure air, nitrogen or 5% CO_2 source equipped with pressure gauge.

Pressure sources from laboratory air lines and pumps must be equipped with an oil filter. The source must not deliver more pressure to the pressure vessel than is recommended by the manufacturer.

2.1.4 Dispensing pressure vessel — 5 or 20 L capacity (Millipore Corp., product nos. XX67 00P 05 and XX67 00P 20, or equivalent).

2.1.5 Disc filter holders — 142 mm or 293 mm diameter (Millipore Corp., product nos. YY30 142 36 and YY30 293 16, or equivalent).

Use only pressure-type filter holders.

2.1.6 Sterilizing filter stacks — 0.22 μm pore size (Millipore Corp., product nos. GSWP 142 50 and GSWP 293 25, or equivalent). Fiberglass prefilters (Millipore Corp., product nos. AP15 142 50 or AP15 293 25 and AP20 142 50 or AP20 293 25, or equivalent).

Stack AP20 and AP15 prefilters and 0.22 μm membrane filter into a disc filter holder with AP20 prefilter on top and 0.22 μm membrane filter on bottom.

Always disassemble the filter stack after use to check the integrity of the 0.22 μm filter. Refilter any media filtered with a damaged stack.

2.1.7 Positively-charged cartridge filter — 10 inch (Zeta plus TSM, Cuno Div., product no. 45134-01-600P, or equivalent). Holder for cartridge filter with adaptor for 10 inch cartridge (Millipore Corp., product no. YY16 012 00, or equivalent).

2.1.8 Culture capsule filter (Gelman Sciences, product no. 12140, or equivalent).

2.1.9 Cell culture vessels — Pyrex, soda, or flint glass or plastic bottles and flasks or roller bottles (e.g., Brockway, product no. 1076-09A, 1925-02, Corning, product no. 25100-25, 25110-75, 25120-150, 25150-1750, or equivalent).

Vessels must be made from clear glass or plastic to allow observation of the cultures and be equipped with airtight closures. Plastic vessels must be treated by the manufacturer to allow cells to adhere properly.

2.1.10 Screw caps, black with rubber liners (Brockway, product no. 24-414 for 6 oz bottles,[6] or equivalent).

Caps for larger culture bottles usually supplied with bottles.

2.1.11 Roller apparatus (Belco, product no. 7730, or equivalent).

2.1.12 Incubator capable of maintaining the temperature of cell cultures at 36.5 ± 1°C.

2.1.13 Waterbath, equipped with circulating device to ensure even heating at 36.5 ± 1°C.

2.1.14 Light microscope, with conventional light source, equipped with lenses to provide 40X, 100X, and 400X total magnification.

2.1.15 Inverted light microscope equipped with lenses to provide 40X, 100X, and 400X total magnification.

2.1.16 Cornwall syringe pipettors, 2, 5, and 10 mL sizes (Curtin Matheson Scientific, product nos. 221-861, 221-879, and 221-887, or equivalent).

2.1.17 Brewer-type pipetting machine (Curtin Matheson Scientific, product no. 138-107, or equivalent).

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[6]Size is given in oz only when it is commercially designated in that unit.

2.1.18 Phase contrast counting chamber (hemocytometer) (Curtin Matheson Scientific, product no. 158-501, or equivalent).

2.1.19 Conical centrifuge tubes, sizes 50 mL and 250 mL.

2.1.20 Rack for tissue culture tubes (Bellco Glass, product no. 2028, or equivalent).

2.1.21 Bottles, aspirator-type with tubing outlet, size 2,000 mL.
Bottles for use with pipetting machine.

2.1.22 Storage vials, size 2 mL.
Vials must withstand temperatures to -70°C.

2.2 Media and Reagents

2.2.1 Sterile fetal calf, gamma globulin-free newborn calf, or iron-supplemented calf serum, certified free of viruses, bacteriophage and mycoplasma (GIBCO BRL, or equivalent).
Test each lot of serum for cell growth and toxicity before purchasing. Serum should be stored at -20°C for long-term storage. Upon thawing, each bottle must be heat-inactivated at 56°C for 30 minutes and stored at 4°C for short-term use.

2.2.2 Trypsin, 1:250 powder (Difco Laboratories, product no. 0152-15-9, or equivalent) or trypsin, 1:300 powder (BBL, Microbiology Systems, product no. 12098, or equivalent).

2.2.3 Sodium (tetra) ethylenediamine tetraacetate powder (EDTA), technical grade (Fisher Scientific, product no. S657-500, or equivalent).

2.2.4 Thioglycollate medium (Difco Laboratories, product no. 0257-01-9, or equivalent).

2.2.5 Fungizone (amphotericin B, Sigma Chemical, product no. A-9528, or equivalent), penicillin G (Sigma Chemical, product no. P-3032, or equivalent), and dihydrostreptomycin sulfate (ICN Biomedicals, product no. 100556, or equivalent), tetracycline (ICN Biomedicals, product no. 103011, or equivalent).
Use antibiotics of at least tissue culture grade.

2.2.6 Eagle's minimum essential medium (MEM) with Hanks' salts and L-glutamine, without sodium bicarbonate (GIBCO BRL, product no. 410-1200, or equivalent).

2.2.7 Leibovitz's L-15 medium with L-glutamine (GIBCO BRL, product no. 430-1300, or equivalent).

2.2.8 Trypan blue (Sigma Chemical Co., product no. T-6146, or equivalent).
Note: This chemical is on the EPA list of proven or suspected carcinogens.

2.2.9 Dimethyl sulfoxide (DMSO; Sigma Chemical Co., product no. D-2650, or equivalent).

2.2.10 Mycoplasma testing kit (Irvine Scientific, product no. T500-000, or equivalent).

3. PREPARATION OF CELL CULTURE MEDIA

3.1 General Principles

3.1.1 Equipment care — Carefully wash and sterilize equipment used for preparing media before each use.

3.1.2 Disinfection of work area — Thoroughly disinfect surfaces on which the medium preparation equipment is to be placed. Many commercial disinfectants do not adequately kill enteroviruses. To ensure thorough disinfection, disinfect all surfaces and spills with either a solution of 0.5% (5 g per liter) I_2 in 70% ethanol or 0.1% HOCl. HOCl can be prepared by adding 19 mL of household bleach (Clorox, The Clorox Co., or equivalent) to 981 mL of ddH_2O and adjusting the pH of the solution to 6-7 with 1 M HCl.

3.1.3 Aseptic technique — Use aseptic technique when preparing and handling media or medium components.

3.1.4 Dispensing filter-sterilized media — To avoid post-filtration contamination, dispense filter-sterilized media into storage containers through clear glass filling bells in a microbiological laminar flow hood. If a hood is unavailable, use an area restricted solely to cell culture manipulations.

3.1.5 Coding media — Assign a lot number to and keep a record of each batch of medium or medium components prepared. Place the lot number, the date of preparation, the expiration date, and the initials of the person preparing the medium on each bottle.

3.1.6 Sterility test — Test each lot of medium and medium components to confirm sterility as described in Subsection 4 before the lot is used for cell culture.

3.1.7 Storage of media and medium components — Store media and medium components in clear, airtight containers at 4°C or -20°C as appropriate.

3.1.8 Sterilization of $NaHCO_3$-containing solutions — Sterilize media and other solutions that contain $NaHCO_3$ by positive pressure filtration.

Negative pressure filtration of such solutions increases the pH and reduces the buffering capacity.

3.2 Media Preparation Recipes

3.2.1 Sources of cell culture media.

Commercially prepared liquid cell culture media and medium components are available from several sources. Cell culture media can also be purchased in powder form that requires only dissolution in ddH_2O and sterilization. Media from commercial sources are quality controlled. The conditions specified by the supplier for storage and expiration dates should be strictly observed. However, media can also be prepared in the laboratory directly from chemicals. Such preparations are labor intensive, but allow quality control of the process at the level of the preparing laboratory.

3.2.2 Procedure for the preparation of EDTA-trypsin.

The procedure described is for the preparation of 10 L of EDTA-trypsin reagent. It is used to dislodge cells attached to the surface of culture bottles and flasks. This reagent, when stored at 4°C, retains its working strength for at least 4 months. The amount of reagent prepared should be based on projected usage over a 4 month period.

(a) Add 30 g of trypsin (1:250) or 25 g of trypsin (1:300) and 2 L of ddH_2O to a 6 L flask containing a 3 inch stir bar. Place the flask onto a magnetic stirrer and mix the trypsin solution rapidly for a minimum of 1 hour.
Trypsin remains cloudy.

(b) Add 4 L of ddH_2O and a 3 inch stir bar into 20 L clear plastic carboy. Place the carboy onto a magnetic stirrer and stir at a speed sufficient to develop a vortex while adding the following chemicals: 80 g NaCl, 12.5 g EDTA, 50 g dextrose, 11.5 g $Na_2HPO_4 \cdot 7H_2O$, 2.0 g KCl, and 2.0 g KH_2PO_4.
Each chemical does not have to be completely dissolved before adding the next one.

(c) Add 4 more liters of ddH_2O to carboy.
Continue mixing until all chemicals are completely dissolved.

(d) Add the 2 L of trypsin from step (a) to the prepared solution in step (c) and mix for a minimum of 1 hour. Adjust the pH of the EDTA-trypsin reagent to 7.5-7.7.

(e) Filter reagent under pressure through a disc filter stack and store the filtered reagent in tightly stoppered or capped containers at 4°C.
The cartridge prefilter (Subsection 2.1.7) can be used in line with the culture capsule sterilizing filter (Subsection 2.1.8) as an alternative to a filter stack (Subsection 2.1.6).

3.2.3 Procedure for the preparation of MEM/L-15 medium.
The procedure described is for preparation of 10 L of MEM/L-15 medium.

(a) Place a 3 inch stir bar and 4 L of ddH_2O into 20 L carboy.

(b) Place the carboy onto a magnetic stirrer. Stir at a speed sufficient to develop a vortex and then add the contents of a 5 L packet of L-15 medium to the carboy. Rinse the medium packet with 3 washes of 200 mL each of ddH_2O and add the rinses to the carboy.

(c) Mix until the medium is evenly dispersed.
L-15 medium may appear cloudy as it need not be totally dissolved before proceeding to step (d).

(d) Add 3 L of ddH_2O to the carboy and the contents of a 5 L packet of MEM medium to the carboy. Rinse the MEM medium packet with three washes of 200 mL each of ddH_2O and add the rinses to the carboy. Add 800 mL of ddH_2O and 7.5 g of $NaHCO_3$ and continue mixing for an additional 60 minutes.

(e) Transfer the MEM/L-15 medium to a pressure can and filter under positive pressure through a 0.22 μm sterilizing filter. Collect the medium in volumes appropriate for the culturing of BGM cells (e.g., 900 mL in a 1 L bottle) and store in tightly stoppered or capped containers at 4°C.
Medium may be stored for periods of up to 2 months.

3.2.4 Procedure for preparation of trypan blue solution.
The procedure described is for the preparation of 100 mL of trypan blue solution. It is used in the direct determination of the viable cell counts of the BGM stock cultures. Since trypan blue is on the EPA suspect carcinogen list, particular care should be taken in its preparation and use so as to avoid skin contact or inhalation. The wearing of rubber gloves during preparation and use is recommended.

(a) Add 0.5 g of trypan blue to 100 mL of ddH_2O in a 250 mL flask. Swirl the flask until the trypan blue is completely dissolved.

(b) Sterilize the solution by autoclaving at 121°C for 15 minutes and store in a screw-capped container at room temperature.

3.2.5 Procedure for preparation of stock antibiotic solutions.
If not purchased in sterile form, stock antibiotic solutions must be filter-sterilized by the use of 0.22 μm membrane filters. It is important that the recommended antibiotic levels not be exceeded when planting cells as the cultures are particularly sensitive to excessive concentrations at this stage.
Antibiotic stock solutions should be placed in screw-capped containers and stored at -20°C until needed. Once thawed, they may be refrozen; however, repeated freezing and thawing of these stock solutions should be avoided by distributing them in quantities that are sufficient to support a week's cell culture work.

(a) Preparation of penicillin-streptomycin stock solution.

The procedure described is for preparation of ten 10 mL aliquots of penicillin-streptomycin stock solution at concentrations of 1,000,000 units of penicillin and 1,000,000 μg of streptomycin per 10 mL unit. The antibiotic concentrations listed in step (a.1) may not correspond to the concentrations obtained from other lots or from a different source.

(a.1) Add appropriate amounts of penicillin G and dihydrostreptomycin sulfate to a 250 mL flask containing 100 mL of ddH_2O. Mix the contents of the flasks on magnetic stirrer until the antibiotics are dissolved.
For penicillin supplied at 1,435 units per mg, add 7 g of the antibiotic.
For streptomycin supplied at 740 mg per g, add 14 g of the antibiotic.

(a.2) Sterilize the antibiotics by filtration through 0.22 μm membrane filters and dispense in 10 mL volumes into screw-capped containers.

(b) Preparation of tetracycline stock solution. Add 1.25 g of tetracycline hydrochloride powder and 3.75 g of ascorbic acid to a 125 mL flask containing 50 mL of ddH_2O. Mix the contents of the flask on a magnetic stirrer until the antibiotic is dissolved. Sterilize the antibiotic by filtration through a 0.22 μm membrane filter and dispense in 5 mL volumes into screw-capped containers.

(c) Preparation of amphotericin B (fungizone) stock solution. Add 0.125 g of amphotericin B to a 50 mL flask containing 25 mL of ddH_2O. Mix the contents of the flask on a magnetic stirrer until the antibiotic is dissolved. Sterilize the antibiotic by filtration through 0.22 μm membrane filter and dispense 2.5 mL volumes into screw-capped containers.

4. PROCEDURE FOR VERIFYING STERILITY OF LIQUIDS

There are many techniques available for verifying the sterility of liquids such as cell culture media and medium components. The two techniques described below are standard in many laboratories. The capabilities of these techniques are limited to the detection of microorganisms that grow unaided on the test medium utilized. Viruses, mycoplasma, and microorganisms that possess fastidious growth requirements or that require living host systems will not be detected. Nonetheless, with the exception of a few special contamination problems, the test procedures and microbiological media listed below should prove adequate. Do not add antibiotics to media or medium components until after sterility of the antibiotics, media, and medium components has been demonstrated. The BGM cell line used should be checked every 6 months for mycoplasma contamination according to test kit instructions. Cells that are contaminated should be discarded.

4.1 Procedure for Verifying Sterility of Small Volumes of Liquids. Inoculate 5 mL of the material to be tested for sterility into 5 mL of thioglycollate broth. Shake the mixture and incubate at 36.5 ± 1°C. Examine the inoculated broth daily for 7 days to determine whether growth of contaminating organisms has occurred.

Vessels that contain thioglycollate medium must be tightly sealed before and after medium is inoculated.

4.2 Visual Evaluation of Media for Microbial Contaminants. Incubate media at 36.5 ± 1°C for at least 1 week prior to use. Visually examine and discard any media that lose clarity.

A clouded condition that develops in the media indicates the occurrence of contaminating organisms.

5. PROCEDURES FOR PREPARATION AND PASSAGE OF BGM CELL CULTURES

A laminar flow biological safety cabinet should be used to process cell cultures. If a biological safety cabinet is not available, cell cultures should be prepared in controlled facilities used for no other purposes. Viruses or other microorganisms must not be transported, handled, or stored in cell culture transfer facilities.

5.1 Vessels and Media for Cell Growth

5.1.1 The BGM cell line grows readily on the inside surfaces of glass or specially treated, tissue culture grade plastic vessels. 16 to 32 oz (or equivalent growth area) flat-sided, glass bottles, 75 or 150 cm^2 plastic cell culture flasks, and 690 cm^2 glass or 850 cm^2 plastic roller bottles are usually used for the maintenance of stock cultures. Flat-sided bottles and flasks that contain cells in a stationary position are incubated with the flat side (cell monolayer side) down. If available, roller bottles and roller apparatus units are preferable to flat-sided bottles and flasks because roller cultures require less medium than flat-sided bottles per unit of cell monolayer surface. Roller apparatus rotation speed should be adjusted to one-half revolution per minute to ensure that cells are constantly bathed in growth medium.

5.1.2 Growth and maintenance media should be prepared on the day they will be needed. Prepare growth medium by supplementing MEM/L-15 medium with 10% serum and antibiotics (100 mL of serum, 1 mL of penicillin-streptomycin stock, 0.5 mL of tetracycline stock, and 0.2 mL of fungizone stock per 900 mL of MEM/L-15). Prepare maintenance medium by supplementing MEM/L-15 with antibiotics and 2% or 5% serum (20 or 50 mL of serum, antibiotics as above for growth medium, and 70 or 50 mL of ddH_2O, respectively).

5.2 General Procedure for Cell Passage

Pass stock BGM cell cultures at approximately 7 day intervals using growth medium.

5.2.1 Pour spent medium from cell culture vessels, and discard the medium.

To prevent splatter, a gauze-covered beaker may be used to collect spent medium.

Before discarding, autoclave all media that have been in contact with cells or that contain serum.

5.2.2 Add to the cell cultures a volume of warm EDTA-trypsin reagent equal to 40% of the volume of medium replaced.

See Table 1 for the amount of reagents required for commonly used vessel types.

To reduce shock to cells, warm the EDTA-trypsin reagent to 36.5 ± 1°C before placing it on cell monolayers. Dispense the EDTA-trypsin reagent directly onto the cell monolayer.

5.2.3 Allow the EDTA-trypsin reagent to remain in contact with the cells at either room temperature or at 36.5 ± 1°C until cell monolayer can be shaken loose from inner surface of cell culture vessel (about 5 minutes).

If necessary, a sterile rubber policeman (or scraper) may be used to physically remove the cell sheet from the bottle. However, this procedure should be used only as a last resort because of the risk of cell culture contamination inherent in such manipulations. The EDTA-trypsin reagent should remain in contact with the cells no longer than necessary as prolonged contact can alter or damage the cells.

5.2.4 Pour the suspended cells into centrifuge tubes or bottles.

To facilitate collection and resuspension of cell pellets, use tubes or bottles with conical bottoms. Centrifuge tubes and bottles used for this purpose must be able to withstand the g-force applied.

5.2.5 Centrifuge cell suspension at 1,000 x g for 10 minutes to pellet cells. Pour off and discard the supernatant.

Do not exceed this speed as cells may be damaged or destroyed.

5.2.6 Suspend the pelleted cells in growth medium (see Subsection 5.1.2) and perform a viable count on the cell suspension according to procedures in Subsection 6.

Resuspend pelleted cells in sufficient volumes of medium to allow thorough mixing of the cells (to reduce sampling error) and to minimize the significance of the loss of the 0.5 mL of cell suspension required for the cell counting procedure. The quantity of medium used for resuspending pelleted cells varies from 50 to several hundred mL, depending upon the volume of the individual laboratory's need for cell cultures.

Table 1. Guide for Preparation of BGM Stock Cultures

Vessel Type	Volume of EDTA-Trypsin Used to Remove Cells (mL)	Volume of Medium (mL)*	Total No. Cells to Plate per Vessel
16 oz** glass flat bottles	10	25	2.5×10^6
32 oz glass flat bottles	20	50	5.0×10^6
75 cm^2 plastic flat flask	12	30	3.0×10^6
150 cm^2 plastic flat flask	24	60	6.0×10^6
690 cm^2 glass roller bottle	40	100	7.0×10^7
850 cm^2 plastic roller bottle	50	120	8.0×10^7

*Serum requirements: growth medium contains 10% serum; maintenance medium contains 2-5% serum.
Antibiotic requirements: penicillin-streptomycin stock solution, 1.0 mL/L; tetracycline stock solution, 0.5 mL/L; fungizone stock solution, 0.2 mL/L.
**Size is given in oz only when it is commercially designated in that unit.

5.2.7 Dilute the cell suspension to the appropriate cell concentration with growth medium and dispense into cell culture vessels with either a Cornwall-type syringe or Brewer-type pipetting machine dispenser.

Calculate the dilution factor requirement using the cell count established in Subsection 6 and the cell and volume parameters given in Table 1 for stock cultures and in Table 2 for virus assay cultures.

As a general rule, the BGM cell line can be split at a 1:3 ratio. However, a more suitable inoculum is obtained if low passages of the line (passages 100-150) are split at a 1:2 ratio and higher passages (generally above passage 250) are split at a 1:4 ratio. To plant 200 25 cm^2 cell culture flasks weekly from a low-level passage of the line would require the preparation of 6 roller bottles (surface area 690 cm^2 each): 2 to prepare the 6 roller bottles and 4 to prepare the 25 cm^2 flasks.

5.2.8 Except during handling operations, maintain BGM cells at 36.5 $\pm$ 1°C in airtight cell culture vessels.

5.3 Procedure for Changing Medium on Cultured Cells

Cell monolayers normally become 95-100% confluent 3-4 days after seeding with an appropriate number of cells, and growth medium becomes acidic. Growth medium on confluent stock cultures should then be replaced with maintenance medium containing 2% serum. Maintenance medium with 5% serum should be used when monolayers are not yet 95-100% confluent but the medium in which they are immersed has become acidic. The volume of maintenance medium should equal the volume of discarded growth medium.

Table 2. Guide for Preparation of Virus Assay Cell Cultures

Vessel Type	Volume of Medium* (mL)	Final Cell Count per Bottle
1 oz** glass bottle	4	9.0×10^5
25 cm^2 plastic flask	10	3.5×10^6
6 oz glass bottle	15	5.6×10^6
75 cm^2 plastic flask	30	1.0×10^7
16 mm x 150 mm tubes	2	4.0×10^4

*Serum requirements: growth medium contains 10% serum.
Antibiotic requirements: penicillin-streptomycin stock solution, 1.0 mL/L; tetracycline stock solution, 0.5 mL/L; fungizone stock solution, 0.2 mL/L.
**Size is given in oz only when it is commercially designated in that unit.

6. Procedure for Performing Viable Cell Counts

With experience, a fairly accurate cell concentration can be made based on the volume of packed cells. However, viable cell counts should be performed periodically as a quality control measure.

6.1 Add 0.5 mL of cell suspension (or diluted cell suspension) to 0.5 mL of 0.5% trypan blue solution in a test tube.

To obtain an accurate cell count, the optimal total number of cells per hemocytometer section should be between 20 and 50. This range is equivalent to between 6.0 x 10^5 and 1.5 x 10^6 cells per mL of cell suspension. Thus, a dilution of 1:10 (0.5 mL of cells in 4.5 mL of growth medium) is usually required for an accurate count of a cell suspension.

6.2 Disperse cells by repeated pipetting.

Avoid introducing air bubbles into the suspension, because air bubbles may interfere with subsequent filling of the hemocytometer chambers.

6.3 With a capillary pipette, carefully fill a hemocytometer chamber on one side of a slip-covered hemocytometer slide. Rest the slide on a flat surface for about 1 minute to allow the trypan blue to penetrate the cell membranes of nonviable cells.

Do not under or over fill the chambers.

6.4 Under 100X total magnification, count the cells in the four large corner sections and the center section of the hemocytometer chamber.

Include in the count cells lying on the lines marking the top and left margins of the sections, and ignore cells on the lines marking the bottom and right margins. Trypan blue is excluded by living cells. Therefore, to quantify viable cells, count only cells that are clear in color. Do not count cells that are blue.

6.5 Calculate the average number of viable cells in each mL of cell suspension by totaling the number of viable cells counted in the five sections, multiplying this sum by 4,000, and where necessary, multiplying the resulting product by the reciprocal of the dilution.

7. PROCEDURE FOR PRESERVATION OF BGM CELL LINE

An adequate supply of BGM cells must be available to replace working cultures that are used only periodically or become contaminated or lose virus sensitivity. Cells have been held at -70°C for more than 15 years with a minimum loss in cell viability.

7.1 Preparation of Cells for Storage

The procedure described is for the preparation of 100 cell culture vials. Cell concentration per mL must be at least 1 x 10^6.

Base the actual number of vials to be prepared on usage of the line and the anticipated time interval requirement between cell culture start-up and full culture production.

7.1.1 Prepare cell storage medium by adding 10 mL of serum and 10 mL of DMSO to 80 mL of growth medium (see Subsection 5.1.2). Sterilize cell storage medium by passage through an 0.22 μm sterilizing filter.

Collect sterilized medium in a 250 mL flask containing a stir bar.

7.1.2 Harvest BGM cells from cell culture vessels as directed in Subsections 5.2.1 through 5.2.5. Count the cells according to the procedure in Subsection 6 and resuspend them in the cell storage medium at a concentration of 1 x 10^6 cells per mL.

7.1.3 Place the flask containing suspended cells on a magnetic stirrer and slowly mix for 30 minutes. Dispense 1 mL volumes of cell suspension into 2 mL vials.

7.2 Procedure for Freezing Cells

The freezing procedure requires slow cooling of the cells with the optimum rate of -1°C per minute. A slow cooling rate can be achieved using the following method or by using the recently available freezing containers (e.g., Nalge Company, product no. 5100-0001, or equivalent) as recommended by the manufacturers.

7.2.1 Place the vials in a rack and place the rack in refrigerator at 4°C for 30 minutes, in a -20°C freezer for 30 minutes, and then in a -70°C freezer overnight. The transfers should be made as rapidly as possible.

To allow for more uniform cooling, wells adjoining each vial should remain empty.

7.2.2 Rapidly transfer vials into boxes or other containers for long-term storage.

To prevent substantial loss of cells during storage, the temperature of cells should be kept constant after -70°C has been achieved.

7.3 Procedure for Thawing Cells

Cells must be thawed rapidly to decrease loss in cell viability.

7.3.1 Place vials containing frozen cells into a 36°C water bath and agitate vigorously by hand until all ice has melted. Sterilize the outside surface of the vials with 0.5% I_2 in 70% ethanol.

7.3.2 Add BGM cells to either 6 oz tissue culture bottles or 25 cm^2 tissue culture flasks containing an appropriate volume of growth medium (see Table 2). Use two vials of cells for 6 oz bottles and one vial for 25 cm^2 flasks.

7.3.3 Incubate BGM cells at 36.5 ± 1°C. After 18 to 24 hours replace the growth medium with fresh growth medium and then continue the incubation for an additional five days. Pass and maintain the new cultures as directed in Subsection 5.

V. CELL CULTURE PROCEDURES FOR ASSAYING PLAQUE-FORMING VIRUSES[6]

1. INTRODUCTION

This section outlines procedures for the detection of viruses in sludge by use of the plaque assay system, as described by Dulbecco (1952), Dulbecco and Vogt (1954), Hsiung and Melnick (1955), and Dahling and Wright (1986). The system uses an agar medium to localize virus growth following attachment of infectious virus particles to a cell monolayer. Localized lesions of dead cells (plaques) developing some days after viral infection are visualized with the vital stain (neutral red), which stains only live cells. The number of circular unstained plaques are counted and reported as plaque-forming units (PFU), whose number is proportional to the amount of infectious virus particles inoculated. The procedures outlined below describe the plaque assay technique for enterovirus enumeration of sludge sample concentrates using the BGM cell line.

The detection methodology presented in this section is geared toward laboratories with a small-scale virus assay requirement. Where the quantities of cell cultures, media, and reagents set forth in the section are not sufficient for processing the test sample concentrates, the prescribed measures may be increased proportionally to meet the demands of more expansive test regimes.

2. PLAQUE ASSAY PROCEDURE

2.1 Apparatus and Materials

2.1.1 Waterbath set at 50 ± 1°C.
Used for maintaining the agar temperature (see Subsection 2.2.10).

2.2 Media and Reagents

2.2.1 ELAH — 0.65% lactalbumin hydrolysate in Earle's base (Gibco BRL, product no. 320-1250, or equivalent).
ELAH can be purchased in liquid form or prepared by dissolving 6.5 g per liter of tissue culture, highly soluble grade lactalbumin hydrolysate in Earle's base (Gibco BRL, product no. 310-4010, or equivalent) prewarmed to 50-60°C. Sterilize ELAH prepared in-house through a 0.22 μm filter stack. ELAH prepared in-house may be stored for 2 months at 4°C.

2.2.2 Maintenance medium — Add 1 mL of penicillin-streptomycin stock (see Section IV, Subsection 3.2.5 for preparation of antibiotic stocks), 0.5 mL of tetracycline stock, and 0.2 mL of fungizone stock per liter to ELAH immediately before washing of cells.

2.2.3 HEPES — 1 M (Sigma Chemical Co., product no. H-3375, or equivalent).
Prepare 50 mL of a 1 M solution by dissolving 11.92 g of HEPES in a final volume of 50 mL ddH_2O. Sterilize by autoclaving at 121°C for 15 minutes.

2.2.4 Sodium bicarbonate ($NaHCO_3$) — 7.5% solution.
Prepare 50 mL of a 7.5% solution by dissolving 3.75 g of sodium bicarbonate in a final volume of 50 mL ddH_2O. Sterilize by filtration through a 0.22 μm filter.

2.2.5 Magnesium chloride ($MgCl_2 \cdot 6H_20$) — 1.0% solution.
Prepare 50 mL of a 1.0% solution by dissolving 0.5 g of magnesium chloride in a final volume of 50 mL ddH_2O. Sterilize by autoclaving at 121°C for 15 minutes.

2.2.6 Neutral red solution — 0.333%, 100 mL volume (GIBCO BRL, product no. 630-5330, or equivalent).
Procure one 100 mL bottle.

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[6]Modified for EPA/600/4-84/013(R11), March 1988 Revision.

2.2.7 Bacto skim milk (Difco Laboratories, product no. 0032-01, or equivalent).
Prepare 100 mL of 10% skim milk in accordance with directions given by manufacturer.

2.2.8 Preparation of Medium 199.
The procedure described is for preparation of 500 mL of Medium 199 (GIBCO BRL, product no. 400-1100, or equivalent) at a 2X concentration. This procedure will prepare sufficient medium for at least fifty 6 oz glass bottles or eighty 25 cm^2 plastic flasks.

(a) Place a 3 inch stir bar into a 1 L flask. Add the contents of a 1 L packet of Medium 199 into the flask. Add 355 mL of ddH_2O. Rinse medium packet with 3 washes of 20 mL each of ddH_2O and add the washes to the flask.
Note that the amount of ddH_2O is 5% less than desired for the final volume of the medium.

(b) Mix on a magnetic stirrer until the medium is completely dissolved. Filter the reagent under pressure through a filter stack (see Section IV, Subsection 2.1.6).
Test each lot of medium to confirm sterility before the lot is used (see Section IV, Subsection 4). Each batch may be stored for 2 months at 4°C.

2.2.9 Preparation of overlay medium for plaque assay.
The procedure described is for preparation of 100 mL of overlay medium and will prepare sufficient media for at least ten 6 oz glass bottles or twenty 25 oz plastic flasks when mixed with the agar prepared in Subsection 2.2.10.

(a) Add 79 mL of Medium 199 (2X concentration) and 4 mL of serum to a 250 mL flask.

(b) Add the following to the flask in the order listed, and swirl after each addition: 6 mL of 7.5% $NaHCO_3$, 2 mL of 1% $MgCl_2$, 3 mL of 0.333% neutral red solution, 4 mL of 1 M HEPES, 0.2 mL of penicillin-streptomycin stock (see Section IV, Subsection 3.2.5 for a description of antibiotic stocks), 0.1 mL of tetracycline stock, and 0.04 mL of fungizone stock.

(c) Place flask with overlay medium in waterbath set at 36 $\pm$ 1°C.

2.2.10 Preparation of overlay agar for plaque assay.

(a) Add 3 g of agar (Sigma Chemical Co., product no. A-9915, or equivalent) and 100 mL of ddH_2O to a 250 mL flask. Melt by sterilizing the agar solution in an autoclave at 121°C for 15 minutes.

(b) Cool the agar to 50°C in waterbath set at 50 $\pm$ 1°C.

2.2.11 Preparation of agar overlay medium.

(a) Add 2 mL of 10% skim milk to overlay medium prepared in Subsection 2.2.9.

(b) Mix equal portions of overlay medium and agar by adding the medium to the agar flask.
To prevent solidification of the liquified agar, limit the portion of agar overlay medium mixed to that volume which can be dispensed in 10 minutes.

2.3 Procedure for Inoculating Test Samples.
Section IV, Subsection 5 provides the procedures for the preparation of cell cultures used for the virus assay in this section.
Cell cultures used for virus assays are generally found to be at their most sensitive level between the third and sixth days after initiation. Those older than seven days should not be used.

2.3.1 Decant and discard the growth medium from previously prepared cell culture test vessels.

To prevent splatter, a gauze-covered beaker may be used to collect spent medium.

The medium is changed from 1-4 hours before cultures are to be inoculated and carefully decanted so as not to disturb the cell monolayer.

2.3.2 Replace discarded medium with an equal volume of maintenance medium on the day the cultures are to be inoculated.

To reduce shock to cells, prewarm the maintenance medium to 36.5 ± 1°C before placing it onto the cell monolayer.

To prevent disturbing cells with the force of the liquid against the cell monolayer, add the maintenance medium to the side of the cell culture test vessel opposite the cell monolayer.

2.3.3 Identify cell culture test vessels by coding them with an indelible marker. Return the cell culture test vessels to a 36.5 ± 1°C incubator and hold at that temperature until the cell monolayer is to be inoculated.

2.3.4 Decant and discard the maintenance medium from cell culture test vessels.

Do not disturb the cell monolayer.

2.3.5 Thaw (if frozen) the remaining sample from Section III, Subsection 4.2.10 and inoculate each BGM cell monolayer with a volume of test sample concentrate appropriate for the cell surface area of the cell culture test vessels used.

Inoculum volume should be no greater than 1 mL for each 40 cm^2 of surface area. Use Table 3 as a guide for inoculation size.

Avoid touching either the cannula or the pipetting device to the inside rim of the cell culture test vessels to avert the possibility of transporting contaminants to the remaining culture vessels.

(a) Inoculate 2 BGM cultures with an appropriate volume of 0.15 M $Na_2HPO_4 \cdot 7H_2O$ (see Section III, Subsection 4.1.1) preadjusted to pH 7.0-7.5. These cultures will serve as negative controls.

(b) Inoculate 2 BGM cultures with an appropriate volume of 0.15 M $Na_2HPO_4 \cdot 7H_2O$ preadjusted to pH 7.0-7.5 and spiked with 20-40 PFU of poliovirus. These cultures will serve as a positive control for the plaque assay.

(c) Remove a volume of the test sample concentrate exactly equal to 1/6th (i.e., 1 g of total dry solids) of the volume recorded in Section III, Subsection 4.2.10. Spike this subsample with 20-40 PFU of poliovirus. Inoculate the subsample onto one or more BGM cultures using a inoculum volume per vessel that is appropriate for the vessel size used. These cultures will serve as controls for cytotoxicity (see Section VI).

(d) Record the volume of the remaining 5/6th portion of the test sample. This remaining portion represents a total dry solids content of 5 g. Inoculate the entire remaining portion (even if diluted to reduce cytotoxicity) onto BGM cultures. Inoculation of the entire volume is necessary to demonstrate a virus density level of less than 1 PFU per 4 g total dry solids.

2.3.6 Rock the inoculated cell culture test vessels gently to achieve uniform distribution of inoculum over the surface of the cell monolayers. Place the cell culture test vessels on a level, stationary surface at room temperature (22-25°C) so that the inoculum will remain distributed evenly over the cell monolayer.

Table 3. Guide for Virus Inoculation, Suspended Cell Concentration and Overlay Volume of Agar Medium

Vessel Type	Volume of Virus Inoculum (mL)	Volume of Agar Overlay Medium (mL)	Total Numbers of Cells
1 oz* glass bottle	0.1	5	1×10^7
25 cm^2 plastic flask	0.1-0.5	10	2×10^7
6 oz glass bottle	0.5-1.0	20	4×10^7
75 cm^2 plastic flask	1.0-2.0	30	6×10^7

*Size is given in oz only when it is commercially designated in that unit.

2.3.7 Incubate the inoculated cell cultures at room temperature for 80 minutes to permit viruses to adsorb onto and infect cells and then proceed immediately to Subsection 2.4.

It may be necessary to rock the vessels every 15-20 minutes during the 80 minute incubation to prevent cell death in the middle of the vessels from dehydration.

2.4 Procedure for Overlaying Inoculated Cultures with Agar

If there is a likelihood that a test sample will be toxic to cell cultures, the cell monolayer should be treated in accordance with the method described in Section VI.

2.4.1 To each cell culture test vessel, add the volume of warm (42-46°C) agar overlay medium appropriate for the cell surface area of the vessels used (see Table 3).

The preparation of the overlay agar and the agar overlay medium must be made far enough in advance that they will be at the right temperature for mixing at the end of the 80 minute inoculation period.

To prevent disturbing cells with the force of the liquid against the cell monolayer, add the agar overlay medium to the side of the cell culture test vessel opposite the cell monolayer.

2.4.2 Place cell culture test vessels, monolayer side down, on a level, stationary surface at room temperature (22-25°C) so that the agar will remain evenly distributed as it solidifies. Cover the vessels with a sheet of aluminum foil, a tightly woven cloth, or some other suitable cover to reduce the light intensity during solidification and incubation. Neutral red can damage or kill tissue culture cells by light-induced the crosslinking of nucleic acids.

Care must be taken to ensure that all caps on bottles and flasks are tight; otherwise, the gas seal will not be complete and an erroneous virus assay will result.

Agar will fully solidify within 30 minutes.

2.4.3 After 30 minutes, invert the cell culture test vessels and incubate them covered in the dark at 36.5 ± 1°C.

2.5 Virus Quantitation

2.5.1 Plaque counting technique.

(a) Count, mark, and record plaques in cell culture test vessels on days two, three, four, six, eight, and twelve after adding the agar overlay medium. Plaques should be counted quickly using a lightbox (Baxter, product no. B5080-1, or equivalent) in a darkened room. Most plaques should appear within 1 week.

Depending on the virus density and virus types present in the inoculated sample, rescheduling of virus counts at plus or minus one day may be necessary. Virus titers are calculated from the total plaque count. Note that not all plaques will be caused by viruses. The presence of virus must always be confirmed using the procedure in Section VII.

(b) Examine cell culture test vessels on day sixteen.

If no new plaques appear at 16 days, discard the vessels; otherwise, continue to count, mark, and record plaques every 2 days until no new plaques appear between counts.

Inoculated cultures should always be compared to uninoculated control cultures so that the deterioration of the cell monolayers is not recorded as plaques.

Samples giving plaque counts that are greater than 2 plaques per cm^2 should be diluted and replated.

2.5.2 Calculation of virus titer.

(a) If the entire remaining portion of the test sample was inoculated onto BGM cultures as described in Subsection 2.3.5(d), sum the total number of plaques in all test vessels for each sample. Multiply the sum by 0.8 to obtain the titer in PFU per 4 g of total dry solids. Average the titer of all composite samples and report the average titer and the standard deviation for each lot of sludge tested.

(b) If the sample was diluted due to high virus levels (e.g., when the virus density of the input to a process is being determined), calculate the virus titer (V) in PFU per 4 g total dry solids with the formula: $V = 0.8 \times (P/I) \times D \times S$, where P is the total number of plaques in all test vessels for each sample, I is the volume (in mL) of the dilution inoculated, D is reciprocal of the dilution made on the inoculum before plating, and S is the volume of the remaining portion of the test sample (as recorded in Subsection 2.3.5(d)). Average the composite samples and report as in step (a).

VI. METHOD FOR THE REDUCTION OF CYTOTOXICITY IN SAMPLE CONCENTRATES[7]

The procedure described in this section reduces the cytotoxicity of cytotoxic sludge samples. However, the procedure may result in a titer reduction of up to 30% and should be applied only to inocula known to be or expected to be toxic.

1. VIRUS RECOVERY AND ASSAY OF SAMPLES

Process and store sludge samples as described in Section III. Inoculate samples onto cell monolayers as described in Section V, Subsection 2.3.

2. REDUCTION OF TOXICITY OF SAMPLE CONCENTRATE

2.1 Apparatus and Materials

2.1.1 Cell culture bottles.

See Section IV for the preparation of cell culture bottles.

[7]Modified from EPA/600/8-84/013(R8), April 1986 Revision.

2.2 Media and Reagents

2.2.1 Washing solution.

(a) To a flask containing a stir bar and an appropriate volume of ddH_2O, add NaCl to a final concentration of 0.85% (weight/volume; e.g., 0.85 g in 100 mL). Mix the contents of the flask on a magnetic stirrer at a speed sufficient to dissolve the salt. Remove the stir bar and autoclave the solution at 121°C for 15 minutes. Cool to room temperature.
The volume of the NaCl washing solution required will depend on the number of bottles to be processed and the cell surface area of the bottles used for the plaque assay.

(b) Add 2% (volume/volume; e.g., 2 mL per 100 mL) serum to the sterile salt solution. Mix thoroughly and store at 4°C.
Although the washing solution may be stored at 4°C for an extended time period, it is advisable to prepare solutions on a weekly basis, thereby lessening the possibility of microbial contamination.

2.3 Procedure for Cytotoxicity Reduction

2.3.1 Decant and discard the inoculum from inoculated cell culture bottles after the 80 minute inoculation period. Add 0.25 mL of the washing solution (Subsection 2.2.1(b)) for each cm^2 of cell surface area into each bottle.
To reduce thermal shock to cells, warm the washing solution to 36.5 ± 1°C before placing it on the cell monolayer.
To prevent disturbing cells with the force of liquid against the cell monolayer, add washing solution to the side of the cell culture bottle opposite the cell monolayer. Also, avoid touching either the cannula or syringe needle of the pipette or the pipetting device to the inside rim of the cell culture bottles to avert the possibility of transporting contaminants to the remaining culture bottles.

2.3.2 Rock the washing solution gently across the cell monolayer a minimum of 2 times. Decant and discard the spent washing solution in a manner that will not disturb the cell monolayer.
It may be necessary to gently rock washing solution across the monolayer more than twice if the sample is oily and difficult to remove from the cell monolayer surface.

2.3.3 Continue by performing the agar overlay procedure for plaque assay in Section V, Subsections 2.4 and 2.5.
If this procedure fails to reduce cytotoxicity with a particular type of sludge sample, eluates prepared from another lot of the sludge sample may be diluted 1:2 to 1:4 before repeating the procedure. This dilution requires that 2-4 times more culture vessels be used.

3. DETERMINATION OF CYTOTOXICITY

Uninoculated cell cultures should always be processed to serve as controls in later comparisons to determine the reduction in sensitivity or survival of the BGM cells attributable to the toxicity of the samples.

3.1 Determine cytotoxicity by macroscopic examination of the appearance of the cell culture monolayer (compare control from Section V, Subsection 2.3.5(a) with test samples) after 3-5 days of incubation at 36.5 ± 1°C. Cytotoxicity should be suspected when the agar color is more subdued, generally yellow to yellow-brown. This change in color results in a mottled or blotchy appearance instead of the evenly diffused "reddish" color observed in "healthy" cell monolayers.

3.2 Cytotoxicity may also cause viral plaques to be reduced in number or to be difficult to distinguish from the surrounding monolayer. To determine if this type of cytotoxicity is occurring, compare the two types of positive controls (see Section V, Subsection 2.3.5(c) and (d)).

VII. VIRUS PLAQUE CONFIRMATION PROCEDURE

1. INTRODUCTION

This section describes a procedure for confirming virus plaques in cell cultures adhering to glass or plastic surfaces. Where large numbers of plaques are observed and confirmation of each plaque is not practical, select at least 10 well-separated plaques per sample or 10% of the plaques in a sample, whichever is greater.

2. RECOVERY OF VIRUS FROM PLAQUE

2.1 Apparatus, Materials, and Reagents

2.1.1 Pasteur pipettes, disposable, cotton plugged — 229 mm (9 inches) tube length and rubber bulb — 1 mL capacity.

Flame each pipette gently about 2 cm from the end of the tip until the tip bends to an approximate angle of 45°. Place the pipettes into a 4 L beaker covered with aluminum foil and dry heat sterilize for not less than 1 hour at 170°C.

2.1.2 16 x 150 mm cell culture tubes containing BGM cells.

See Section IV, Subsection 5 for the preparation of cell culture tubes.

2.1.3 Tissue culture roller apparatus — 1/5 rpm speed (New Brunswick Scientific, product no. TC-1, or equivalent) with culture tube drum for use with roller apparatus (New Brunswick Scientific, product no. ATC-TT16, or equivalent).

2.1.4 Freezer vial, screw-capped (with rubber insert) or cryogenic vial — 0.5-1 dram capacity.

2.2 Procedure for Virus Confirmation

2.2.1 Procedure for obtaining viruses from plaque.

A decision to test the plaque material for viruses immediately or to store the material at -70°C for later testing must be made before proceeding further. Whenever possible, the plaque material should be tested immediately, because storage at -70°C may result in some reduction in confirmation counts.

(a) Place a rubber bulb onto the upper end of a cotton-plugged pasteur pipette and then remove the screw-cap or stopper from a plaque bottle.

(b) Squeeze the rubber bulb on the pasteur pipette to expel the air and penetrate the agar directly over the edge of a plaque with the tip of the pipette. Gently force the tip of the pipette through the agar to the surface of the vessel, and scrape some of the cells from the edge of the plaque.

Repeatedly scratch the surface and use gentle suction to ensure that the virus-cell-agar plug enters the pipette.

(c) Remove the pipette from the plaque bottle and tightly replace the cap or stopper.

If the sample is to be tested in cell culture immediately, proceed to Subsection 2.2.2(b). If sample must be stored, proceed to Subsection 2.2.2(c).

2.2.2 Procedure for inoculating viruses obtained from plaques onto cell cultures.

(a) Cell culture processing.

If at all feasible, use a laminar flow hood while processing cell cultures. Otherwise, use an area restricted solely to cell culture manipulations. Viruses or other microorganisms must not be transported, handled, or stored in cell culture transfer facilities.

(a.1) Prepare plaque confirmation maintenance medium by adding 5 mL of serum and 5 mL of ddH_2O per 90 mL of antibiotic-supplemented ELAH (see Section V, Subsection 2.2.2) on day samples are to be tested.

(a.2) Pour the spent medium from cell culture tubes and discard the medium. Replace the discarded medium with 2 mL of the plaque confirmation maintenance medium. Label the tubes with sample and plaque isolation identification information.

To prevent splatter, a gauze-covered beaker may be used to collect spent medium.

To reduce shock to cells, warm the maintenance medium to 36.5 ± 1°C before placing it on the cell monolayer.

To prevent disturbing cells with the force of the liquid against the cell monolayer, add the maintenance medium to the side of the cell culture test tube opposite the cell monolayer. Note that cells will be only on the bottom inner surface of the culture tube relative to their position during incubation.

(b) Procedure for samples tested immediately.

(b.1) Remove the cap from a cell culture tube and place the tip of a pasteur pipette containing the virus-cell-agar plug from Subsection 2.2.1(c) into the maintenance medium in the cell culture tube. Force the virus-cell-agar plug from the pasteur pipette by gently squeezing the rubber bulb. Withdraw and discard the pipette, and replace and tighten down the screw-cap on the culture tube.

Tilt cell culture tube as necessary to facilitate the procedure and to avoid scratching the cell sheet with the pipette.

Squeeze bulb repeatedly to wash contents of pipette into the maintenance medium.

(b.2) Place the cell culture tube in the drum used with the tissue culture roller apparatus along with three additional culture tubes which have not been inoculated with agar sample to serve as negative controls. Add 0.1 mL of ELAH containing 20-40 PFU of polivirus to each of three more culture tubes to serve as positive controls.

(b.3) Incubate the cell cultures at 36.5 ± 1°C while rotating at a speed of 1/5 rpm. Examine the cells daily microscopically for 1 week, starting with day 3, for evidence of cytopathic effects (CPE).

CPE may be identified as cell disintegration or as changes in cell morphology. Rounding-up of infected cells is a typical effect seen with enterovirus infections. However, uninfected cells round up during mitosis and a sample should not be considered positive unless there are significant clusters of rounded-up cells over and beyond what is observed in the uninfected controls. If there is any doubt about the presence of CPE or if CPE appears late (i.e., on day 6 or 7), the conformation process should be repeated by transferring 0.2 mL of the medium in the culture tube to a freshly prepared tube.

Incubation of BGM cells in roller apparatus for periods greater than 1 week is not recommended as cells under these conditions tend to die-off if held longer.

Tubes developing CPE may be stored in a -70°C freezer for additional optional tests (e.g., the Lim Benyesh-Melnick identification procedure).[8]

(c) Procedure for samples to be stored at -70°C before testing.

Place 0.1 mL of antibiotic supplemented ELAH from Section V, Subsection 2.2.2 in a freezer vial for each plaque to be confirmed.

[8]For more information see EPA/600/4-84/013(R12), May 1988 Revision.

(c.1) Remove cap from vial containing ELAH. Place the tip of the pasteur pipette containing the virus-cell-agar plug from Subsection 2.2.1 Step (c) into the vial. Force the virus-cell-agar plug from the pasteur pipette into the ELAH by gently squeezing the rubber bulb.
Squeeze the bulb repeatedly to wash the contents of the pipette into the ELAH.

(c.2) Withdraw the pipette from the vial, replace and tighten down the screw-cap, and discard the pipette. Store the vial at -70°C. Place 3 additional vials containing 0.1 mL of ELAH that have not been inoculated with an agar sample to serve as negative controls and 3 vials containing 0.1 mL of ELAH with 20-40 PFU of poliovirus to serve as positive controls at -70°C.

(c.3) When confirmation is to be completed, prepare cell culture tubes in accordance with Step (a) above and thaw each frozen sample quickly in warm water (30-37°C).

(c.4) Remove the caps from each tube and transfer the entire contents of the vial containing the thawed sample into a cell culture tube using a pipette.
Tilt cell culture tube as necessary to facilitate the procedure and to avoid scratching the cell sheet with the pipette.
Place the tip of the pipette into the maintenance medium in the cell culture tube. Squeeze the bulb repeatedly to wash any of the remaining test sample into the maintenance medium.

(c.5) Withdraw the pipette from the cell culture tube. Replace and tighten down the screw-cap on the tube, discard the pipette and sample vial, and continue with Step (b3) above.

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Appendix I
Analytical Method for Viable Helminth Ova*

388, 389, 390, 391, 392, 393. HELMINTH OVA

INTRODUCTION

Parasitic infections present a potential health risk associated with use of sludge due to the existence of highly resistant stages of the organisms and low infective doses. Ascaris ova are the most commonly isolated nematode ova in sludge. Others may include Trichuris, Toxocara, Hymenolepis and Taenia. In 1973, ascariasis was estimated to affect four million people in the United States. Ova from the parasitic helminths enter sewage from the feces of infected individuals.

Ascaris ova are probably the most resistant of the ova or cysts found in sludge. This fact, along with the common occurrence of Ascaris ova, make them a good indicator for the fate of parasites as a group. The described test procedure was developed for solid and semi-solid samples. It is not suitable for water or sewage. A total solids analysis is also required to express the final results as ova/g dry weight.

Procedure 388,389,390,391,392,393: Helminth Ova (11-25-91)

1. Scope and Application

   1.1 This procedure determines CSDLAC parameter numbers 388, Total Parasites; 389, Total Ascaris; 390, Viable Ascaris; 391, Trichuris; 392, Hymenolepis; 393, Toxocara.

   1.2 This procedure is applicable to composted sewage sludge and other solid and semi-solid materials.

2. Summary of Procedure

   2.1 This procedure identifies, quantifies and determines the viability of several types of ova from intestinal parasites. Solid samples are processed by blending with buffered water containing a surfactant. The blend is screened to remove large particles. The solids in the screened portion are allowed to settle out and the supernatant decanted off. The sediment is subjected to density gradient centrifugation using zinc sulfate (specific gravity 1.20). This flotation procedure yields a layer most likely to contain Ascaris and some other parasitic ova. Proteinaceous material is removed using an acid-alcohol/ether extraction step and the resulting concentrate is incubated at 26°C until control ova of Ascaris lumbricoides var. suum are fully embryonated. The concentrate is then microscopically examined for parasite ova using a Sedgwick-Rafter counting chamber.

*This is an expanded version of the Yanko (1987) method referenced in the Part 503 regulation. This expanded version provides additional detail and presents the method in a step-by-step fashion as provided in pages 393-1 to 393-6 of "Laboratory Section Procedures for the Characterization of Water and Wastes," 4th edition, published by the Sanitation Districts of Los Angeles County, Los Angeles, California, 1989.

3. Sample Handling and Preservation

3.1 Solid samples are collected in sterile bags such as Whirl-Pak bags. Liquid sludge samples are collected in clean screw cap containers such as Nalgene bottles or jars.

3.2 Samples not analyzed promptly are stored at 0°C to 4°C.

4. Adavantages and Limitations

4.1 Concentration of the sample increases the probability that ova will be detected if they are in the sample.

4.2 Ascaris ova as an indicator is advantageous since they are relatively large and easy to identify.

4.3. Seeded studies have indicated the recovery for this test to be approximately ninety per cent.

4.4 The test uses standard microbiological equipment.

4.5 The test requires intensive training to enable the analyst to identify ova in a complex mixture of debris and to determine viability.

4.6 The test may take up to 5 weeks to complete including sample processing and incubation.

4.7 Numerous transfers of the sample to new vessels may decrease the recovery of indigenous ova.

5. Apparatus

5.1 Standard light microscope.

5.2 Sedgwick-Rafter cell.

5.3 2 L Pyrex beakers.

5.4 Table top centrifuge.

5.5 Rotor to hold four 100 mL centrifuge tubes, preferably glass or teflon.

5.6 Rotor to hold eight 15 mL conical centrifuge tubes, preferably glass or teflon.

5.7 48 mesh Tyler sieve.

5.8 Large plastic funnel to support sieve.

5.9 Teflon spatula.

5.10 Large test tube rack to accommodate 100 mL centrifuge tubes.

5.11 Small test tube rack to accommodate 15 mL conical centrifuge tubes.

5.12 Number "0" rubber stoppers.

5.13 Wooden applicator sticks.

5.14 Vacuum source.

5.15 Vacuum flask, 2 L or larger.

5.16 Stopper to fit vacuum flask fitted with glass or metal tubing as a connector for 1/4 inch tygon tubing.

5.17 Pasteur pipets.

5.18 Incubator at 26°C.

6. Reagents

6.1 Phosphate-buffered water. Prepare stock phosphate buffer solution by dissolving 34.0 g potassium dihydrogen phosphate (KH_2PO_4) in 500 mL distilled water, adjusting to pH 7.2 $\pm$ 0.5 with 1 N NaOH, and diluting to 1 L with distilled water.

Add 1.25 mL stock phosphate buffer solution and 5.0 mL magnesium chloride solution (81.1 g $MgCl_2.6H_2O$/L distilled water) to 1 L distilled water.

Prepare phosphate buffer working solution containing 0.1% (v/v) Tween 80. Adjust the pH to 7.2 $\pm$ 0.1 with 1 N NaOH.

6.2 Tween 80.

6.3 Zinc sulfate solution, sp. gr. 1.20. Weigh 454 g $ZnSO_4$ into 1 L DI H_2O. Dissolve and check specific gravity with a hydrometer. Adjust specific gravity to 1.2 as necessary.

6.4 0.1 N H_2SO_4 in 35% ethyl alcohol.

6.5 Ethyl ether, reagent grade.

7. Procedure

7.1 Weigh 50 g (wet weight) of compost and blend at high speed for 1 min. with 450 mL phosphate buffered water (PBW) containing 0.1 percent Tween 80 to achieve a ten percent suspension. If the sample is a liquid sludge, pour it directly into a blender jar (400 to 500 mL) and add Tween 80 to 0.1% v/v prior to blending as above. Record volume tested.

7.2 The % moisture of the sample is determined by the Analytical group on a separate portion of the sample for use in the final calculation of ova/g dry weight. The concentration of ova in liquid sludge samples may be expressed as ova per unit volume.

7.3 Pour the homogenized sample through a 48 mesh Tyler sieve held on a large funnel over a 2 L beaker.

7.4 Wash the sample through the sieve with several rinses of warm tap water. Washings are caught in the beaker.

7.5 Allow the screened and washed sample to settle overnight.

7.6 Siphon off the supernatant to just above the settled layer of solids.

7.7 Mix the settled material by swirling and then pour it into two 100 mL centrifuge tubes.

7.8 Rinse the beaker two or three times and pour the rinsings into two 100 mL centrifuge tubes.

7.9 Balance the tubes and centrifuge at 1250 RPM (400 x G) for 3 min.

7.10 Pour off the supernatant and resuspend the pellet thoroughly in zinc sulfate solution, specific gravity 1.20.

7.11 Centrifuge the zinc sulfate suspension at 1250 RPM for 3 min.

7.12 Pour the zinc sulfate supernatant into a 500 mL Erlenmeyer flask, dilute to at least half the concentration with deionized water, cover and allow to settle 3 h or overnight.

7.13 Aspirate the supernatant to just above the settled material.

7.14 Resuspend the sediment by swirling and pipette into two to four 15 mL conical centrifuge tubes.

7.15 Rinse the flask two to three times with deionized water and pipette the rinse water into the tubes.

7.16 Centrifuge the tubes at 1400 RPM (480 x G) for 3 min.

7.17 Combine the pellets into one tube and centrifuge at 1400 RPM for 3 min.

7.18 Resuspend the pellets in 7 mL acid alcohol solution (0.1 N H_2SO_4 in 35% EtOH) and add 3 mL of ether.

7.19 Cap the tube with a rubber stopper and invert several times, venting each time.

7.20 Centrifuge the tube at 1800 RPM (660 x G) for 3 min.

7.21 Resuspend the pellet in 4 mL 0.1 N H_2SO_4 and pour into Nalgene tubes with loose caps.

7.22 Incubate the tubes at 26°C for three to four weeks.

7.22.1 Simultaneously incubate control ova dissected from an adult Ascaris lumbricoides var. suum.

7.22.2 When the majority of control ova are embryonated, samples are ready to be examined.

7.23 Examine concentrates microscopically using a Sedgwick-Rafter cell to enumerate detected ova.

7.23.1 Note viability based on the presence of embryonated ova whose larval forms can be induced to move when the light intensity is increased.

7.23.2 Identify the ova and report as ova/g dry weight.

8. Calculation

8.1 Calculate % total solids using the % moisture result:

% Total Solids = 100% - % moisture

8.2 Calculate ova/g dry weight in the following manner:

$$\text{Ova/g dry wt} = \frac{(\text{No. ova}) \times (\text{Cell factor}/\#\text{ transects}) \times (\text{mL final volume})}{(\%\text{ conc}) \times (\text{mL sample screened}) \times}$$

(% total solids)

Where: Transect = One microscope field diameter width across the length of a Sedgwick-Rafter cell

Cell Factor = # of transects to examine entire Sedgwick-Rafter. This is dependent on microscope model and magnification.

Cell factor is also equal to the number of transects per mL since the Sedgwick-Rafter cell contains 1 mL.

9. Quality Assurance Guidelines

9.1 Run duplicate tests every tenth sample.

10. Precision and Accuracy

10.1 Precision criterion was established as per Standard Methods, 17th Ed., 1989, 9020B.4b, pp. 9-17 to 9-18.

10.2 The current established precision criterion is 0.5702.

10.3 There is currently no means of assessing the accuracy of the method.

11. References

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