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Pathogens and Vector Attraction in Sewage Sludge

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PATHOGENS AND VECTOR ATTRACTION IN SEWAGE SLUDGE

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FOREWORD

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Gregory Sayles, Ph.D., Director
Center for Environmental Solutions and Emergency Response

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ACRONYMS AND ABBREVIATIONS

EPA	Environmental Protection Agency
POTW	Publicly Owned Treatment Works
CWA	Clean Water Act
CFR	Code of Federal Register
PSRP	Processes that Significantly Reduce Pathogens
PFRP	Processes to Further Reduce Pathogens
EQ	Exceptional Quality
VAR	Vector Attraction Reduction
MPN	Most Probable Number
CFU	Colony Forming Units
PFU	Plaque Forming Units
QAPP	Quality Assurance Project Plan
SRT	Solids Retention Time
MCRT	Mean Cell Residence Time

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This document has been organized differently from the previous versions to help remove redundancy and duplication that existed in those versions, as well as improve the flow and allow the reader an easier time navigating through the document. In addition to formatting changes this document has been updated to clarify Alternatives 3 and 4 specifically for Class A pathogen reduction. The methods section has been updated to include the U.S. EPA methods for testing biosolids for the presence of fecal coliforms and *Salmonella.*

1: INTRODUCTION

1.1 Sewage Sludge and Regulations

Sewage sludge (often termed "biosolids") results from the treatment of domestic sewage in a wastewater treatment facility. When applied to land at the appropriate agronomic rate biosolids provide several benefits including nutrient and water addition, and soil structure enhancement[1]. Land application of biosolids also can have economic and waste management benefits including conservation of landfill space, reduction of demand on non-renewable resources like phosphorus and reduction of farm costs for fertilizers. There are over 14,600 publicly owned treatment works (POTWs) servicing over 238 million people across the U.S.[2] Additionally, there are more than 60 million people in the U.S. that have private sewage systems (septic systems)[3]. While data reported to EPA are limited, Figure 1 is based on 2019[4] electronic reporting to EPA[5] by over 2,200 facilities.

Biosolids Use & Disposal from POTWs in 2019

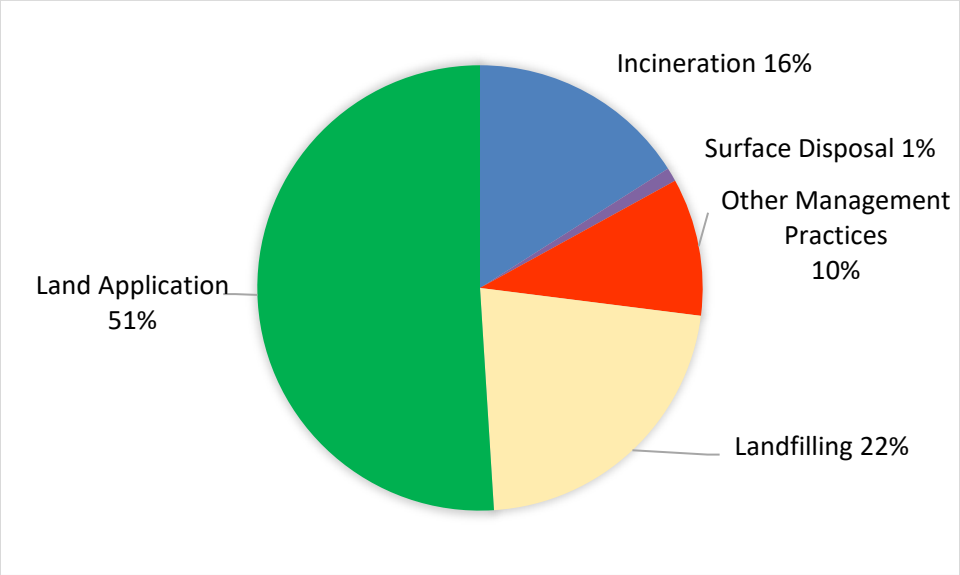

Figure 1. Distribution of biosolids use and disposal based on 2019 EPA electronic reporting data. It should be noted that smaller facilities treating less than 1 million gallons per day and private wastewater treatment facilities are not represented in Figure 1. However, according to a 2007 North East Biosolids and Residuals Association (NEBRA) report these facilities generate about eight percent of the total flow

[1] Various studies sited at: U.S. Department of Agriculture Agricultural Research Service.
[2] EPA Clean Watersheds Needs Survey 2012: Report to Congress.
[3] EPA Office of Wastewater Management.
[4] For facilities with a (1) Class 1 management facilities (any publicly owned treatment works (POTW) with approved pretreatment program); (2) major POTWs (POTWs with a design flow rate greater than or equal to one million gallons per day); and (3) POTWs that serve 10,000 people or more or otherwise required to report by EPA or permitting authority.
[5] EPA Enforcement and Compliance History Online.

generated in the United States[6,7]. These smaller treatment facilities tend to store solids in lagoons, transport untreated solids to larger wastewater treatment plants, and generally use the lowest-cost and easiest methods of disposal such as landfilling given the economies of scale involving beneficial use of biosolids.

Biosolids can be applied to agricultural land (e.g., pastures and cropland), disturbed areas (e.g., mined lands and construction sites), plant nurseries, forests, recreational areas (e.g., parks and golf courses), cemeteries, highway and airport runway medians, and home lawns and gardens.

Section 405(d) of the Clean Water Act (CWA) requires EPA to:

- *Establish numeric limits and management practices that protect public health and the environment from the reasonably anticipated adverse effects of chemical and microbial pollutants during the use or disposal of sewage sludge.*
- *Review biosolids (sewage sludge) regulations every two years to identify additional toxic pollutants that occur in biosolids (i.e., biennial reviews) and set regulations for those pollutants if sufficient scientific evidence shows they may harm human health or the environment.*

As required by Section 405(d) of the CWA, EPA developed a regulation to protect public health and the environment from any reasonably anticipated adverse effects of pollutants that might be present in sewage sludge. This regulation, *The Standards for the Use or Disposal of Sewage Sludge,* was published on February 19, 1993 (58 FR 9248).

The Standards for the Use or Disposal of Sewage Sludge, found in 40 CFR Part 503 or "Part 503", establishes requirements for the final use or disposal of sewage sludge when it is: 1) applied to land as a fertilizer or soil amendment; 2) placed in a surface disposal site, e.g. sewage sludge-only landfills; or 3) incinerated in a sewage sludge incinerator. Sewage sludges that are used as alternative daily, intermediate, or final cover at municipal solid waste landfills are regulated under 40 CFR Part 258. 40 CFR Part 503, Subpart B land application requirements include:

- Applicability (§ 503.10)
- Special definitions (§ 503.11)
- General requirements (§ 503.12)
- Pollutant limits (§ 503.13)
- Management practices (§ 503.14)

[6] NEBRA (July 20, 2007) 'A national biosolids regulation, quality, end use & disposal survey', North East Biosolids and Residuals Association, Tamworth, NH. Available at: https://static1.squarespace.com/static/54806478e4b0dc44e1698e88/t/5488541fe4b03c0a9b8ee09b/1418220575693/NtlBiosolidsReport-20July07.pdf

[7] https://www.biosolidsdata.org

- Operational standards – pathogens and vector attraction reduction (§ 503.15)
- Frequency of monitoring (§ 503.16)
- Recordkeeping (§503.17)
- Reporting (§ 503.18)

All Part 503 requirements apply to publicly- and privately-owned treatment works that generate or treat domestic sewage sludge and to anyone who uses or disposes of sewage sludge. The requirements of Part 503 are self-implementing and must be followed even without the issuance of a permit. In addition, persons using or disposing biosolids are subject to state and potentially county or local biosolids management regulations.

Complete information on requirements for pathogen and vector attraction reduction can be found in 40 CFR Part 503, Subpart D. These requirements are designed to reduce the presence of, and potential for contact with, the disease bearing microorganisms (pathogens) in sewage sludge applied to the land or placed on a surface disposal site. Requirements are divided into two areas:

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

This document is intended as a resource to pathogen and vector attraction reduction for anyone involved with the treatment of sewage sludge for land application. For information on other land application requirements (applicability, special definitions, general requirements, pollutant limits, management practices, frequency of monitoring, recordkeeping, and reporting) please consult Part 503.

1.2 Definitions

Terms used throughout this guidance are consistent with Part 503, however terminology not defined by Part 503 also may be used. Examples include, but are not limited to, the term "biosolids", operational parameters and biosolids management. The following glossary provides common terms used in this document:

Applier - The applier is the individual or party that land applies treated sewage sludge (biosolids). This may include farmers, municipalities, private enterprises and contractors of these parties.

Biosolids - Biosolids are *treated* sewage sludge that meet Part 503 requirements for land application. Typically intended to be land applied as a soil amendment or fertilizer.

Exceptional Quality (EQ) Biosolids - Class A "Exceptional Quality" or "EQ" biosolids is treated sewage sludge that meets the pollutant concentrations in § 503.13(b)(3), the Class A pathogen requirements in § 503.32(a) and one of the vector attraction reduction requirements in §§ 503.33(b)(1) through (b)(8). As such, Class A EQ biosolids meets the most stringent pollutant, pathogen, and vector attraction reduction requirements under EPA's regulations. Biosolids in this category are not subject to the Part 503 general

requirements (§ 503.12) and management practices (§ 503.14) for land application. It should be noted that the term "Exceptional Quality (EQ)" is not used in Part 503 (Table 1.0), however it is commonly understood within the regulatory and regulated sectors.

Table 1.0 Minimum Requirements for Land Application

	Pollutant Limit Requirement §503.13(a)(1)-(a)(4) and §503.13(b)(1)-(b)(4)		**Pathogen Requirements §503.13(a) §503.32(a)(3)-(a)(8) and §503.32(b)(2)-(b)(4)**	**Vector Attraction Reduction Requirement §503.15(c) §503.33(b)(1)-(b)(10)**
Class A Exceptional Quality (EQ)	Ceiling Concentrations (a)(1)	Pollutant Concentration (b)(3)	Any Class A Alternative (a)(3)-(a)(8)	Any Alternative 1-(b)(1)-(b)(8)
Class A	Ceiling Concentrations (a)(1)	Pollutant Concentration (a)(2)-(a)(4)or(b)(3)	Any Class A Alternative (a)(3)-(a)(8)	Alternative 9 or 10 (b)(9)or (b)(10)
		Cumulative Pollutant Loading Rates (a)(4) or (b)(4)		Any Alternative 1-10(b)(1)-(b)(10)
		Annual Pollutant Loading Rates (a)(4) or (b)(4)		Any Alternative 1-8 (b)(1)-(b)(8)
Class B	Ceiling Concentrations (a)(1)	Pollutant Concentration (a)(2)-(a)(4) or (b)(3)	Any Class B Alternative (b)(2)-(b)(4)	Any Alternative 1-10 (b)(1)-(b)(10)
		Cumulative Pollutant Loading Rates (a)(2) or (b)(2)		Any Alternative 1-10 (b)(1)-(b)(10)

Class A Biosolids – Sewage sludge intended for land application that has been either treated or tested for the presence of microbial pathogens through the use of process indicator organisms e.g. fecal coliforms or salmonella, enteric viruses, and viable helminth ova. The term Class A biosolids typically refers only to the pathogen monitoring and / or treatment of the sewage sludge.

Class B Biosolids – Sewage sludge intended for land application that has been either treated or tested for the presence of microbial pathogens. Class B materials when land applied must adhere to management practices, including public access, crop harvest, and animal grazing restrictions once they are land applied because they could contain pathogens. The term Class B biosolids typically refers to the level of pathogen destruction or treatment.

Detectable Limits - Minimum measured concentration at which an analyte can be detected. The detectable limit for any given analyte varies depending on the lab methodology used and the volume of material analyzed.

Domestic Septage - Domestic septage is either liquid or solid material removed from a septic tank, cesspool, portable toilet, Type III marine sanitation device, or similar treatment works that receives only domestic sewage. Domestic septage does not include liquid or solid material removed from a septic tank,

holding tank, cesspool, or similar treatment works that receives either commercial wastewater or industrial wastewater and does not include grease removed from a grease trap at a restaurant.

Domestic Sewage - Domestic sewage is waste and wastewater from humans or household operations that is discharged to or otherwise enters a treatment works.

Indicator Organism - A bacterium, group of bacteria, virus, or protozoa that are used to estimate the level of pathogens present. These organisms are used to help determine if the sewage sludge treatment was able to sufficiently reduce the microbial populations including potential pathogens. Fecal coliform is an indicator used in sewage sludge regulations.

Non-Public Contact Sites – Sites that are not frequently visited or used by the public such as agricultural land, forests, and reclamation sites.

Pathogen - A bacterium, virus, or other microorganism that can cause disease.

Preparer - The entity who prepares sewage sludge is either the person who generates biosolids during the treatment of domestic sewage in a treatment works or the person who changes the quality of sewage sludge received from a treatment works prior to land application or derives a material from sewage sludge (§ 503.9(r)). Any time the quality of sewage sludge is changed, a material is derived from sewage sludge and the preparer becomes a generator. Examples of materials derived from biosolids include, but are not limited to, the mixing of multiple sources of sewage sludge (as from wastewater treatment facilities), biosolids treated by composting (where sewage sludge is mixed with bulking agents or other admixtures), pelletizing, or drying, and mixtures of non EQ biosolids with other materials (e.g., biosolids blended with soil).

Process to Further Reduce Pathogens (PFRP) - PFRP terminology was used in the original sewage sludge regulations prior to the Part 503 regulations. A PFRP is a process that can consistently reduce the density of the microbial population including all bacterial, viral, and protozoan pathogens to below detectable levels. PFRP processes follow Class A criteria for pathogens, vector attraction reduction, and land application.

Process to Significantly Reduce Pathogens (PSRP) - PSRP terminology was used in the original sewage sludge regulations prior the Part 503 regulations. A PSRP is a process that can consistently reduce the density of the microorganisms in sewage sludge by 2 log or greater, or by a factor of 100. PSRP processes establish treatment parameters for Class B biosolids.

Product – The term “product” is sometimes used in this document in discussions regarding material distribution. A product is the treated sewage sludge (biosolids) for final use or disposal that has met Part 503 requirements. Product can refer to any biosolids that meet land application requirements. Treatment includes, but is not limited to, thickening, stabilization, and dewatering of sewage sludge.

Sewage Sludge – Sewage sludge is defined in Part 503 as “solid, semi-solid, or liquid residue generated during the treatment of domestic sewage in a treatment works. Sewage sludge includes, but is not limited to, domestic septage; scum or solids removed in primary, secondary, or advanced wastewater treatment processes; and any material derived from sewage sludge. Sewage sludge does not include ash generated

during the firing of sewage sludge in a sewage sludge incinerator or grit and screenings generated during preliminary treatment of domestic sewage in a treatment works". The distinction between untreated sewage sludge and treated sewage sludge (biosolids) is made throughout this document.

Vectors – Organisms such as insects and rodents, that can spread disease by carrying and transferring pathogens.

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2: SEWAGE SLUDGE PATHOGENS

2.1 Introduction

This chapter provides general information on pathogens associated with sewage sludge, biosolids and wastewater treatment processes. Biosolids preparers should have a basic knowledge of microbiology so that they can understand what is expected to meet Part 503 requirements. Basic knowledge will also help address questions regarding pathogens and the protection of public health and the environment. The Part 503 regulations are designed to significantly reduce or eliminate the risk from pathogens present in treated sewage sludges.

2.2 Pathogens

A pathogen is an organism capable of causing disease to a host. For that capability to be realized, three main criteria must be met: a susceptible host, a route of exposure, and an infectious agent. Without meeting these criteria, exposure to a pathogen will have no effect on human health. Treatment processes and management practices described in this document protect public health by addressing one or more of the criteria required for disease to occur. Throughout this document, "pathogen" refers only to living organisms, except where specified.

Pathogens that propagate in the enteric or urinary systems of humans and are discharged in feces or urine pose the greatest risk to public health regarding the management of sewage sludge and/or biosolids. Pathogens are also found in the urinary and enteric systems of other animals and may propagate in non-enteric settings. However, because this document is concerned with the regulation of sewage sludge and biosolids, this chapter focuses on the pathogens most found in the human enteric system.

2.3 Pathogens in Sewage Sludge

The four major types of human pathogenic organisms (bacteria, viruses, protozoa, and helminths) all may be present in domestic sewage. The actual species and quantity of pathogens present in the domestic sewage from a particular municipality (and the sewage sludge produced when treating the domestic sewage) depend on the health status of the local community and may vary substantially at different times. The level of pathogens present in treated sewage sludge (biosolids) also depends on the reductions achieved by the wastewater and sewage sludge treatment processes.

Wastewater treatment processes typically have 2 treatment trains which consist of the liquids also known as effluents, and the solids called sewage sludges. Pathogens can occur in both the liquids as well as settle out with the solids during wastewater treatment. Biological wastewater treatment processes such as lagoons, trickling filters, and activated sludge treatment may substantially reduce the number of pathogens in the wastewater (USEPA, 1989). These processes may also reduce the number of pathogens in sewage sludge by creating adverse conditions for pathogen survival.

Nevertheless, the resulting biological sewage sludges may still contain sufficient levels of pathogens to pose a public health and environmental concern. Table 2.1 lists some principal pathogens of concern that may be present in wastewater and 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.

Table 2.1 Principal Pathogens of Concern in Domestic Sewage 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
Ascaris suum	May produce symptoms such as coughing, chest pain, and fever due to worms migrating within the body
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 USEPA (1989).

2.4 Survivability of Pathogens

Wastewater generally contains significantly high concentrations of pathogens which may enter the wastewater system from industries, hospitals, and infected individuals. Wastewater treatment removes many of these pathogens from the influent through processes like solids settling and thereby

concentrating the pathogens in the sewage sludge. Pathogens that do not settle out with the solids remain in the liquid phase of wastewater and are further subjected to other processes used in secondary and tertiary treatments of wastewater. Like any other living organisms, pathogens thrive only under certain conditions. Outside of these set conditions survivability decreases. Each pathogen species has a different tolerance to different conditions; pathogen reduction requirements are therefore based on the need to reduce all pathogenic populations. Some of the factors that influence the survival of pathogens include pH, temperature, competition from other microorganisms, sunlight, contact with host organisms, proper nutrients, and moisture level. Table 2.2 shows a comparison of the survival of bacteria, viruses, and parasites in different sewage sludge treatments.

Table 2.2 Summary of the Effects of Sewage Sludge Treatment on Pathogens (Log Reductions Shown*)

PSRP Treatment	Bacteria	Viruses	Parasites (protozoa and helminths)
Anaerobic Digestion	0.5-4.0	0.5-2.0	0.5
Aerobic Digestion	0.5-4.0	0.5-2.0	0.5
Composting (PSRP)	2.0-4.0	2.0-4.0	2.0-4.0
Air Drying	0.5-4.0	0.5-4.0	0.5-4.0
Lime Stabilization	0.5-4.0	4.0	0.5

*A 1-log reduction (10-fold) is equal to a 90 percent reduction.
Class B processes are based on a 2-log reduction.

2.5 Units for Measuring Microorganisms

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. While the use of units of volume is sensible for wastewater, it is less sensible for sewage sludge. Many 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 requires that sewage sludge solids content always be determined when measuring microorganism densities.

Under Part 503, the density limits for the microorganisms are expressed as numbers of plaque forming units (PFUs), colony forming units (CFUs), or most probable number (MPNs) per 4 grams dry weight sewage sludge. 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 (e.g., 0.25/g or 0.75/g). However, density limits for fecal coliforms, the indicator organisms, are given on a "per gram" basis because these organisms are much more numerous than pathogens.

2.6 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 (numbers) 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 a sample. The sample is diluted at least once into tubes containing nutrient medium. The tubes are maintained under conditions favorable for bacterial growth. The original bacterial density in the sample is estimated based on the number of tubes that show growth and the level of dilution in those tubes.

2.7 Pathogen Reduction

Pathogen reduction can be achieved by treating sewage sludge prior to use or disposal and through natural attenuation. 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). Processes vary significantly in their effectiveness. For example, some processes (e.g., lime stabilization) may effectively reduce 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.

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

Treatment Approach	Effectiveness	Process Examples[a]
Application of 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 (biological and physical heat)[b]
Application of 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.
Application of chemical disinfectants	Substantially reduces bacteria and viruses and vector attraction. Probably reduces protozoan cysts. Does not effectively reduce helminth ova unless combined with heat.	Lime stabilization
Reduction of the sewage sludge's volatile organic content (the microbial food source).	Reduces bacteria. Reduces vector attraction.	Aerobic digestion Anaerobic digestion Composting[b]
Removal of 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 5 and 7 for a description of these processes. Many processes use more than one approach to reduce pathogens.

[b]Effectiveness depends on design and operating conditions.

2.8 Monitoring Indicator Species

Sewage sludge may contain numerous species of pathogenic organisms and conducting an analysis for each species is impractical and unnecessary. Therefore, the Part 503 microbiological requirements are largely based on the use of indicator organisms for the possible presence of pathogens. These requirements test for both the representative and hardiest of known viral, helminth, and bacterial species to represent the larger set of pathogens. The indicator and representative organisms are ones having been found to respond to treatment processes and environmental conditions in a similar manner to other organisms. Monitoring the levels of these organisms, therefore, provides information about the survival of the larger group.

The microbiological indicators that are required for monitoring under Part 503 include helminth ova, enteric virus, fecal coliforms and *Salmonella spp*. Helminth ova, enteric viruses, and *Salmonella spp*. are considered pathogens. These organisms are not always found in untreated or treated sewage sludge. Even though these organisms are not always present in sewage sludge, they remain as process indicators because they represent microbes that are diverse and can be environmentally resistant to treatments.

Fecal coliforms are enteric bacteria that are used as indicators for the presence of bacterial pathogens. Although fecal coliforms themselves are usually not harmful to humans, their presence indicates that pathogens may be present. They are abundant in human feces and therefore are always present in untreated sewage sludge. They are easily and inexpensively measured, and their densities decline in about the same proportion as enteric bacterial pathogens during sludge processing (USEPA, 1992).

Salmonella sp. may be found in untreated sewage sludges. *Salmonella* typically has a low infectious dose and can cause disease with ingestion of a lower amount of these organisms than many other bacterial pathogens. The problem with using *Salmonella sp.* as an indicator of treatment is that in many instances *Salmonella* occurs variably, and in such low numbers that it is below the detection limits for the methods used to isolate it from sewage sludge or biosolids. Using this indicator can give a false sense that the sewage sludge was adequately treated when there is no way of knowing if *Salmonella* was in the untreated material.

Tests required by Part 503 for helminth ova are employed to determine their presence and viability. The only helminth ova viability that can be determined is that of *Ascaris sp.,* the hardiest of known helminth species. It follows that if conditions are such that *Asacaris* cannot survive, the same conditions would prevent the survival of other helminth species such as *Toxacara*, *Trichuris*, and *Hymenolepis*.

Enteric viruses represent a class of many diverse different viruses that can be found in the intestinal system of humans. Collectively they are known as enteric viruses. Part 503 testing for viruses simultaneously monitors for several enterovirus species that are presumed to be good representatives for other types of enteric viruses. Enteric viruses are not the only viruses that are found in sewage sludge.

2.9 Bacteria Regrowth

One of the primary concerns for biosolids preparers is regrowth of pathogenic bacteria. Some bacteria are unique in that they can multiply outside of a host. The processes outlined in Part 503 and in this document have been demonstrated to reduce pathogens, but even very small populations of certain

bacteria can rapidly proliferate under the right conditions. For example, regrowth can occur in sewage sludge in which the competitive bacterial populations have been essentially eliminated through treatment. Some examples of these treatments include pasteurization, radiation, and some drying technologies. During these treatments many, if not all, the bacteria can be effectively killed off, and during those processes the carbon biomass is often broken down into smaller fragments which can become a food source for other and or surviving microorganisms. If these food sources come into contact with pathogens, they can cause exponential growth of the bacterial population (Chen et al. 2011, Gibbs, 2007, Qi, 2008, Sidu et al. 2001, Zaleski et al. 2005). Regrowth does not occur for viruses, helminths, and protozoa because they cannot regrow outside their specific host organism(s). Once reduced by treatment, their populations do not increase. Part 503 contains specific requirements designed to ensure that regrowth of bacteria has not occurred prior to use or disposal.

2.10 Potential Exposure to Pathogens

Humans and animals could be exposed to pathogens directly by contact with untreated or improperly treated sewage sludge, mishandled biosolids, or indirectly by consuming drinking water or food contaminated by sewage sludge pathogens. Insects, birds, rodents, and even farm workers could contribute to these exposure routes by transporting sewage sludge and biosolids including pathogens away from the site. Potential routes of exposure include:

2.11 Direct Contact

Examples of direct contact include:

- Touching the sewage sludge or biosolids which may lead to infection of the skin, or pathogens may find their way into the mouth through contaminated hands.
- Walking through an area such as a field, forest, or reclamation area shortly after biosolid application.
- Handling soil from fields where biosolids have been applied.

Direct contact could occur through an inhalation exposure of microbes that become airborne (via aerosols and dust) during sewage sludge spreading or by strong winds, plowing, or cultivating the soil after application (Pillai, 2007). However, additional studies show the exposure of inhalation due to land application of biosolids is minimal. (Herrmann et al. 2017, Tanner et al. 2008, Brooks et al. 2005)

2.12 Indirect Contact

Examples of indirect contact include:

- Consumption of pathogen-contaminated crops grown on biosolid-amended soil.
- Consumption of pathogen-contaminated milk or meat from animals contaminated by grazing in pastures or fed crops grown on biosolid-amended fields.
- Ingestion of drinking water or recreational waters contaminated by runoff from nearby land application sites or by organisms from biosolid migrating into ground-water aquifers.

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

Part 503 requirements place barriers in the pathway of exposure either by reducing the number of pathogens in biosolids or by preventing direct or indirect contact with any pathogens possibly present in the biosolids.

2.13 Site Restrictions

Treatment of Class A biosolids results in biosolid material that is pathogen free (see Chapter 4), therefore it can be distributed, and land applied without any restrictions to the public. Class B biosolids are not treated to the same extent as Class A, therefore a significantly reduced, but measurable number of pathogens may be present in these materials (see Chapter 6),Therefore management practices and site restrictions are required for Class B land application such that they can still be safely land applied. Class B materials used in conjunction with proper management practices provides the same safety level as land application with Class A biosolids.

While the site restrictions required in Part 503 are sufficient to protect the public from health impacts, workers exposed to Class B biosolids might benefit from several additional precautions. For example, dust masks should be worn for the spreading of dry materials and workers should wash their hands carefully after working with Class B biosolids. Other recommended practices for workers handling biosolids include (CDC, 2002):

- Wash hands before eating, drinking, smoking, or using the restroom.
- Use gloves when touching biosolids or sewage sludge or surfaces exposed to biosolids or sewage sludge.
- Remove excess sewage sludge or biosolids from shoes prior to entering an enclosed vehicle.
- Keep wounds covered with clean, dry bandages.
- If contact with biosolids or sewage sludge occurs, wash contact area thoroughly with soap and water.
- Trucks that are used in the transportation of Class B biosolids and then immediately used for transport of harvested crops should be thoroughly cleaned prior to loading the crops.

2.14 Vector Attraction Reduction

Insects, birds, rodents, and domestic animals may transport sewage sludge and pathogens from sewage sludge to humans, unless properly mitigated as per the regulations. Vectors may be attracted to sewage sludge as a food source, and the reduction of the attraction of vectors to sewage sludge are designed to prevent the spread of pathogens. This requirement is equally important as pathogen reduction and is a focus of Part 503. Vector attraction reduction (VAR) can be accomplished in two ways: 1) treating the sewage sludge to the point at which vectors will no longer be attracted to the sewage sludge; and 2) placing a barrier between the sewage sludge and vectors. VAR must occur simultaneously to the

pathogen reduction processes or after pathogen reduction. VAR is not permitted to be done prior to pathogen reduction to meet the requirements of Part 503. The technological and management options for vector attraction reduction are discussed in Chapter 9.

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3: DOMESTIC SEPTAGE

3.1 Introduction

This chapter discusses Part 503 pathogen reduction and vector attraction requirements for domestic septage. States and local entities may have additional and/or more stringent requirements.

3.2 Domestic Septage

Domestic septage is either liquid or solid material removed from a septic tank, holding tank, cesspool, portable toilet, Type III marine sanitation device, or similar treatment works that receives only domestic sewage. Domestic septage does not include liquid or solid material removed from a septic tank, cesspool, or similar treatment works that receives either commercial wastewater or industrial wastewater and does not include grease removed from a grease trap at a restaurant. If domestic septage is mixed with commercial septage it is no longer regulated under Part 503. Domestic septage contains a variety of the same pathogens present in wastewater treatment facilities, is typically more liquid in nature, and usually contains lower levels of heavy metals and other pollutants (USEPA 1996). Septage can contain valuable resources such as nitrogen and phosphrous that make land application of these residuals acceptable.

3.3 Pathogen Reduction

The Part 503 regulations cover land application of domestic septage to ensure further protection of public health and the environment. 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 treated sewage sludge (biosolids) applied to these types of land (Class A requirements). Separate, pathogen reduction requirements exist for domestic septage applied to non-public contact sites (e.g. agricultural land, forests, or reclamation sites). These requirements include site restrictions to reduce the potential for human exposure to domestic septage and to allow for pH adjustment or environmental attenuation with site restrictions only on harvesting crops. No pathogen requirements apply if domestic septage is placed on a surface disposal site.

Under Part 503.32(c), pathogen reduction in domestic septage applied to these non-public contact sites: agricultural land, forest, or reclamation sites[8] may be reduced in one of two ways:

- If no treatment of the septage is done prior to application the material must be directly injected into the soil or incorporated within six hours of application. This allows for the natural microbial population to further reduce the pathogens present in these materials.
- 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, The Part 503 regulation uses the term alkali in the broad sense to mean any substance that causes an increase in pH.

[8] Class 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.

If the pH is below 12, either initially or after 30 minutes, additional alkali material needs to 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 at least or greater than 12.

If domestic septage is not applied soon after pH adjustment and 30-minute latent period, it is recommended that the pH be retested and additional alkali be added to the domestic septage to raise the pH to 12, if necessary.

3.4 Vector Attraction Reduction

Domestic septage also must meet vector attraction reduction requirements (VAR). 40 CFR Part 503 lists 12 different options for meeting the VAR component of biosolids land application (Chapter 9). The choice of vector attraction options may affect the duration of site restrictions in some cases. Specifically, if Option 9 or 10 (injection or incorporation) is used to reduce vector attraction, the restriction on harvesting for food crops grown below the soil surface (e.g., potatoes and carrots) is increased from 20 months to 38 months for domestic septage.

Option 12 for vector attraction reduction applies to materials that are treated with alkali substances for pathogen reduction. This vector attraction reduction requirement is slightly less stringent than the alkali addition requirement 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 in 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. 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.

3.5 Site Restrictions

Domestic septage that is land applied outside of Class A treatments must also maintain site restrictions where the land is restricted for public access and other activities as outlined in Part 503. See chapter 6 for further details on site restrictions.

3.6 References

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4: CLASS A PATHOGEN REQUIREMENTS

4.1 Introduction

This chapter discusses the Class A pathogen requirements in Subpart D of 40 CFR Part 503. The implicit goal of the Class A pathogen requirements is to reduce all the pathogens present in sewage sludge (including enteric viruses, pathogenic bacteria, and viable helminth ova) to below detectable levels. Once Class A requirements are met along with pollutant limits and a suitable vector attraction reduction option materials are suitable for unrestricted use, including biosolids that are sold or given away in a bag or other container, bulk biosolids applied to a lawn or home garden and bulk biosolids applied to other types of land.

There are six alternative requirements for demonstrating Class A pathogen reduction. Two of these alternatives provide continuity with 40 CFR Part 257 (the regulation that governed sewage sludge prior to Part 503) 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 by using the organisms as listed in Table 4.1:

Table 4.1 Microbial indicators for Class A biosolids treatment

Microbial Indicator	Class A regulatory Limit
Fecal coliforms or *Salmonella* sp.	less than 1000 MPN per gram total solids (dry weight basis) less than 3 MPN per 4 grams total solids (dry weight basis)
Enteric viruses[9]	less than 1 PFU per 4 grams total solids (dry weight basis)
Viable helminth ova	less than 1 viable helminth ova per 4 grams total solids (dry weight basis)

For the following sections, the title of each section provides the number of the Subpart D requirement discussed in the section. The regulatory language can be found in Appendix B. Chapter 10 provides guidance on the sampling and analysis needed to meet the Class A microbiological monitoring requirements.

[9] 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 Part 503 regulation is to reduce all enteric viruses to less than 1 PFU per 4 grams total solids sewage sludge, this document refers to "enteric viruses" when discussing this requirement, although the detection method enumerates only enteroviruses.

Class A Product or Process

A product is considered Class A once it meets the pollutant limits, pathogen monitoring standards and one of the vector attraction reduction requirements.

Pathogen reduction methods must be met before vector attraction reduction or simultaneously. This order can't be modified.

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

Although vector attraction reduction (VAR) and pathogen reduction are separate requirements, they are often related steps of a process. Chapter 9 discusses the VAR options in greater detail.

The order of Class A pathogen reduction requirements in relation to VAR is a critical component of the regulations. Part 503.32(a)(2) requires that Class A pathogen reduction be accomplished prior or simultaneously to VAR. This requirement on the order of VAR and pathogen reduction is necessary to prevent the growth of bacterial pathogens after sewage sludge is treated. Contamination of biosolids with a bacterial pathogen after one of the Class A pathogen reduction alternatives has been conducted may result in extensive bacterial regrowth unless: a) an inhibitory chemical is present, b) the biosolids are too dry to allow bacterial growth, c) little food remains for the microorganisms to consume, or d) an abundant population of non-pathogenic bacteria is present. Vegetative cells of non-pathogenic bacteria suppress the growth of pathogenic bacteria by "competitive inhibition" due to competition for nutrients. It should be noted that vector attraction reduction by alkali addition [503.3(b)(6)] or drying [503.3(b)(7) and (8)] is based on the characteristic of the biosolids (pH or total solids) remaining elevated. Should the pH drop or the biosolids absorb moisture, the biosolids may be more hospitable to microorganisms, and pathogenic bacteria, if introduced, may grow. Therefore, it is required that biosolids treated with these methods be stored appropriately to maintain dryness and/or elevated pH.

Biological treatment processes like anaerobic digestion, aerobic digestion, and composting produce changes in the sewage sludge so that it satisfies one of the vector attraction reduction requirements [503.3(b)(1) through (5)]. These processes repress bacterial growth by minimizing the food supply and providing competition for the remaining food possibly used by non-pathogenic organisms. The pathogen reduction alternative must precede the vector attraction reduction process; otherwise, the large number of beneficial non-pathogenic bacteria would be killed, and growth of pathogenic bacteria could occur. Certain pathogen reduction processes such as composting accomplish VAR by a biological process simultaneously with thermal reduction of pathogens. A non-pathogenic bacterial community survives which adequately suppresses growth of pathogenic bacteria.

4.3 Monitoring of Fecal Coliform or Salmonella sp. to Detect Growth of Bacterial Pathogens [503.32(a)(3)-(8)]

The goal of Class A processes is to reduce the level of pathogens to below detectable levels. The Class A processes listed in 40 CFR Part 503 have been shown to sufficiently reduce pathogen levels in biosolids. Favorable conditions for the regrowth of pathogenic bacteria following Class A treatments include adequate moisture, absence of an inhibitory chemical, and inadequate reduction of nutrients in the treated sewage sludge.

Because Class A biosolids may be used without site restrictions, all Class A material must be tested to show that the microbiological requirements are met ***at the time when it is ready to be used or disposed***. In addition to meeting process requirements, Class A biosolids must meet one of the following requirements:

Either the density of fecal coliforms in the sewage sludge be less than 1,000 MPN[10] per gram total solids (dry weight basis), ***or*** the density of *Salmonella* sp. bacteria in the sewage sludge be less than 3 MPN per 4 grams of total solids (dry weight basis).

Although Part 503 does not specify the number of samples that should be taken to show compliance with Class A density requirements, sampling programs should provide adequate representation of the biosolids generated. Chapter 9 provides guidance for calculating the number of samples that should be taken per sampling event. Unlike Class B biosolids, compliance with Class A requirements is not based on a geometric mean value. ***Each sample analyzed must comply with the numerical requirements.***

The microbiological requirement must be met either:

- At the time of use or disposal[11], or
- At the time the biosolids are prepared for sale or give away in a bag or other container for land application, or
- At the time Class A EQ biosolids or material that meets Class A EQ requirements are derived from sewage sludge[12]

When a facility stores material before it is distributed for use or disposal, microbiological testing should take place after storage, just prior to application or distribution. This will ensure that the material meets the microbiological requirements for Class A materials upon application.

In each case, the timing represents the last practical monitoring point before the biosolids are applied to the land or placed on a surface disposal site. Biosolids that are sold or given away cannot be monitored

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

[11] Minus the time needed to test the biosolids and obtain the test results prior to use or disposal (see Chapter 10).

[12] See the applicability requirements in 40 CFR § 503.10 and the EPA memorandum *Land Application Requirements for Class A Exceptional Quality Treated Sewage Sludge* (November 5, 2020) for more information. See appendix J for the EPA memo.

just prior to actual use or disposal instead, monitoring is required immediately prior to bagging or distribution.

As discussed in Chapter 10, the timing of pathogen sampling is also a function of laboratory turnaround time. Obtaining results for fecal coliform and *Salmonella* sp. analysis may take several days if tests are performed in-house, but commercial labs may require more time to process and report results. It is not unusual for laboratories to have a turnaround time of 2 weeks, even for simple tests such as fecal coliform analysis. If this is the case, this time should be factored into the sampling program so that results can be obtained before biosolids are distributed for use or disposal.

4.4 Monitoring Fecal Coliforms or Salmonella sp.

Fecal coliforms are used in Part 503 as an indicator organism because reduction in fecal coliforms correlates to reduction in *Salmonella* sp. and other organisms. The requirements were based on experimental work by Yanko (1987) and correlations developed from Yanko's data by Farrell (1993) which show that low levels of fecal coliforms correlate with a very low level of *Salmonella* sp. detection in composted sewage sludge (USEPA, 1992).

Anecdotal reports suggest that some composting facilities may have difficulty meeting this requirement even when *Salmonella* sp. are not detected. This might be expected under several circumstances. For example, severe thermal treatments of sewage sludge during composting can eliminate *Salmonella* sp. yet leave residual fecal coliforms. If the sewage sludge has been poorly composted and thus is a good food source, fecal coliforms may grow after the compost cools down from thermophilic temperatures, however because the *Salmonella* sp. are absent, they cannot grow.

Chapter 2 mentions that *Salmonella* in most situations is not an ideal treatment indicator organism because it is found in low densities in most raw sludges, and therefore its absence in the treated sewage sludge does not correlate to proper treatment. Also, the methods for *Salmonella sp.* are not as robust and are variable compared to the methods for measuring fecal coliforms. While it may be possible to meet the regulatory requirements by testing and meeting the regulatory limits for *Salmonella* sp., it is recommended that the pathogen reduction process be reviewed to determine at what point fecal coliforms potentially are not being reduced or are being reintroduced into treated biosolids and ensure that process requirements are being fulfilled. Also, treated sewage sludge should not be tested for both *Salmonella* and fecal coliform and subsequently only report *Salmonella* values if fecal coliform levels are too high. Both values should be reported. High fecal coliform values would suggest the process is not robust enough to kill off all the bacterial pathogens.

4.5 Monitoring for Enteric Virus and Viable Helminth Ova

Alternatives 3, 4, and 6 require additional microbial indicator testing for enteric viruses and viable helminth ova. The specific methods that must be used for these tests are outlined in Chapter 10, as well as referenced in Appendices F and G. The method required for enteric viruses only enumerates enteroviruses which are presumed to be a good indicator for all enteric viruses. Since the objective of the Part 503 regulation is to reduce all enteric viruses to less than 1PFU per 4 grams total solids within the sewage sludge, this document refers to "enteric viruses" when discussing the method for this requirement. Tests for enteric viruses and viable helminth ova take a substantial amount of time. It can

take four weeks to determine whether helminth ova are viable, and two weeks or longer for enteric virus analysis results. In situations where analyses of these organisms are required by Part 503, the biosolids must be held and not land applied until the test results show compliance. This will require the ability to store material until the test results are available. Finding laboratories that perform these tests can also be a challenge, laboratories must be familiar with the tests allowed in the regulation. Deviation from these methods can invalidate the results and render the material to be out of compliance with the regulations.

4.6 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. Four different time-temperature regimes are included 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 (USEPA, 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 4.2. 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 Celsius degrees (5.4 Fahrenheit degrees) lower than the temperature calculated for the Regimes A through C equation.

The time-temperature relationships described for Alternative 1 are based on research conducted to correlate the reduction of various pathogens in sewage sludge to varying degrees of thermal treatment. The resulting time-temperature relationship which is the basis for Alternative 1 is shown in Figure 4-1. These requirements are similar to the FDA requirements for treatment of eggnog, a food product with flow characteristics similar to those of liquid sewage sludge. The Regimes A through D differ depending on the characteristics of sewage sludge treated and the type of process used because of the varying efficiency of heat transfer under different conditions.

Therefore, testing of temperatures throughout the sewage sludge mass and agitating the material to ensure uniformity is appropriate. For processes such as thermophilic digestion, it is important that the digester design not allow for short circuiting of untreated sewage sludge as this would render the digestor process out of compliance with Alternative 1.

These time-temperature regimes are not intended to be used for composting (the time-temperature regime for composting is covered in Alternative 5: Processes to Further Reduce Pathogens).

It is mandatory for all sewage sludge particles to meet the time-temperature regime.

Table 4.2 The Four Time-Temperature Regimes for Alternative 1 (Thermally Treated Sewage Sludge) [503.32(a)(3)]

Regime	Part 503 Section	Applies to	Required Time-Temperature[1] Relationship
A	503.32(a)(3)(ii)(A)	Sewage sludge with at least 7% solids (except those covered by Regime B)	$D = 131{,}700{,}000/10^{0.1400t}$ $t \geq 50^{o}C$ $(122^{o}F)$[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]	$D = 131{,}700{,}000/10^{0.1400t}$ $t \geq 50^{o}C$ $(122^{o}F)$[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	$D= 131{,}700{,}000/10^{0.1400t}$ $D \geq 1.74 \times 10^{-4}$ (i.e., 15 seconds) and $D \leq 0.021$ (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	$D = 50{,}070{,}000/10^{0.1400t}$ $t \geq 50^{o}C$ $(122^{o}F)$[2] $D \geq 0.021$ (i.e. 30 minutes)[7]

'D = time in days; t = temperature (^{o}C).

[2]The restriction to temperatures of at least $50^{o}C$ $(122^{o}F)$ 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 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.

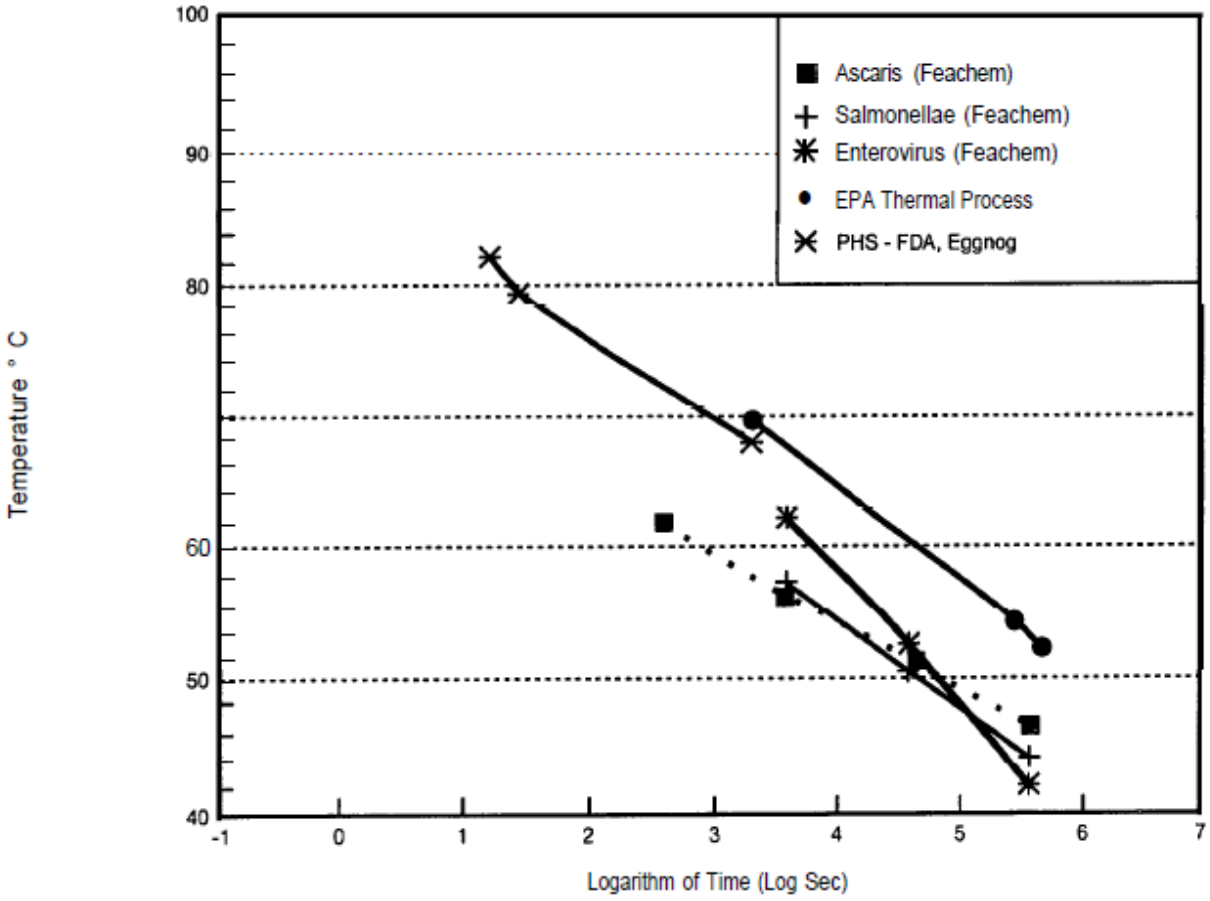

Figure 4.1 EPA's time-temperature relationship for thermal disinfection compared with time-temperature relationships.

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 a non-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 or plug flow operations, or even laminar flow in pipes.

Vector Attraction Reduction

Thermally treated sewage sludge must be treated by an additional vector attraction reduction process since thermal treatment does not necessarily break down the volatile solids in sewage sludge. Vector attraction reduction can be met by further processing the sewage sludge with pH adjustment or heat drying (Options 6 and 7), or by meeting one of the other options (Options 8 – 11). Options 1 through 5 are not applicable to thermally treated sludge unless the sludge was subject to biological digestion after or during thermal treatment (for example: Option 1 could be utilized for a thermophilic anaerobic or aerobic digestion if volatile solids reduction is satisfied across the digestion process).

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

This alternative describes conditions of a high temperature-high pH process that has proven effective in reducing pathogens to below detectable levels. The process conditions required by Part 503 are:

- Elevating pH to greater than 12 and maintaining the pH for more than 72 hours.
- Maintaining the temperature above 52°C (126°F) throughout the sewage sludge 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, high temperature, and reduced moisture 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 sewage sludge is measured at 25°C (77°F) or an appropriate correction is applied (see Section 10.7).

Because the elevated pH and temperature regimes must be met by the entire sewage sludge mass, operational protocols include monitoring pH and temperature at various points in a batch and agitating the sewage sludge during operations to ensure consistent temperature and pH are appropriate.

Vector Attraction Reduction

The pH requirement of VAR Option 6 is met when the pathogen requirement by Alternative 2 is achieved. Compliance with the pathogen requirement by Alternative 2 exceeds the pH requirements of VAR Option 6.

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

This alternative applies to sewage sludge treated by processes that do not meet the process conditions required by Alternatives 1 and 2 and can be used while testing under Alternative 6. 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 coliforms in the sewage sludge must be less than 1000 MPN per gram of total solids (dry weight basis), or the *Salmonella sp.* bacteria in sewage sludge must be less than three MPN per four grams of total solids (dry weight basis) at the time the sewage is used or disposed, at the time the sewage sludge is prepared for sale or given away in a bag or other container for land application, or at the time Class A EQ biosolids or material that meets Class A EQ requirements are derived from sewage sludge.
- The density of enteric viruses in the sewage sludge after pathogen treatment must be less than one PFU per four grams of total solids (dry weight basis).
- The density of viable helminth ova in the sewage sludge after pathogen treatment must be less than one per four grams of total solids (dry weight basis).

Testing for enteric viruses and viable helminth ova can be complicated by the fact that they are often 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 one PFU per four grams total solids (dry weight basis) and/or the density of viable helminth ova is greater than or equal to one per four grams total solids (dry weight basis), see section 4.15 for testing frequency. At this point, the treated sewage sludge is analyzed to see if these organisms survived treatment. If enteric virus densities are below detection limits, the sewage sludge meets Class A requirements for enteric viruses and will continue to do so if 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 helminth ova and will continue to do so if 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 helminth ova reduction have been successfully demonstrated (see Section 7.4). The minimum frequency of monitoring under this alternative is the same as the times listed in Table 1.1, however, it may be necessary to sample more frequently to adequately capture the operational conditions of the treatment process.

Vector Attraction Reduction

Meeting vector attraction reduction depends on the process by which pathogen reduction is met. For example, sewage sludge subjected to long-term storage may meet vector attraction reduction through volatile solids reduction (Options 1-3). Sewage sludges may also undergo additional processing or be applied following the requirement in Options 8-11.

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

Alternative 4 is intended to be utilized for treatment processes that are “unknown”. In these situations, there is no specific treatment regime that is applied that meets one of the other Class A Alternatives. This may include lagoons, or other processes with an undefined or inconsistent treatment process. Under this alternative the sewage sludge must meet the following limits at the time the biosolids (or material derived from sludge) are used or disposed, at the time the sewage sludge is prepared for sale or given away in a bag or other container for land application, or at the time Class A EQ biosolids or material that meets Class A EQ requirements are derived from sewage sludge.

- The density of enteric viruses in the sewage sludge must be less than one PFU per four grams of total solids (dry weight basis).
- The density of viable helminth ova in the sewage sludge must be less than one per four grams of total solids (dry weight basis).
- In addition, as for all Class A biosolids, the sewage sludge must meet fecal coliform or *Salmonella* sp. limits.

4.10 Vector Attraction Reduction

Like Alternative 3, meeting vector attraction reduction for Alternative 4 depends on the process by which pathogen reduction is met. For example, sewage sludge subject to long-term storage may meet vector attraction reduction through volatile solids reduction (Options 1-3). Sewage sludge may also undergo additional processing or be applied following the requirement in Options 8-11.

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

Alternative 5 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 Table 4.3. The material must also be tested for either fecal coliforms or *Salmonella sp*. The density of fecal coliforms in the sewage sludge must be less than 1,000 MPN per gram total solids (dry weight basis), or the density of *Salmonella* sp. bacteria in the sewage sludge less than 3 MPN per 4 grams total solids (dry weight basis). These tests need to be conducted at the time the sewage sludge is used or disposed or at the time the sewage sludge is prepared for sale or given away in a bag or other container for land application, or at the time Class A EQ biosolids or material that meets Class A EQ requirements are derived from sewage sludge.

Table 4.3 describes the sewage sludge treatment processes allowed under Alternative 5.

For all PFRP processes, the goal of temperature monitoring should be to represent all areas of a batch or pile and to ensure that temperature profiles from multiple points in the process all meet mandated temperatures. In some instances, it may be possible to monitor representative areas of a batch, pile, or reasonable worst-case area to ensure compliance. Chapter 5 contains more guidelines about the operation of PFRP processes.

Table 4.3. Processes to Further Reduce Pathogens (PFRPs) Listed in Appendix B of 40 CFR Part 503[1]

PFRP	PFRP Description
Composting	Using either the within-vessel composting method or the static aerated pile composting method, the temperature of sewage sludge is maintained at 55°C (131°F) or higher for 3 consecutive days. Using the windrow composting method, the temperature of the sewage sludge is maintained at 55°C (131°F) or higher for 15 consecutive 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.
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 bulb temperature of the gas in contact with the sewage sludge as the sewage sludge leaves the dryer exceeds 80°C (176°F).
Heat Treatment	Liquid sewage sludge is heated to a temperature of 180°C (356°F) or higher for 30 minutes.
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).
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]).
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]).
Pasteurization	The temperature of the sewage sludge is maintained at 70°C (158°F) or higher for 30 minutes or longer.

[1] Chapter 5 provides a detailed description of these technologies.

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

Under Alternative 6, sewage sludge is considered Class A sewage sludge if:

- It is treated by any process equivalent to a PFRP, and either the density of fecal coliforms 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 three MPN per four 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 Class A EQ biosolids or material that meets Class A EQ requirements are derived from sewage sludge[13].

Facilities that meet Alternative 6 for pathogen reduction must still meet vector attraction reduction requirements.

[13] See the applicability requirements in 40 CFR § 503.10 and the EPA memorandum *Land Application Requirements for Class A Exceptional Quality Treated Sewage Sludge* (November 5, 2020) for more information. See appendix J for the EPA memo.

4.13 Processes Recommended as Equivalent

Processes recommended to be equivalent to PFRP are shown in Table 8.1. Products of all equivalent processes must still meet the Class A fecal coliform or *Salmonella* sp. requirements.

4.14 Equivalency Determination

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 8).

4.15 Frequency of Testing

Part 503 sets minimum sampling and monitoring requirements. Table 1.1 in Chapter 1 describes the minimum frequency at which the sewage sludge must be sampled and analyzed for pathogens or vector attraction reduction to meet regulatory requirements. In addition to meeting these requirements, EPA recommends that sewage sludge generators consider the potential public health impacts and possible liability issues when designing a sampling program. In some cases, it may be appropriate to sample more frequently than what is required under Part 503.

4.16 Important Considerations for Class A treated Materials

Care must be taken that once pathogen reduction treatments are performed no other additives or additional treatments to the material that may potentially contain pathogenic microorganisms encounter the treated material. This includes common practices where wastewater influent or effluent is added back to the treated solids in the case of dewatering or prior to digestion. Influent or effluent is sometimes used as a diluent to decrease the solids content prior to or afterdigestion, or as a matrix that is used to reconstitute polymers that aid in dewatering. Typical wastewater influents or effluents are either not treated or treated using a process that would not comply with Class A sewage sludge pathogen requirements, as such they may contain pathogens or indicators that are above the allowable limits for Class A materials. In fact, wastewater effluent regulatory requirements use different process indicator organisms and different testing requirements from sewage sludges. Therefore, effluent that is mixed with Class A treated materials can compromise the integrity of the treated biosolids, by contaminating the product with pathogenic organisms. It is also not acceptable to use “treated wastewater effluent” for these purposes, unless the effluent is treated in a Class A manner as outlined in 40 CFR Part 503.

If the generator assumes they have a Class A product for pathogens, but recent testing results show that the product meets Class B standards for pathogens instead, then distribution of that product as Class A would constitute a violation of Part 503. Therefore, it is recommended that the product remains on site until lab results are available. Also, it is advisable to store biosolids in discrete batches and take multiple samples per sampling event. This will allow better identification of piles that may be out of compliance and will allow for the distribution of material that is correctly identified as Class A.

If pathogen testing shows that a product distributed as Class A material is actually a Class B product, entities that received the product should be notified. The facility may even consider recalling the biosolids from the users of that product.

If the biosolids product has already been distributed to public access areas, including homes, gardens, parks, or other public areas, the biosolids preparer may consider testing the soil. If the testing indicates unacceptable levels of pathogens, corrective actions may be necessary.

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5: PROCESSES TO FURTHER REDUCE PATHOGENS (PFRP)

5.1 *Introduction*

Processes to Further Reduce Pathogens (PFRPs) is terminology that was used in the previous sewage sludge regulations prior 40 CFR Part 503. This distinction was given to processes that were able to reduce pathogens below detectable levels. There are seven PFRPs specifically listed in Part 503: 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 the regulations, pathogenic bacteria, enteric viruses, and viable helminth ova are reduced to below detectable levels as defined in 40 CFR Part 503. In addition to these seven PFRP's listed in Part 503, the Pathogen Equivalency Committee (PEC) recommends alternative processes for PFRP to the proper permitting authority.

This chapter provides detailed descriptions of the seven PFRPs listed in Part 503, Alternative 5. Because the purpose of these processes is to produce Class A biosolids, the pathogen reduction process must be conducted concurrent to or prior to the vector attraction reduction process (see Section 4.2). Table 5.1 includes the PFRP approvals granted under Alternative 6 at the end of this chapter.

Under Part 503.32(a)(7), sewage sludge treated by PFRPs are Class A with respect to helminth ova, enteric viruses, and pathogenic bacteria. In addition, Class A biosolids must be monitored for fecal coliform or *Salmonella* sp. bacteria at the time of use or disposal, at the time the biosolids are prepared for sale or give away in a bag or other container for land application, or at the time the biosolids are prepared to meet the requirements for "exceptional quality" sludge in 503.10(b),(c),(e) or (f) to ensure that growth of bacteria has not occurred (see Section 4.3, Appendix J). As mentioned earlier in Chapter 4 microbial sampling for all Class A products including PFRPs must occur as close to the time of use and disposal as possible. Part 503 doesn't list exact times for testing because sewage sludge processing and land application events vary, and this allows for flexibility that is necessary to ensure public health is maintained during land application events. It is also worth noting that the addition of all additives to the sewage sludge process such as anti-dust sprays, polymers, and dewatering methods should occur prior to sampling events, because the addition of these products or processes may impact the microbial quality of the material.

5.2 Composting

Composting is the controlled, aerobic decomposition of organic matter which produces a humic-like material. Sewage sludge that is composted is generally mixed with a bulking agent such as wood chips to allow air to pass more easily through the composting material, thereby creating aerobic conditions. 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 three consecutive days.

- Using the windrow composting method, the temperature of the sewage sludge is maintained at 55°C (131°F) or higher for 15 consecutive 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.

For aerated static pile and in-vessel composting processes, temperatures should be taken at multiple points at a range of depths throughout the composting medium. Points which are likely to be slightly cooler than the center of the pile, such as the toes of piles, also should be monitored. Because the entire mass of sewage sludge must attain the required temperatures for the required duration, the temperature profiles from every monitoring point, not just the average of the points, should reflect PFRP conditions.

It has been found that points within 0.3 m (1 foot) of the surface of aerated static piles may be unable to reach PFRP temperatures, and for this reason it is recommended that a 0.3 m (1 foot) or greater layer of insulating material be placed over all surfaces of the pile. Finished compost is often used for insulation. It must be noted that because the insulation will most likely be mixed into the composted material during post-processing or curing, compost used as an insulation material must be a Class A material so as not to reintroduce pathogens into the composting sewage sludge.

For windrow composting, the operational requirements are based on the same time-temperature relationship as aerated static pile and in-vessel composting. The material in the core of the windrow attains at least 55°C and must remain at that temperature for three consecutive days. Windrow turning moves new material from the surface of the windrow into the core so that this material may also undergo pathogen reduction. After five turnings, all material in the windrow must have spent 3 days at the core of the pile. The time-temperature regime takes place over a period of at least 15 consecutive days during which time the temperature in the core of the windrow is at least 55°C. See Appendix H for additional guidance.

Pathogen reduction is a function of three parameters:

- Ensuring that all sewage sludge is mixed into the core of the pile at some point during active composting

- Ensuring that all sewage sludge particles spend 3 consecutive days in the core during which time the temperatures are at 55°C

- Preventing growth of pathogenic bacteria in composted material

Ensuring that all material is mixed into the core of the pile, depends on the configuration of the windrows and the turning methodology. Pile size and shape as well as material characteristics determine how much of the pile is in the “hot zone” at any given time. Additional turning and maintenance of temperatures

after the mandated 15 days are recommended, depending on the windrow configuration. For example, the Los Angeles County Sanitation District found that as many as 12-15 turnings were necessary to reduce pathogens in windrow composted sewage sludge (Personal Communication, Ross Caballero, Los Angeles County Sanitation District, 1998).

It is important that once that material is in the pile core it be subject to the full time-temperature regime necessary to reduce pathogens. Therefore, the turning schedule and the recovery of the core zone to 55°C are important factors. If pile turning is not evenly distributed throughout the 15-day period, some material may not spend adequate time in the core of the pile. Additionally, pile temperatures generally drop off immediately after turning; if temperatures in the pile core do not quickly recover to 55°C (within 24 hours), *the necessary pathogen reduction period of 3 days will not be achieved.*

Because of the operational variability, pathogen reduction in windrow composting has been found to be less predictable than pathogen reduction in aerated static pile or in-vessel composting. In order to improve pathogen reduction, the following operational guidelines are recommended.

- Windrow turning should take place after the pile core has met pathogen reduction temperatures for three consecutive days. Windrow turnings should be evenly spaced within the 15 days so that all material remains in the core zone for three consecutive days; allowing additional time as needed for the core temperature to come up to >55°C.

- Pathogen reduction temperatures (55°C) must be met for 15 *consecutive* days at the pile core.

- Temperatures should be taken at approximately the same time each day in order to demonstrate that 55°C has been reached in the pile core within 24 hours after pile turning.

- Testing frequency should be increased; a large sewage sludge windrow composting operation recommends testing each windrow for *Salmonella* sp. before piles are distributed (Personal Communication, Ross Caballero, Los Angeles County Sanitation District, 1998). Samples are taken after turning is completed, and piles which do not comply with Class A requirements are retained on site for further composting.

Vector Attraction Reduction

VAR Option 5 is the most appropriate for composting operations. This option requires aerobic treatment (e.g., composting) of the sewage sludge for at least 14 consecutive days at over 40°C (104°F) with an average temperature of over 45°C (113°F). This is usually easily attained by sewage sludge composting.

The PFRP and VAR requirements can be met concurrently in composting. For within-vessel or aerated static pile composting, the temperature profile should show PFRP temperatures at each of the temperature monitoring points for three consecutive days, followed by a minimum of 11 more days during which time the average temperature of the pile complies with VAR requirements. For windrow piles, the compliance with PFRP temperatures will also fulfill VAR requirements.

PFRP temperatures should be met before or at the same time that VAR requirements are fulfilled in order to reduce the potential for pathogen regrowth. However, continued curing of the composting material will most likely further prevent the growth of pathogenic bacteria from taking place.

Like all microbiological processes, composting can only take place with sufficient moisture (45-60%). Excessive aeration of composting piles or arid ambient condition may dry composting piles to the point at which microbial activity slows or stops. The cessation of microbial activity results in lowered pile temperatures which can easily be mistaken for the end-point of composting. Although composting may appear to have ended, and compost may even meet vector attraction reduction via Option 7, overly dried compost can cause both odor problems and vector attraction if moisture is reintroduced into the material and microbial activity resumes. It is therefore recommended that the composting process be maintained at moisture levels between 45-60% (40-55% total solids) (Epstein, 1997).

Although not mandated by Part 503, compost is usually maintained on site for longer than the required PFRP and VAR duration. In order to produce a high-quality, marketable product, it has been found that a curing period, or the period during which the volatile solids in the sewage sludge continue to decompose, odor potential decreases, and temperatures decrease into the mesophilic (40-45°C) range, is necessary. Depending on the feedstock and the PFRP selected, the curing period may last an additional 30 - 50 days after regulatory requirements are met.

In general, compost is not considered marketable until the piles are no longer self-heating. It is important to note that compost piles that are cooled by excessive aeration or that do not self-heat because the material is too dry to support microbial activity may not actually be fully decomposed.

It has been found that further reduction of organic material takes place during the curing phase of composting (Epstein, 1997). Therefore, microbiological testing should take place at the end of the curing process when compost is prepared for sale or distribution. Compost which is stored on site for extended periods of time until it can be sold or distributed must be tested for compliance with microbiological limits when it is to be used or disposed.

5.3 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 steam dryers.

Vector Attraction Reduction

No further processing is required because the PFRP requirements for heat drying also meet the requirements of VAR Option 8 for vector attraction reduction (the percent solids must be at least 90% before mixing the sewage sludge with other materials). This fulfills the requirement of Option 7 if the sewage sludge being dried contains no unstabilized solids.

Drying of sewage sludge to 90% solids deters the attraction of vectors, however unstabilized dried biosolids that are rewetted may become odorous and attract vectors. Therefore, it is recommended that materials be used or disposed while the level of solids remains high and that dried material be stored and maintained under dry conditions.

Some operators have found that maintaining stored material at solids levels above 95% helps to deter reheating because microbiological activity is halted. However, storage of materials approaching 90% total solids can lead to spontaneous combustion with subsequent fires and risk of explosion. While there is little likelihood of an explosion occurring with storage of materials like pellets, precautionary measures such as maintaining proper oxygen levels and minimizing dust levels in storage silos and monitoring temperatures in material can reduce the risk of fires.

5.4 Heat Treatment

Heat treatment processes are used to disinfect sewage sludge and reduce pathogens to below detectable levels. 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 PFRP description for heat treatment is:

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

Vector Attraction Reduction

Heat treatment in most cases must be followed by vector attraction reduction. VAR Options 6 to 11 (pH adjustment, heat drying, or injection, incorporation, or daily cover) may be used (see Chapter 8). Options 1 through 5 would not typically be applicable to heat treated sludge unless the sludge was digested or otherwise stabilized during or after heat treatment (e.g., through the use of wet air oxidation during heat treatment).

5.5 Thermophilic Aerobic Digestion

Thermophilic aerobic digestion is a refinement of the conventional aerobic digestion processes discussed in Section 7.2. 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 by up to 70% in a relatively short time. The digested sewage sludge is effectively pasteurized due to the high temperatures. Pathogenic viruses, bacteria, viable helminth ova and other parasites are reduced to below detectable limits if the process is carried out at temperatures exceeding 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 consecutive 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. This is due to the dramatic increase in temperatures compared to mesophilic aerobic digestion. Residence time is normally determined by dividing the volume of sewage sludge in the vessel by the volumetric flow rate. Facility operation should minimize the potential for bypassing by withdrawing treated sewage sludge before feeding, and feeding no more than once a day.

Complete-mix reactors with continuous feeding may not be adequate to meet Class A pathogen reduction because of the potential for bypassing or short-circuiting of untreated sewage sludge. These types of systems are difficult to demonstrate complete mix, as well as account for the short circuiting that will occur. Since every particle must be treated in a Class A process any short circuiting would invalidate this process.

Vector Attraction Reduction

Vector attraction reduction must be demonstrated. Although all options, except VAR Options 2, 4, and 12 are possible, VAR Options 1 and 3 which involve the demonstration of volatile solids loss are the most suitable. VAR Option 2 is appropriate only for anaerobically digested sludge, and VAR Option 4 is not possible because it is not yet known how to translate SOUR measurements obtained at high temperatures to 20°C [68°F].

5.6 Beta Ray and Gamma Ray Radiation

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 million 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 (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 (20°C [68°F]).

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. Since organic matter is not destroyed with the use of this process, sewage sludge must be properly stored after processing to prevent contamination.

Vector Attraction Reduction

Radiation treatment must be followed by vector attraction reduction. The appropriate options for demonstrating vector attraction reduction are the same as for heat treatment (see Section 9.4), namely VAR Options 6 to 11. Options 1-5 are not applicable unless the sewage sludge is subsequently digested.

5.7 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 viable helminth ova to below detectable values. Sewage sludge can be heated by heat exchangers or by steam injection. 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 growth of pathogenic bacteria can occur if sewage sludge is re-inoculated.

In theory, quicklime can be used to meet the requirements for pasteurization of sewage sludge. The water in the sludge slakes the lime, forming calcium hydroxide, and generates heat. However, it is difficult to ensure that the entire mass of sewage sludge comes into contact with the lime and achieves the required 70oC for 30 minutes. This is particularly true for dewatered sewage sludges. Processes must be designed to 1) maximize contact between the lime and the sewage sludge, 2) ensure that adequate moisture is present, 3) ensure that heat loss is minimal, and 4) if necessary, provide an auxiliary heat source. Pasteurization cannot be accomplished in open piles.

In addition, in order for pasteurization to be conducted properly, facility operators must be trained with regard to 1) the proper steps to be taken to ensure complete hydration of the alkaline reagent used, 2) the evaluation of the slaking rate of the lime based alkaline material required for their particular process,

specifying the reactivity rate required, 3) the proper measurement of pH, 4) an awareness of the effect of ammonia gassing off and how this affects the lime dose, and 5) the necessity for maintaining sufficient moisture in the sewage sludge/alkaline mixture during the mixing process to ensure the complete hydration of the quicklime and migration of hydroxyl ions throughout the sewage sludge mass. This is done to ensure that the entire sewage sludge mass is disinfected.

EPA -sponsored studies showed that pasteurization of liquid sewage sludge at 70°C (158°F) for 30 minutes inactivates parasite ova and cysts and reduces the population of measurable viruses and pathogenic bacteria to below detectable levels (USEPA, 1979). This process is based on the pasteurization of milk which must be heated to at least 63°C (145°F) for at least 30 minutes.

Vector Attraction Reduction

Pasteurization must be followed by a vector attraction reduction process unless the vector attraction reduction conditions of VAR Option 6 (pH adjustment) have been met. The VAR options appropriate for demonstrating vector attraction reduction are the same as those for heat treatment (see Section 9.4), namely VAR Options 6 to 11. VAR Options 1 to 5 are not applicable unless the sludge is subsequently digested.

5.8 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 Processes because they consistently demonstrated microbial removal and as well as pathogen levels below detection. Chapter 8 discusses how the Pathogen Equivalency Committee makes a recommendation of equivalency. Table (5.1) lists all the PFRP processes found to be equivalent and approved for use in the US, either on a national or site-specific basis.

Table 5.1 EPA Approved PFRP Equivalencies

Applicant / Process Name	Equivalency Type / Date Received	Process Description
Magna Management (Tucson, AZ) MagnaGrow Process	Site-Specific PFRP Equivalency September 2013 Green Valley Wastewater Treatment facility located at 2201 North Old Nogales Highway Green Valley, AZ 85614	Metam sodium is added to sludge based on sludge total solids in Gallons per dry ton; and Sodium or Potassium Hydroxide is added until the pH of the mixture in the reactor is above 12. Percent total solids of sludge can range between 5% and 25%. The batch reactor is a specially designed rotomixer. The sludge is kept in the tightly sealed rotomixer for 24 hours from the starting time, after which the generated product is neutralized for beneficial reuse. Since the sludge is kept above pH of 12 for 2 hours and the pH does not fall below 11.5 for at least 22 hours, then the treatment process satisfies the requirements of Part 503.33(b) (6) (i.e., add base to pH greater than 12, not to fall below this pH for two hours, and not to fall below pH 11.5 for 22 more hours, with only a single application of base).
BCR Environmental (Jacksonville, FL) Neutralizer Process	Conditional National Equivalency November 2010	Waste activated sludge total solids concentration of less than or equal to 4% is treated at temperatures greater than or equal to 15° C (59° F) with an oxidizing agent (chlorine dioxide) at an oxidation reduction potential (ORP) of greater than or equal to +100 mV with a contact time at +100 mV ORP ≥ 1 hour. Such treatment in followed by addition of sodium nitrite at 1500 mg/L and acid to

Applicant / Process Name	Equivalency Type / Date Received	Process Description
		achieve a pH of ≤ to 2.3 s.u. for six hours. The ORP during this second treatment stage must be > +100 mV and nitrite contact time at specified pH and ORP is at least six hours.
Columbus Water Works (Columbus, GA) Columbus Biosolids Flow-Through Thermophilic Treatment (CBFT³) Process	Conditional Site-Specific PFRP Equivalency [Conditionality: 1) once built the fluid dynamics of the full-scale CFMD must be verified to be consistent with that of the laboratory-scale CFMD; 2) helminth and enteric viruses must continue to be monitored on the full-scale process in addition to and at the same frequency as, the regulatory requirement for fecal coliform or *Salmonella* spp. monitoring until data statistically supports the required 2 and 3-log reductions, respectively.] October 2005	The process consists of four stages: 1) sludge preheat tank; 2) continuously fed, mixed digester (CFMD) operated at a minimum temperature of 53°C and a residence time of > 6.0 days; 3) a plug-flow reactor or series of batch tanks that provide a contact time of at least 30 minutes at a temperature of ≥ 60°C; 4)a mesophilic digester. It is further necessary that the limited conditions under which the process was tested are maintained at full-scale operation, namely that: 1) Columbus's co-thickened mixed primary and waste activated sludge contains 6.0 & ± 1.0 to 2.0% total solids of which ≥ 50% is volatile; 2) the average ammonium-nitrogen content of the digesting sludge in the CFMD is ≥ 920 mg/L; 3) the pH in the digester is ≥ 7.3 and ≤ 8.3; 4) total volatile acid concentrations are between 1,000 and 2,250 mg/L; 5) thorough heating of the sludge is verified throughout process operations by continuous temperature monitoring.
Burch Biowave, Inc. (Fredericktown, OH)[1] Burch Biowave™ Process	Acknowledgement as a Class A, Alt. 1 process March 2005	A thin layer of dewatered sludge (> 7% total solids) is conveyed through a system of microwave generators (75-100 kW) which heat sludge to > 80°C for 6-14 minutes. These conditions exceed the time and temperature requirements for Class A, Alternative 1 [$D = 131{,}700{,}000/10^{0.1400t}$ where D = time required in days; t = temperature in °C (Regime B)]. Heated air and an exhaust blower assist in drying the sludge to 75 – 90% solids.
Schwing Bioset, Inc. (Houston, TX) Bioset Process	National PFRP Equivalency August 2011	Dewatered municipal sludge solids between six to thirty-five percent total solids by weight are mechanically mixed with calcium oxide (quicklime) to achieve a pH of greater than or equal to twelve standard units. Sulphamic acid is added to, and mixed with the sludge/quicklime to promote an exothermic reaction which increases the temperature of the mixture to equal to or greater than 55°C (131°F). The sludge/quicklime/sulphamic acid mixture is then directed to a pressurized plug flow reactor for a minimum solids retention time of forty minutes at a minimum temperature of 55°C (131°F).
ONDEO Degremont (Richmond, VA) (formerly held by Lyonnaise des Eaux (Le Pecz-Sur-Seine,France)) Two-Phase Thermo-Meso Feed Sequencing Anaerobic Digestion (2PAD™)	Conditional National PFRP Equivalency [Conditionality: Helminth and enteric viruses must continue to be monitored on the full-scale process in addition to and at the same frequency as, the regulatory requirement for fecal coliform or *Salmonella* spp. monitoring until data statistically supports the required 2 and 3-log reductions, respectively.] September 2002	Sewage sludge is treated in the absence of air in an acidogenic thermophilic reactor and a mesophilic methanogenic reactor connected in series. The mean cell residence time shall be at least 2.1 days (± 0.05 d) in the acidogenic thermophilic reactor followed by 10.5 days (± 0.3 d) in the mesophilic methanogenic reactor. Feeding of each digester shall be intermittent and occurring 4 times per day every 6 hours. The mesophilic methanogenic reactor shall be fed in priority from the acidogenic thermophilic reactor. Between two consecutive feedings temperature inside the acidogenic thermophilic reactor should be between 49°C and 55°C with 55°C maintained during at least 3 hours. Temperature inside the mesophilic methanogenic reactor shall be constant at least 37°C.

Applicant /	Equivalency Type /	Process Description
Ultraclear, Marlboro, NJ	Microbiological Conditioning and Drying Process (MVCD)	In this process, sludge cake passes through several aerobic-biological type stages (Composting is an example) where different temperatures are maintained for varying times. Stage 1 occurs at 35°C for 7-9 hours; stage 2 occurs at 35-45°C for 8-10 hours; stage 3 occurs at 45-65°C for 7-10 hours; and the last stage is pasteurization at 70-80°C for 7-10 hours. In addition one of two conditions described below must be met: **Condition 1:** Dewatered sludge cake is dried by direct or indirect contact with hot gases, and moisture content is reduced to 10% or lower. Sludge particles reach temperatures we// in excess of 80°C or the wet bulb temperature of the gas stream in contact with the sludge at the point where it leaves the dryer is in excess of 80°C. OR **Condition 2**: A) Using the within-vessel, static aerated pile, or windrow composting methods, the sludge is maintained at minimum operating conditions of 40°C for 5 days. For 4 hours during the period the temperature exceeds 55°C; {Note: another PSRP-type process should be substituted for that of composting}; and B) Sludge is maintained for at least 30 minutes at a minimum temperature of 70°C.
Synox Corp.	National PFRP Equivalency	Operation occurs in a batch mode under the following conditions:
Pori International, Inc. (Baltimore, MD) Pori Process	National PFRP Equivalency May 1992	Sludge is preheated to 82°C (180°F) using recovered steam. Sulfuric acid is added to reduce the pH to 3. The mixture is then pressurized to 100 psig achieving temperatures of ≥ 165°C (330°F) for a treatment time of 1 hour. Lime slurry is used to neutralize pH
CBI Walker, Inc.	Conditional National PFRP Equivalency	Sludge is introduced intermittently into a vessel, amounting to 5 to 20% of its volume, where it is heated by both external heat exchange and by the bio-oxidation which results from vigorously mixing air with the sludge (pasteurized) and has a nominal residence time of 18 to 24 hours. Time between feedings of unprocessed sludge can range from 1.2 (@ ~ 65°C) to 4.5 (@ ~ 60°C) hours. Exiting sludge is heat exchanged with incoming unprocessed sludge. Thus, the sludge is cooled before it enters a mesophilic digester.
Fuchs Gas Und Wassertechnik, Gmbh (Mayen, Germany)[2] Autothermal Thermophilic Aerobic Digestion (ATAD)	Conditional National PFRP Equivalency [Conditionality: 1)Time and temperature in the first vessel must be ≥ 30 minutes and ≥ 50°C, and controlled by the equation $D = 50{,}070{,}000/10^{0.1400t}$(where D = time required in days; t = temperature in °C) for sludges of ≤ 7%	ATAD is a two-stage autothermal aerobic digestion process. The stages are of equal volume. Treated sludge amounting to 1/3 the volume of a stage is removed every 24 hours from the second stage as a product. An equal amount then is taken from the first stage and fed to the second stage. Similarly, an equal amount of untreated sludge is then fed to the first stage. In the 24-hour period between feedings, the sludge in both stages is vigorously agitated

	Equivalency Type / Date Received	Process Description
	solids; 2) Operations of the reaction vessel during the time-temperature periods must be either plug flow or batch mode.] November 1992	and contacted with air. Bio-oxidation takes place and the heat produced increase the temperature. Sludge temperature in the reactors averages between 56 and 57°C for ≥ a 16-hour period, while the overall hydraulic residence time is 6 days
K-F Environmental Technologies, Inc. (Pompton Plains, NJ)[1] Type of Sludge Drying Process	Acknowledgement of PFRP by meeting current regulations under 40 CFR 257, App. II November 1992	Sludge is heated to a minimum temperature of 100°C and indirectly dried to below 10% moisture using oil as a heat transfer medium. The final discharge product has exceeded a temperature of 80°C and is a granular, dry pellet that can be land applied, incinerated, or landfilled. In addition the following conditions must be met: Dewatered sludge cake is dried by direct or indirect contact with hot gases, and moisture content is reduced to 10% or lower. Sludge particles reach temperatures well in excess of 80°C or the wet bulb temperature of the gas stream in contact with the sludge at the point where it leaves the dryer is in excess of 80°C.
International Process Systems, Inc. (Glastonbury, CT)[1] Type of Composting Process	Conditional National PFRP Equivalency [Conditionality: Process operation is to be controlled so that the composting mass passes through a zone in the reactor in which the temperature of the compost is at least 55°C throughout the entire zone, and the time of contact in this zone is at least three days.] April 1991	IPS developed a unique within-vessel composting reactor using forced-aeration and bed-agitation to create an optimal aerobic environment. Long rectangular vessels are loaded at one end. An agitator/mixer assembly rides across the top of the vessel, mixing & conveying material down the vessel at a rate of approximately 12 ft/day. Finished compost reaches the opposite end of the vessel in 18 days having passed through five zones of treatment with average temperatures > 60°C.
ATW, Inc. (Santa Barbara, CA) Alkaline Stabilization / Pasteurization	PFRP Equivalency Prior to 1989	Manchak process uses quicklime to simultaneously stabilize and pasteurize biosolids. Quicklime, or a combination of quicklime and fly ash, is mixed with dewatered sludge at a predetermined rate in a confined space. An instant exothermic reaction is created in the product wherein the pH is raised in excess of 12 after two hours of contact, in addition, the temperature is raised in excess of 70°C for > 30 minutes
N-Viro Energy Systems, Ltd. (Toledo, OH) Advanced Alkaline stabilization with subsequent accelerated drying	National PFRP Equivalency January 1988	*Method 1:* Fine alkaline materials (cement kiln dust, lime kiln dust, quicklime fines, pulverized lime, or hydrated lime) are uniformly mixed by mechanical aeration mixing into liquid or dewatered sludge to raise the pH to > 12 for 7 days. If the resulting sludge is liquid, it is dewatered. The stabilized sludge cake is then air dried (while pH remains > 12 for ≥ 7 days) for > 30 days and until the cake is ≥ 65% solids. A solids concentration of ≥ 60% is achieved before the pH drops below 12. The mean temperature of the air surrounding the pile is > 5°C (41°F) for the first 7 days. *Method 2:* Now in 40 CFR 503 as Class A, Alternative 2
Scarborough Sanitation District (Scarborough, ME)[1] Fly ash composting	Site-Specific PFRP Equivalency March 1987	Traditional static aerated pile composting using fly ash as the bulking agent. Thus, heat (at least in part) is generated through chemical reaction with the fly ash and not through biological reactions as would a typical composting process. Time and temperature requirements for Class A static aerated piles were exceeded with operating conditions of 60 to 70°C reached within 24 hours and maintained for 14 days. Equivalency was recommended on this basis.

5.9 References

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6: CLASS B PATHOGEN REQUIREMENTS FOR MATERIAL APPLIED TO AGRICULTURAL LAND, A FOREST, OR A RECLAMATION SITE

6.1 Introduction

This chapter discusses the Class B requirements in Subpart D of 40 CFR Part 503. The implicit goal of the Class B pathogen requirements is to reduce the pathogens load in the sewage sludge. As mentioned in Chapter 2, Part 503 uses microbial indicators as one option to determine if sewage sludges have been properly treated with respect to pathogen destruction. In the case of Class B materials, the assumption is 2 million MPN or CFU of fecal coliforms in the final product equates to a 2 log reduction of the overall fecal coliform population in the raw sludge. It is further assumed that a 2 log reduction in the bacterial population would show a 1 log reduction in the virus population (Kowal 1985). There is no assumption of any protozoan removal using the fecal coliform indicator level.

Land application of these materials whether meeting PSRP requirements or fecal coliform monitoring, includes proper barriers and site restrictions to allow for the natural attenuation of pathogens present in these biosolids. The site restrictions are discussed in further detail in section 6.5 of this chapter. The degree of the site restriction access is dependent upon the particular use of that land that received the Class B material. For instance, land that will be used for planting crops will have different requirements than land that will be used for animal grazing. The purpose of the site restrictions is to allow further reduction of the pathogen population in the applied biosolids through environmental conditions such as sunlight, desiccation, and natural attenuation. It is also important that treatment workers, land appliers and haulers, and farm workers understand the inherent risk with Class B land application and use proper personal protective equipment (PPE) and precautions (CDC 2002).

There are three alternative requirements for demonstrating Class B pathogen reduction. As with Class A biosolids, Class B biosolids must address vector attraction. The choice of vector attraction options may affect the duration of the site restrictions in some cases.

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

Alternative 1 requires that seven samples of treated sewage sludge be collected and that the geometric mean fecal coliform density of these samples be less than 2 million CFU or MPN per gram of biosolids (dry weight basis). This approach uses fecal coliform density as an indicator of the average density of bacterial and viral pathogens.

A geometric mean of at least seven samples is required with this alternative. The use of seven samples is expected to reduce the standard error to a reasonable value. The standard deviation can be a useful predictive tool. A standard deviation of greater than 1 log for the fecal coliform density indicates a wide range in the densities of the individual samples. This may be due to sampling variability or variability in

the laboratory analysis, or it may indicate that the treatment process is not consistent in its reduction of pathogens. A high standard deviation can therefore alert the preparer that the sampling, analysis, and treatment processes should be reviewed.

Each of the multiple samples taken for fecal coliform analysis should be taken at the same point in the process so that treatment of each sample is equal. Generally, a log standard deviation between duplicate samples under 0.3 is acceptable for lab analyses (see Table 6.1).

Table 6.1 Calculating the Geometric Mean for Class B Alternative 1

Directions for performing geometric mean analysis on Class B Biosolids		
• Take seven samples over a 2-week period. • Analyze samples for fecal coliform using the membrane filter or MPN dilution method. • Take the log (Base 10) of each result. • Take the average (arithmetic) of the logs. • Take the anti-log of the arithmetic average. This is the geometric mean of the results. Example: The results of analysis of seven samples of sewage sludge are shown below. The second column of the table shows the log of each result.		
Sample Number	Fecal Coliform (MPN/dry gram sewage sludge)	Log
Sample 1	6.4×10^6	6.81
Sample 2	4.8×10^4	4.68
Sample 3	6.0×10^5	5.78
Sample 4	5.7×10^5	5.76
Sample 5	5.8×10^5	5.76
Sample 6	4.4×10^6	6.64
Sample 7	6.2×10^7	7.80
Average (Arithmetic)		6.18
Antilog (geometric mean)		1.5×10^6*
Log standard deviation		1.00*

*Note that this sewage sludge would meet Class B fecal coliform requirements even though several of the analysis results exceed the 2.0×10^6/dry gram limit. Duplicate analyses on the same sample would give a

much lower standard deviation. Variability is inflated by differences in feed and product over a 2-week sampling period

Process parameters including retention time and temperature should be examined to verify that the process is running as specified. Monitoring equipment should be calibrated regularly.

The seven samples should be taken over a 2-week period in order to represent the performance of the facility under a range of conditions. For small facilities that are required to sample infrequently, sampling should be performed under worst case conditions, for example, during the winter when the climatic conditions are the most adverse.

When lagoons are infrequently dredged, the lagoon can be cordoned off into seven equal sections and a sample taken from each section and the geometric mean calculated. Samples could be composited within each section to increase representativeness.

Vector Attraction Reduction

Meeting the requirements for VAR depends on the process by which the pathogen reduction level is met. Chapter 9 discusses VAR in more detail (Table 9.2).

6.3 Sewage Sludge Alternative 2: Use of a Process to Significantly Reduce Pathogens (PSRP) [503.32(b)(3)]

Under Alternative 2, biosolids are considered to be Class B for pathogen reduction if they are treated using one of the "Processes to Significantly Reduce Pathogens" (PSRPs) Table7.1. The biological PSRP processes are sewage sludge treatment processes that have been demonstrated to result in a 2-log reduction in fecal coliform density (see Chapter 7).

Microbial Monitoring

Unlike the comparable Class A requirement (see Section 4.8), this Class B alternative does not require microbiological monitoring. However, monitoring of process requirements such as time, temperature, and pH required.

Vector Attraction Reduction

Meeting the requirements for VAR depends on the process by which the pathogen reduction level is met. Chapter 9 discusses VAR in more detail.

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

Under Class B Alternative 3, sewage sludge treated by any process determined to be equivalent to a PSRP is considered to be Class B biosolids. A list of processes that have been recommended as equivalent to PSRP are shown in Table 8.1.

Part 503 gives the regulatory authority responsibility for determining equivalency. The Pathogen Equivalency Committee is available as a resource to provide guidance and recommendations on equivalency determinations to the regulatory authorities (see Chapter 8).

Microbial Monitoring

*Unlike the comparable Class A requirement (see Se*ction 4.8), this Class B alternative does not require microbiological monitoring. However, monitoring of process requirements such as time, temperature, and pH is required.

Vector Attraction Reduction

Meeting the requirements for VAR depends on the process by which the pathogen reduction level is met. Chapter 9 discusses VAR in more detail.

6.5 Site Restrictions for Land Application of Biosolids [503.32(b)(5)]

Potential exposure to pathogens in Class B biosolids via food crops is a function of three factors: 1.) presence of pathogens in land applied biosolids. 2.)Transfer of these pathogens to the harvested crop, and lastly 3.)ingestion of the crop prior to removal of pathogens due to crop processing protocols. Elimination of one of these steps eliminates the pathway by which public health may be affected. As stated previously, biosolids that meet the Class B requirements may contain reduced but still significant densities of pathogenic bacteria, viruses, protozoans, and viable helminth ova. Thus, site restrictions are used to allow time for further reduction in the pathogen population. Harvest restrictions are imposed to eliminate the possibility that food will be harvested and ingested before pathogens which may be present on the food have died off. Harvest restrictions vary, depending on the type of crop, because the amount of contact a crop will have with biosolids or pathogens in biosolids varies.

The site restrictions are primarily based on the survival rate of viable helminth ova, one of the hardiest pathogens that may be present in sewage sludge. The survival of pathogens, including the helminth ova, depends on exposure to the environment. Some of the factors that affect pathogen survival include pH, temperature, moisture, cations, sunlight, presence of soil microflora, and organic material content. On the soil surface, helminth ova have been found to die off within four months, but survival is longer if pathogens are within the soil. Helminth ova have been found to survive in soil for several years (Smith, 1997; Kowal 1985).

Site restrictions also take the potential pathways of exposure into account. For example, crops that do not contact the soil, such as oat or wheat, may be exposed to biosolids, but pathogens on crop surfaces have been found to be reduced very quickly (30 days) due to exposure to sunlight, desiccation, and other environmental factors. Crops that touch the soil, such as melons or cucumbers, may also come into contact with biosolids particles, but pathogens in this scenario are also subject to the harsh effects of sunlight and rain and will die off quickly. Crops grown in soil such as potatoes are surrounded by biosolids amended soil, and pathogen die-off is much slower below the soil surface.

These pathways should be considered when determining which site restriction is appropriate for a given situation. The actual farming and harvesting practices as well as the intended use of the food crop should also be considered. For example, oranges are generally considered a food crop that does not touch the ground. However, some oranges grow very low to the ground and may come into contact with soil. If the oranges that have fallen to the ground or grew touching the ground are harvested for direct consumption without processing, the 14-month harvest restriction for crops that touch the soil should be followed. Orange crops which *do not* touch the ground at all would not fall under the 14-month harvest restriction;

harvest would be restricted for 30 days under 503.32(b)(5)(iv) which covers food crops that do not have harvested parts in contact with the soil. For similar situations, the potential for public health impacts must be considered. Harvest practices such as the use of fallen fruit or washing or processing crops should be written into permits so that restrictions and limits are completely clear. Figure 6.1 illustrates the steps of exposure that should be considered when making a decision about harvest and site restrictions. In addition, several examples of permit conditions are included. The site restrictions for land applied Class B biosolids are summarized below. Note that the restrictions apply only to the harvesting of food crops, but not to the planting or cultivation of crops and the time periods are from the time of application to the time of harvest.

6.6 Food Crops with Harvested Parts That Touch the biosolid/Soil Mixture

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

This time frame is sufficient to enable environmental conditions such as sunlight, temperature, and desiccation to further reduce pathogens on the land surface. Note that the restriction applies only to harvesting. Food crops can be planted at any time before or after biosolids application, as long as they are not harvested within 14 months after sludge application. Examples of food crops grown on or above the soil surface with harvested parts that typically touch the sewage sludge/soil mixture include lettuce, cabbage, melons, strawberries, and herbs. Land application should be scheduled so that crop harvests are not lost due to harvest restrictions.

6.7 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.

Pathogens on the soil surface will be exposed to environmental stresses which greatly reduce their populations. Helminth ova have been found to die off after 4 months on the soil surface (Kowal, 1994). Therefore, a distinction is made between biosolids left on the soil surface for four months and biosolids which are injected, disced, or plowed into soil more quickly.

6.8 Examples of Site Restrictions for Questionable Food Crop Situations

Tree Nut Crops - Nuts which are washed, hulled, and dehydrated before being distributed for public consumption must follow the 30-day restriction. Nuts which are harvested from the ground and sold in their shell without processing are subject to the 14-month restriction.
Sugar Beets - Sugar beets aren't expected to be eaten raw. If the beets are transported off site and considerable biosolids amended soil is carried off with them, the restrictions apply. If biosolids are left on the soil surface for 4 months or longer before being incorporated, the 20-month restriction applies. If biosolids are incorporated within 4 months of application, the 38-month restriction applies.

Tomatoes (and peppers) - Fruit often comes in contact with the ground. Tomatoes are sold both to processors and to farm stands. Tomatoes may be eaten raw by the public without further processing. The 14-month restriction applies.

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 four months prior to incorporation into the soil.

Exposure of the surface of root crops such as potatoes and carrots to 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 biosolids on the land surface. Class B biosolids incorporated into the soil surface less than four 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. Thirty-eight months after biosolids application is usually sufficient to reduce helminth ova to below detectable levels.

6.9 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) This would include crops with harvested parts that do not typically touch the biosolids/soil mixture and which are not collected from the ground after they have fallen from trees or plants. The restriction also applies to all feed and fiber crops. These crops may be exposed to pathogens when biosolids are applied to the land. Harvesting of these crops could result in the transport of biosolids pathogens from the growing site to the outside environment. After 30 days, however, any pathogens in biosolids that may have adhered to the crop during application will likely have been reduced to non-detectable levels. Hay, corn, soybeans, or cotton are examples of a crop covered by this restriction.

6.10 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.

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

6.11 Turf Harvesting

503.32(b)(5)(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.

The one-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 cases in which the turf is not used on areas with high potential for public access.

6.12 Public Access

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

As with the turf requirement above, a one-year waiting period is necessary to protect public health and the environment in a potential high-exposure situation. A baseball diamond, playground, public park, soccer field, and overflow parking lot are examples of land with a high potential for public exposure. The land gets heavy use and contact with the soil is substantial (children or ball 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 land with low potential for public exposure. Even farm workers and family members walk about very little on such fields. Public access restrictions do not apply to farm workers, but workers should be aware of the public health implications of land application and the land application schedule and should follow good hygiene practice during the 30-day period.

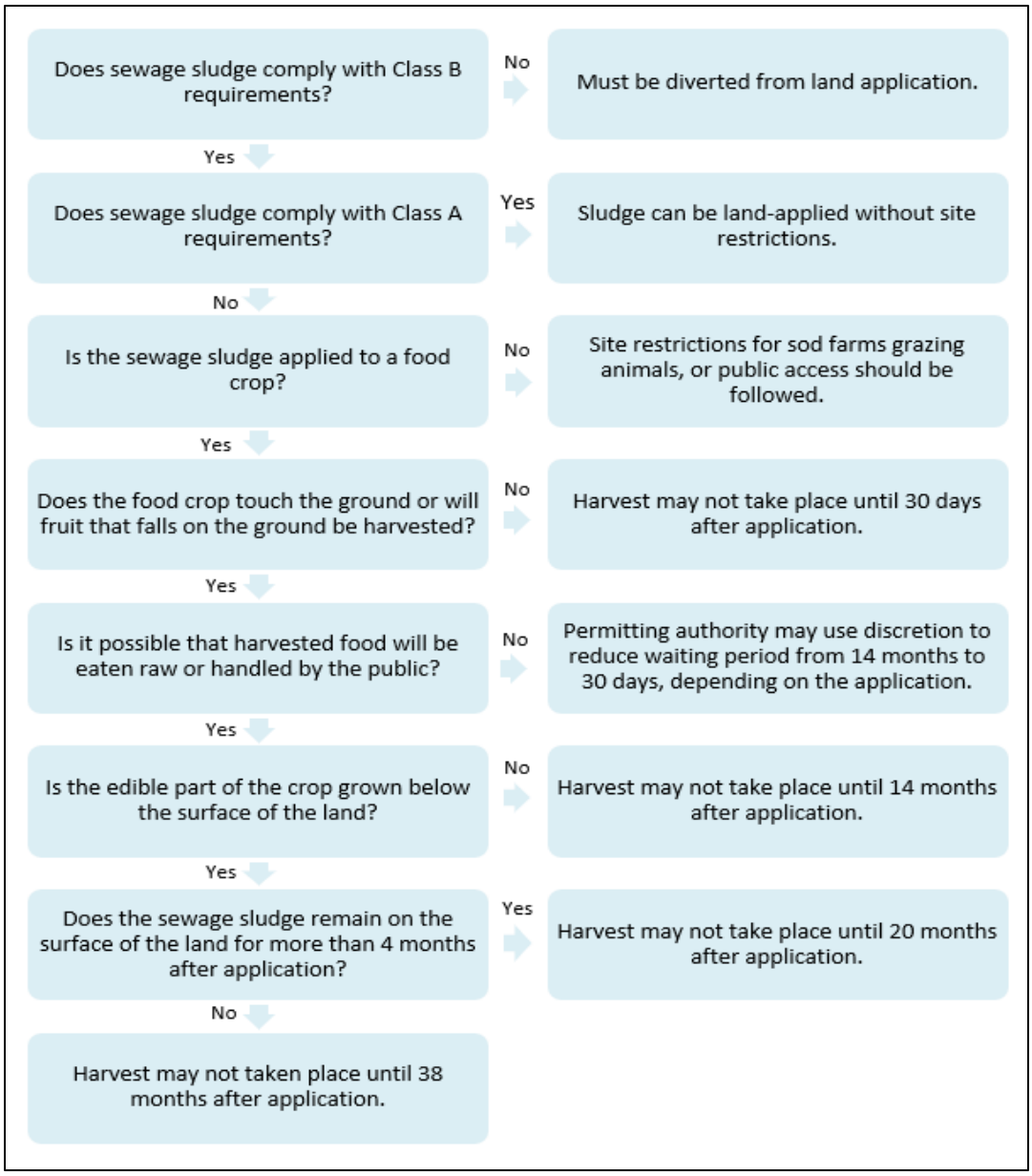

Figure 6.1. Decision tree for harvesting and site restrictions.

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7: PROCESSES TO SIGNIFICANTLY REDUCE PATHOGENS (PSRP)

7.1 Introduction

Processes to Significantly Reduce Pathogens (PSRPs) was a distinction given to processes that can reduce the microbial populations in treated sewage sludge by a factor of 10 or greater. This terminology was first introduced into the sewage sludge regulations prior to 40 CFR Part 503.Once Part 503 was adopted these were added under Alternative 2 and Alternative 3 for Class B materials. There are five PSRPs listed under Alternative 2 (see Table 7.1). When operated under the conditions specified outlined in the regulations, PSRPs reduce fecal coliform densities to less than 2 million CFU or MPN per gram of total solids (dry weight basis) and reduce *Salmonella* sp. and enteric virus densities in sewage sludge by approximately a factor of 10 (Farrell, et al., 1985).

Additionally, the Pathogen Equivalence Committee (PEC) can recommend a process as PSRP equivalent under Alternative 3, more detail on the PEC and PSRP testing is provided in Chapter 8. Table 7.2 lists PSRP equivalencies approved by EPA by under Alternative 3.

Although theoretically two or more PSRP processes, each of which fails to meet its specified requirements, could be combined for effectively reducing pathogens (e.g., partial treatment in digestion followed by partial treatment by air drying) it cannot be assumed that the pathogen reduction contribution of each of the operations will result in the 2-log reduction in fecal coliform necessary to define the combination as a PSRP. Therefore, to comply with Class B pathogen requirements, one of the PSRP processes must be conducted as outlined in this chapter, or fecal coliform testing must be conducted in compliance with Class B Alternative 1. The biosolids preparer also has the option of applying for PSRP equivalency for the combination of processes. Achieving PSRP equivalency enables the preparer to eliminate the requirement of monitoring for fecal coliform density.

Table 7.1. Processes to Significantly Reduce Pathogens (PSRPs) Listed in Appendix B of 40 CFR Part 503

PSRP	Regulatory Conditions to maintain PSRP
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).
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).
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).
Composting	Using either the within-vessel, static aerated pile, or windrow composting methods, the temperature of the sewage sludge is raised to 40°C (104oF) 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).
Lime Stabilization	Sufficient lime is added to the sewage sludge to raise the pH of the sewage sludge to 12 for > 2 hours of contact.

7.2 Aerobic Digestion

In aerobic digestion, sewage sludge is biochemically oxidized by bacteria in an open or enclosed vessel. To supply aerobic microorganisms with enough oxygen, either the sewage sludge must be agitated by a mixer, or air must be forcibly injected. Under proper operating conditions, the volatile solids in sewage sludge are converted to carbon dioxide, water, and 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 are 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:

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).

For temperatures between 15°C (59°F) and 20°C (68°F) use the relationship between time and temperature provided below to determine the required mean cell residence time.

$$\frac{t}{40\ days} = 1.08 - (20 - T)$$

t = Time (units) at temperature (°C)

T = Temperature in °C

The regulation does not differentiate between batch, intermittently fed and continuous operation, so any method is acceptable. The mean cell residence time is considered the residence time of the sewage sludge solids. The appropriate method for calculating residence time depends on the type of digester operation used (see Appendix D).

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 supernatant is 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 completely-mixed reactors in series are more effective in reducing pathogens than is a single well-mixed reactor at the same overall residence time. The residence time required for this type of system to meet pathogen reduction goals may be 30% lower than the residence time required in the PSRP definition for aerobic digestion (see Appendix D). However, since lower residence times would not comply with PSRP conditions required for aerobic digestion in the regulation, approval of the process as a PSRP by the permitting authority would be required.

Other: Digesters are frequently operated in unique ways that do not fall into the categories above. Appendix D provides information that should be helpful in developing a calculation procedure for these cases. Aerobic digestion carried out according to Part 503 requirements typically reduces bacterial organisms by 2-log and viral pathogens by 1-log. 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 sewage sludges is demonstrated either when the percent volatile solids reduction during sewage sludge treatment equals or 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 9).

7.3 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 that may or may not be heated. Because the biological activity consumes most of the volatile solids needed for further bacterial growth, microbial activity in the treated sewage sludge is limited. Anaerobic digestion is one of the most widely used treatments for sewage sludge treatment, 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 accelerate biological activity. High-rate systems use a combination of active mixing and carefully controlled, elevated temperature to increase the rate of volatile solids destruction. 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 oxygen 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).*

- *Straight-line interpolation to calculate mean cell residence time is allowable when the temperature falls between 35 ℃ and 20 ℃.*

Anaerobic digestion that meets the required residence times and temperatures typically reduces bacterial and viral pathogens by 90% or more. Viable 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).

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%, which satisfies vector attraction reduction requirements. 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 sewage sludge for 40 additional days at 30°C to 37°C (86°F to 99°F) be less than 17% (see Section 8.3). The SOUR test is an aerobic test and cannot be used for anaerobically digested sewage sludge.

7.4 Air Drying

Air drying allows partially digested sewage sludge to dry naturally in the open air. Wet sewage sludge is usually applied to a depth of approximately 23 cm (nine 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 air-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. During the drying/storage period in the bed, the sewage sludge is undergoing physical, chemical, and biological changes. These include biological decomposition of organic material, ammonia production, and desiccation.

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 three months. During two of the three months, the ambient average daily temperature is above 0°C (32°F).

Although not required by Part 503, it is advisable to ensure that the sewage sludge drying beds are exposed to the atmosphere 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 by 1-log and bacteria by approximately 2-log. Viable helminth ova also are reduced, except for some hardy species that remain substantially unaffected.

Vector Attraction Reduction

Frequently sand-bed drying follows an aerobic or anaerobic digestion process that does not meet the specified process requirements and does not produce 38% volatile solids destruction. However, it may be that the volatile solids reduction produced by the sequential steps of digestion and drying will meet the vector attraction reduction requirement of 38% volatile solids reduction. If this is the case, vector attraction reduction requirements are satisfied.

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 (VAR Option 1, see Section 9.2), 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 VAR Option 7 in Section 9.8, and VAR Option 8 in Section 9.9).

7.5 Composting

Composting involves the aerobic decomposition of organic material using controlled temperature, moisture and oxygen levels. Composting can yield either Class A or Class B biosolids, 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 reduce pathogens for production of Class B biosolids 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, and the composted biosolids are "cured" for an additional period.

The three most common composting methods in the United States are windrow, aerated static pile, and within-vessel composting. Windrow composting involves stacking the sewage sludge/bulking agent mixture into long piles, or windrows, generally 1.5 to 2.7 meters high (5 to 9 feet) and 2.7 to 6.1 meters wide (9 to 20 feet). These rows are regularly turned or mixed with a turning machine or front-end loader to fluff up the material and increase porosity which allows better convective oxygen flow into the

material. Turning also breaks up compacted material and reduces the moisture content of the composting media. 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 reduction.

Aerated static pile composting uses forced-air rather than mechanical mixing 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 odor control. 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 but they share two basic techniques: the process takes place in a reactor vessel where the operating conditions can be carefully controlled, and active aeration meets the system's high oxygen demand. Agitated bed systems (one type of within-vessel composting) depend on continuous or periodic mixing within the vessel followed by a curing period.

Pathogen reduction during composting depends on time and temperature variables. Part 503 provides the following definition of PSRP requirement for pathogen reduction during 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).

These conditions achieved using either within-vessel, aerated static pile, or windrow methods, reduce bacterial pathogens by 2-log and viral pathogens by 1-log.

A process time of only 5 days is not long enough to fully break down the volatile solids in sewage sludge, so the composted sewage sludge produced under these conditions will not be able to meet any of the requirements for reduced vector attraction. In addition, sewage sludge that has been composted for only 5 days may still be odorous. Breakdown of volatile solids 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 during which time pile turning or active aeration may continue.

Vector Attraction Reduction

Vector attraction reduction must be conducted in accordance with Option 5, or compost must be incorporated into soil (VAR Options 9,10) when land applied. 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).

7.6 Lime Stabilization

The lime stabilization process is relatively straightforward. Lime that is 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 for 2 hours or more after contact. Lime stabilization can reduce bacterial and viral pathogens by 99% or more. However, such alkaline conditions have little effect on helminth ova.

The Part 503 PSRP description for lime stabilization states:

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

For the Class B lime stabilization process, the alkaline material must be a form of lime. Use of other alkaline materials must first be demonstrated to be equivalent to a PSRP. Elevation of pH to 12 for two hours is expected to reduce bacterial and viral density effectively.

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

Mixing must ensure that 1) the entire mass of sewage sludge comes into contact with the lime and undergoes the increase in pH, and 2) samples are representative of the overall mixture (see Chapter10). The pH should be measured at several locations to ensure that itis raised throughout the sewage sludge.

A variety of lime stabilization processes are currently in use. The effectiveness of any lime stabilization process for controlling pathogens depends on maintaining the pH at levels that reduce microorganisms in the sewage sludge. Field experience has shown that the application of lime stablized material after the pH has dropped below 10.5 can create odor problems. Therefore, it is recommended that biosolids application take place while the pH remains elevated. If this is not possible and odor problems develop, alternative management practices in the field including injection, incorporation, or top dressing the applied biosolids with additional lime, may be necessary. Alternate management practices (e.g., adding additional lime, drying, and composting) may also be necessary if the biosolids have not left the wastewater treatment plant

Vector Attraction Reduction

For lime-treated sewage sludge, vector attraction reduction is best demonstrated by Option 6 of the vector attraction reduction requirements. This option requires that the sewage 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 9.7).

7.7 Alternative 3 Equivalent Processes

Table 7.2. below lists the processes that the EPA's Pathogen Equivalency Committee has recommended as being equivalent to PSRP. Information on the PEC and how to apply for equivalency are discussed in Chapter 8.

Table 7.2. EPA Approved PSRP Equivalencies

Applicant/ Process Name	Equivalency Type / Date Received	Process Description
BCR Environmental CleanB™ (Jacksonville, FL)	National PSRP Equivalency October, 2015	The CleanB™ process is a plug-flow, chemical oxidation/aeration process that utilizes chlorine dioxide to achieve Class B PSRP equivalent waste activated sludge disinfection. Chemical addition consists of the addition of site-generated chlorine dioxide into a closed contact chamber and providing 10 minutes of contact time.
East Bay Municipal Utilities District Low MCRT Treatment Process	Site Specific PSRP Equivalency November 2010	Single stage completely mixed thermophilic anaerobic digestion process operated at a temperature of 50 ± 3° C and a mean cell residence time of ≥ 240 hours (10-day simple moving average) for treatment of primary sludge, waste-activated sludge, and high-strength non-hazardous organic materials. Digester feeding is semi-continuous and draw-off is intermittent, both occurring at any time.
Synox Corp. (Jacksonville, FL) OxyOzonation	National PSRP Equivalency August 1989	Batch process where sludge is acidified to pH 3.0 by sulfuric acid; exposed to 1 lb. Ozone/1000 gallons of treated sludge under 60 psig pressure for 60 minutes; 100 mg/L of sodium nitrite and held for ≥ 2 hours; and stored at ≤ pH 3.5. Limitations imposed were for total solids to be ≤ 4%; temperature must be ≥ 20°C; and total solids must be ≤ 6.2% before nitrite addition.
N-Viro Energy Systems, Ltd. (Toledo, OH) Alkaline Addition to achieve Lime Stabilization	National PSRP Equivalency April 1987	Use of cement kiln dust and lime kiln dust (instead of lime) to treat sludge by raising the pH. Sufficient lime or kiln dust is added to sludge to produce a pH of 12 for at least 12 hours of contact.
Comprehensive Materials Management, Inc. (Houston, TX) Cement Kiln Dust to achieve Lime Stabilization	National PSRP Equivalency March 1987	Use of kiln dust (instead of lime) to treat sludge pH to at least 12 after 2 hours of contact.Dewatered sludge is mixed with cement kiln dust in an enclosed system then hauled off for land application.
Ned K. Burleson and Associates, Inc. (Fort Worth, TX) Mid-Range Temperature Aerobic Digestion	National PSRP Equivalency Prior to 1989	Typical aerobic digestion for 20 days at 30°C (86°F) or 15 days at 35°C (95°F). This is above regulation temperatures for PFRP (15 - 20°C), but below regulation temperatures for PFRP (55 - 60°C).

7.8 References

Berg G. and D. Berman. 1980. Destruction by anaerobic mesophilic and thermophilic digestion of viruses and indicator bacteria indigenous to domestic sludges. Appl. Envir. Microbiol. 39 (2):361-368.

Farrell, J.B., G. Stern, and A.D. Venosa. 1990. Microbial destructions achieved by full-scale anaerobic digestion. Paper presented at Municipal Wastewater Sludge Disinfection Workshop. Kansas City, MO. Water Pollution Control Federation, October 1995.

USEPA. 1992. Technical support document for reduction of pathogens and vector attraction in sewage sludge. EPA 822/R-93-004.

8: EQUIVALENCY AND EPA'S PATHOGEN EQUIVALENCY COMMITTEE

8.1 Introduction

One way to meet the pathogen reduction requirements of Part 503 is to treat sewage sludge in a process "equivalent to" the processes to further reduce pathogens (PFRP) or processes to significantly reduce pathogens (PSRP) (see Tables 4.3 and 7.1):

- Under Class A Alternative 6, sewage sludge that is treated in a process equivalent to PFRP and meets the Class A microbiological requirement (see Section 4.3) is considered to be a Class A biosolids 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 biosolids with respect to pathogens (see Section 5.4).

Under Part 503, equivalency pertains only to pathogen reduction. However, like all Class A and B biosolids, sewage sludges treated by equivalent processes must also meet a separate vector attraction reduction requirement (see Chapter 9).

8.2 Equivalency Determination

To be equivalent, a treatment process must be able to consistently reduce pathogens to levels comparable to the reduction achieved by the listed PFRPs or PSRPs. These levels are the same levels required of all Class A and B biosolids. The process continues to be equivalent as long as it is operated under the same conditions (e.g., time, temperature, pH) that produced the Part 503 required reductions. The permitting authority is responsible for determining equivalency under Part 503. The permitting authority and facilities are encouraged to seek guidance from EPA's Pathogen Equivalency Committee (PEC) in making equivalency determinations. The PEC makes both site-specific and national equivalency recommendations. The recommendation is then given to the proper permitting authority, and they will ultimately decide if the process can be deemed equivalent. The PEC's recommendations is not an endorsement of any process by EPA.

8.3 Benefits of Equivalency

A determination of equivalency can be beneficial to a facility because it reduces the microbiological monitoring burden in exchange for greater monitoring of process parameters. For example, a facility meeting Class A requirements by sampling for enteric viruses and viable helminth ova in compliance with Alternative 4 may be able to eliminate this monitoring burden if they are able to demonstrate that their treatment process adequately reduces these pathogens on a consistent basis[14]. Similarly, a facility meeting Class B Alternative 1 requirements by analyzing sewage sludge for fecal coliform may be able to eliminate the need for testing if the process is shown to reduce pathogens to the same extent as all PSRP

[14] A determination of PFRP equivalency will not reduce the monitoring required for *Salmonella* sp. or fecal coliform because all Class A biosolids, even biosolids produced by equivalent processes, must be monitored for *Salmonella* sp. or fecal coliform (see Section 4.3)

processes. Equivalency is also beneficial to facilities which may have low cost, low technology systems capable of reducing pathogen populations. Options such as long-term storage, air drying, or low technology composting have been considered by the PEC.

Because equivalency status allows a facility to eliminate or reduce microbiological sampling, it is imperative that the treatment processes deemed equivalent undergo rigorous review to ensure that the Part 503 requirements are met. Obtaining a recommendation of equivalency necessitates a thorough examination of the process and an extensive sampling and monitoring program. The time needed to review an application is contingent on the completeness of the initial application. Sewage sludge preparers wishing to apply for equivalency should review this chapter carefully and discuss the issue with the regulatory authority to determine if equivalency is appropriate for their situation.

8.4 Recommendation of Site-Specific Equivalency

Equivalency may be site-specific meaning the equivalency applies only to a particular operation run at a specified location under the conditions that are written in the equivalency determination. It cannot be assumed that the same equivalency process will work for all types of sewage sludges or would be effective in other facilities with different geographical characteristics. A facility that would attempt to utilize a site-specific equivalency at a new location would need to go through the equivalency process as a new equivalency determination. Once an equivalency is granted, any modification of the process would require new testing to prove the modification has no effect on the pathogen destruction performance.

8.5 Recommendation of National Equivalency

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. A recommendation of national equivalency can be useful for treatment processes that will be marketed, sold, 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. National equivalencies are granted only after the process is demonstrated successfully in multiple locations, or with different conditions such that it can be determined that the process is not influenced by geographic location.

8.6 Role of the Pathogen Equivalency Committee

The EPA created the PEC in 1985 to make recommendations to EPA management on applications for PFRP and PSRP equivalency under Part 257 (Whittington and Johnson, 1985). The PEC consists of scientists with diverse expertise who review the equivalency determinations. See Appendix I for a copy of the memo.

8.7 Guidance and Technical Assistance on Equivalency Determinations

The PEC continues 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. It is not necessary to consult the PEC on sampling and monitoring programs if

a protocol is already approved under one of the Class A alternatives. The PEC does not write recommendations or approvals for processes that meet an existing alternative. The PEC will evaluate if it is necessary for a process to go through the equivalency or if it already meets one of the existing alternatives thus eliminating the need for an equivalency determination.

Equivalency determinations made by the PEC are RECOMMENDATIONS, the permitting authority has the ultimate decision on granting approval of a process as equivalent. The Equivalency process is lengthy and expensive. A recommendation for Equivalency is not considered an endorsement by EPA.

8.8 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. This is the regional EPA office or the State in cases in which responsibility for the Part 503 program has been delegated to the state. 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 must submit a Quality Assurance Project Plan (QAPP) which includes information on sewage sludge characteristics, process characteristics, climate, and other factors that may affect pathogen reduction or process efficiency as described in Section 8.5. The committee evaluates this information considering current knowledge concerning sewage sludge treatment and pathogen reduction and endorses the QAPP as a proper way to evaluate the process for an equivalency determination.

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

If the PEC 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 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.

8.9 Basis for PEC Equivalency Recommendations

As mentioned in Section 8.1, to be determined equivalent, a treatment process must consistently and reliably reduce pathogens in sewage sludge to the same levels achievable by the listed PFRPs or PSRPs. The applicant must identify the process operating parameters (e.g., time, temperature, pH) that result in these reductions.

8.10 PFRP Equivalency

To be equivalent to a 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 demonstrate that enteric viruses and viable helminth ova have been reduced to below detectable limits. The PEC requires that an equivalent process determination reduce enteric viruses by 3 log(base10), and viable helminth ova by 2 log(base 10). In many situations these organisms are not in a high enough density in raw sewage sludges, therefore it is necessary to spike the untreated material with these indicator organisms to achieve the proper log reductions. The equivalency determination does not require any demonstration of log reduction with the bacterial indicators fecal coliforms or *Salmonella* because Part 503 requires ongoing monitoring of all Class A biosolids for fecal coliform or *Salmonella* sp. (see Section 4.3). Thus, to demonstrate PFRP equivalency, the treatment process must be able to consistently show that enteric viruses and viable helminth ova are below the detectable limits shown below:

PFRP Microbial Indicator	Detection Limit
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)

8.11 PSRP Equivalency

A PSRP equivalency determination requires the demonstration of treatment reducing fecal coliforms by 2 log (base 10) and the density of these organisms cannot exceed 2 million CFU or MPN per dry gram of material. This 2 log reduction is based upon data from conventional biological and chemical treatment processes such as digestion and lime stabilization that a 2 log reduction in the indicator class fecal coliforms is equivalent to at least a 1 log reduction in other pathogenic bacteria, viruses and protozoans that may be present in the sludge. Since these pathogenic organisms are present in lower concentrations than fecal coliforms it is expected that a 1 log reduction is adequate treatment when combined with the other site restrictions imposed on these materials during land application. (Farrell et al., 1985, Farrah et al., 1986,USEPA, 1989c).

The data submitted must be scientifically sound 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. As mentioned in the PFRP section this is achieved by demonstration and different locations.

8.12 Guidance on Demonstrating Equivalency for PEC Recommendations

Many of the applicants seeking equivalency do not receive a recommendation from the PEC. The most common reason for this is incomplete applications or insufficient microbiological data. The review process can be both lengthy and expensive, but it can be expedited and simplified if the applicant is aware of the type of data that will be required for the review and submits a complete plan for demonstrating equivalency in a timely fashion.

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 Part 503 (see Tables 4.3 and 7.1), 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 condition required in Part 503 for lime stabilization) but uses a substance other than lime to raise pH could possibly qualify as a PSRP equivalent. 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., sewage sludge characteristics, process operating parameters, climatic factors) that influence the microbiological characteristics of the treated sewage sludge (see Section 9.5 for more detail on relevant parameters).
- Sampling and analytical data to demonstrate that the process has reduced microbes to the required levels (see Section 8.3 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 that is submitted as the final compliance sample should be sampled using accepted, regulatory approved techniques for sampling and analyzed using the methods required by Part 503 (see Chapter 10). The PEC will consider allowances for alternative methods for enteric virus and viable helminth ova. These methods must be clearly described in the QAPP and endorsed by the PEC prior to testing. The applicant must provide documentation that the modified or alternative method is comparable with the methods prescribed in Part 503. The PEC reserves the right to determine such an allowance. The sampling program should demonstrate the quality of the sewage sludge that will be produced under a range of conditions. Therefore, sampling events should include a sufficient number of samples to adequately represent product quality, and sampling events should be designed to reflect how

the operation might be affected by changes in conditions including climatic and sewage sludge quality variability. Sampling can also include surrogates in addition to the regulatory approved organisms such as coliphage and aerobic endospores, as further demonstration that processes are able to remove pathogens.

Data Quality

The quality of the data provided is an important factor in EPA's equivalency recommendation. The most important step that ensures the proper data quality and collection is by first obtaining QAPP that is reviewed and accepted by the PEC. The QAPP will outline the specific parameters of the process and provide details on testing, and data collection portion of the equivalency determination. This first step will allow the PEC to give feedback on the proposed testing and let an applicant know if the data collected will provide the necessary detail to make an equivalency determination. The analysis should be conducted using an independent and experienced laboratory that is familiar with biosolids sampling and analytical methods.

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.

Equivalency at pilot scale

The PEC will only consider proof at full-scale, however, a pilot-scale operation can be used and in some instances is encouraged for organisms that require spikes (enteric virus, and viable helminth ova). In these situations, the PEC requires that a pilot-scale test occur simultaneously to a full-scale test operation. In these cases, the pilot scale operation will be evaluated for all the organisms and other physical or chemical parameters that would be evaluated in the full scale. The full-scale test may only include a portion of the indicators in addition to all of the physical and chemical parameters. If it is determined that the pilot-scale operates identically to the full-scale system, then the data for the spiked organisms will be accepted from the pilot test and is not needed for full scale demonstration. This testing approach needs to clearly be described in the QAPP, and the permitting authority and the PEC will need to approve this type of testing prior to initiation of equivalency. The QAPP must specify where data is obtained from a pilot-scale operation, and to discuss why and to what extent this simulates full-scale operation.

The conditions of the pilot-scale operation should be at least as rigorous as those of a 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 from the full-scale operation that might reduce the comparability of the pilot-scale test results will invalidate any PEC equivalency recommendations and permitting authority equivalency determinations and will require a retest under more similar conditions.

8.13 Guidance on Application for Equivalency Recommendations

The following outline and instructions are provided as guidance for preparing applications for equivalency recommendations by EPA's PEC.

Applications should include the original PEC endorsed QAPP followed by a summary fact sheet. The fact sheet will summarize key information about the process, and other important facts that will be considered as part of what is required for measuring or monitoring after an equivalency is granted. The report should also include the full name of the treatment facility and the processes used should be provided. The application should indicate whether it is for recommendation of a PFRP or PSRP equivalency. And a site-specific or national equivalency. It is then necessary to give a proper process description which includes the type of sewage sludge used in the process 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 sewage 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.[15] The earliest point at which sewage sludge treatment can be defined is the point at which the sewage sludge is collected from the wastewater treatment process. Sufficient information should be provided for a mass balance calculation (e.g., 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:

Sewage Sludge Characteristics

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

[15] 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.

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 sewage 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 including information on monitoring equipment. Process uniformity and reliability should also be addressed. Actual monitoring data should be provided whenever appropriate.

The report will include the treated sludge characteristics including the type of treated sewage sludge as well as the sewage sludge monitoring program for pathogens. This can be completed by answering the following questions in the report:

- How and when are samples taken?
- Parameters needed for samples analyzed?
- What protocols are used for analysis?

- What are the results?
- How long has this program been in operation?

The report should give a detailed description of the sample techniques that were used during the testing. 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 sewage sludge are usually needed (see Sections 8.3, 4.6, and 11.4). The sampling points should correspond to the beginning and end of the treatment process as defined previously under the section Process Description. Chapters 10 and 11 provide guidance on sampling. Samples should be representative of the sewage sludge in terms of location of collection within the sewage 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.
- Number of composite samples compiled.
- Total solids of each sample.
- Air 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.

The report will also include analytical methods and results section, this is necessary to determine if the proper analytical techniques were used to evaluate the equivalency. This is done by identifying the analytical techniques used and the laboratory(s) performing the analysis. 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.

The report should include a quality assurance section that describes how the quality of the analytical data has been ensured. Subjects appropriate to address are how 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.

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. Lastly 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.

8.14 Pathogen Equivalency Committee Recommendations

The EPA biosolids webpage (https://www.epa.gov/biosolids/examples-equivalent-processes-pfrp-and-psrp) list processes that the PEC has recommended for use nationally as equivalent to PSRP or PFRP respectively. Tables 5.1 and 7.2 list the approved PSRP, and PFRP equivalencies. As such individuals having an interest in any of the processes are encouraged to contact either the PEC or the applicant for greater detail on how the process must be operated to be PFRP or PSRP respectively.

8.15 References

Farrah, S.R., G. Bitton and S.G. Zan. 1986. Inactivation of enteric pathogens during aerobic digestion of wastewater sludge. EPA Pub. No. EPA/600/2-86/047. Water Engineering Research Laboratory, Cincinnati, OH. NTIS Publication No. PB86-183084/A5. National Technical Information Service. Springfield. Virginia.

Farrell, J.B., G. Stern, and A.D. Venosa. 1985. Microbial destructions achieved by full-scale anaerobic digestion. Workshop on control f Sludge pathogens. Series IV. Water Pollution Control Federation. Alexandria, Virginia.

Smith, James E. Jr. and J.B. Farrell. 1996. Current and future disinfection - Federal perspectives. Presented at Water Environment Federal 69th Annual Conference & Exposition.

Whittington, W.A., and E. Johnson. 1985. Application of 40 CFR Part 257 regulations to pathogen reduction preceding land application of sewage sludge or septic tank pumpings. Memorandum to EPA Water Division Directors. USEPA Office of Municipal Pollution Control, November 6.

9: REQUIREMENTS FOR REDUCING VECTOR ATTRACTION

9.1 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.

Suitable methods for measuring vector attraction directly are not available currently. Vector attraction reduction is accomplished by employing one of the following:

- Biological processes that breakdown volatile solids, reducing the available food nutrients for microbial activities and odor producing potential
- Chemical or physical conditions that stop microbial activity
- Physical barriers between vectors and volatile solids in the sewage sludge

40 CFR Part 503 does not provide an option for vector attraction equivalency, therefore there is no equivalency committee comparable to the PEC. As a result, the specific options listed in Part 503 are the only available means for demonstrating vector attraction reduction. In addition to pathogen reduction alternatives, one of these options for VAR must be met in order to fully comply with 40 CFR Part 503 for land application.

The term stability is often used to describe sewage sludge. Although it is associated with vector attraction reduction, stability is not regulated by Part 503. Regarding sewage sludge, stability is generally defined as the point at which food for rapid microbial activity is no longer available. Sewage sludge which is stable will generally meet vector attraction reduction (VAR) requirements. ***The converse is not necessarily true; meeting VAR requirements does not ensure sewage sludge stability***. Because stability is related to odor generation and the continued degradation of sewage sludge, it is often considered an important parameter when producing biosolids for sale or distribution. Table 9.1 lists some of the common methods for measuring stability.

Table 9.1 Stability Assessment

Process	Monitoring Methods
Composting	CO_2 respiration, O_2 uptake
Heat Drying	Moisture content
Alkaline Stabilization	pH; pH change with storage; moisture; ammonia evolution; temperature
Aerobic Digestion	SOUR; volatile solids reduction, additional volatile solids reduction
Anaerobic Digestion	Gas production; volatile solids reduction, additional volatile solids reduction

Part 503 contains 12 options for demonstrating a reduction in vector attraction of sewage sludge. These requirements are designed to either reduce the attractiveness of sewage sludge to vectors (Options 1 through 8 and Option 12) or prevent the vectors from coming into contact with the sewage sludge (Options 9 through 11). VAR options are summarized in Table 9.2. Guidance on when and where to sample sewage sludge to meet these requirements is provided in Chapter 10.

Table 9.2 Vector Attraction Reduction Options

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
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 liquid sewage sludge with 2% or less solids that cannot meet the requirements of Option 1 -- e.g., sewage sludges treated in extended aeration plants. Sludges with > 2% solids must be diluted.

Requirement	What is Required	Most Appropriate For:
Option 4 503.33(b)(4)	SOUR at 20°C (68°F) is 1.5 mg oxygen/hr/g total sewage sludge solids	Liquid sewage sludges from aerobic processes run at temperatures between 10 to 30° C. (should not be used for composted sewage 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 sewage 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 (alkaline materials 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., 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

Requirement	What is Required	Most Appropriate For:
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

9.2 Monitoring for Vector Attraction Reduction

Not all the vector attraction reduction options listed in this chapter require lab testing. Specifically, Options 5,9,10, and 11 are technology descriptions. These technologies must be maintained throughout the year in the manner described in the regulation.

The remaining vector attraction reduction options are based on laboratory testing for volatile solids reduction, moisture content or oxygen uptake reduction. Some of the options can only be used with certain sludge processes. For example, the oxygen uptake rate test is only appropriate for a sludge from any aerobic digestion or wastewater treatment process. Other options such as the 38 percent reduction in volatile solids, can be applied to a variety of biological sludge treatment processes. In any case, the technology aspect of the option or the process by which vector attraction reduction is being attained must be documented. Monitoring for vector attraction reduction should be performed at a minimum according to the required monitoring schedule.

Some tests for vector attraction reduction 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 material tested and the performance of the vector attraction reduction process as affected by the changes in feedstock. It is suggested in Section 9.14 that facilities maintain a sampling program that involves sampling at evenly spaced time intervals throughout an established monitoring period. The on-going samples can be used to calculate running averages of volatile solids reduction which are more representative than single samples or an attempt to correlate feed sludge and sludge product. As is the case 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 for pathogen reduction are being determined.

The longer VAR tests present a similar problem as monitoring for microbiological quality. 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 9.6). In such circumstances, the preparer may wish to run the vector attraction reduction tests more frequently than required to demonstrate ongoing compliance with the requirements. More frequent testing will indicate if the facility is performing consistently and will reduce the need for multiple samples during the sampling period.

The preparer may wish to conduct composite sampling which combines samples taken within a 24-hour period to better represent sludge quality (See Section 10.6). Since some of the bench scale tests may be affected by long-term storage of samples, compositing should be limited to a 24-hour period. If composting is done, the composite should be held at ≤ 5°C during compositing and the assay must begin immediately upon completion of the composite.

Preparers should discuss specific facility parameters with the permitting authority to design a sampling program that is appropriate. It should be notes that Options 1-5 can be met prior to storage and need not be retested after storage.

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

This option is intended for use with biological treatment systems only. Historically, volatile solids reduction has been used as a measure of proper digestion, which is why it should only be used with biological process and not chemical addition or other physical processes. Under Option 1, reduction of vector attraction is achieved if the mass of volatile solids in the sewage sludge is reduced by at least 38%. This is the percentage of volatile solids reduction that can easily be attained by 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 percent volatile solids reduction can include any additional volatile solids reduction that occurs before the biosolids leave the treatment works, such as might occur when the sewage sludge is processed on drying beds or in lagoons.

The starting point for measuring volatile solids in sewage sludge is at the point at which sewage sludge enters a sewage sludge treatment process. This can be problematic for facilities in which wastewater is treated in systems like oxidation ditches or by extended aeration. Sewage sludges generated in these processes are already substantially reduced in volatile solids content by their long exposure to oxidizing conditions in the process. If sewage sludge removed from these processes is further treated by anaerobic or aerobic digestion to meet VAR requirements, it is unlikely that the 38% reduction required to meet Option 1 can be met. In these cases, use of Options 2,3, or 4 is more appropriate.

The end point where volatile solids are measured to calculate volatile solids losses can be at any point in the process. Volatile solids continue to degrade throughout sewage sludge treatment; however it is recommended that samples be taken at the end of treatment. Information on methods of calculation is provided in Appendix C.

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

Under this option, 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. As noted in Appendix D, the material balance method for calculating additional volatile solids reduction will likely show greater reductions than the Van Kleeck method.

Frequently, return activated 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 of time, they are adequately reduced in vector attraction, but because they entered the digester with volatile solids already partially reduced, 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. It is not necessary to demonstrate that Option 1 cannot be met before using Option 2 or 3 to comply with VAR requirements.

This additional anaerobic digestion test may have utility beyond use for sewage sludge from the classical anaerobic digestion process. The regulation states that the test can be used for a previously anaerobically digested sewage sludge. One possible application is for sewage sludge that is to be removed from a wastewater lagoon. Such sewage sludge may have been stored in such a lagoon for many years, during which time it has undergone anaerobic digestion and lost most of its volatile solids. It is only recognized by the regulations as a sewage sludge when it is removed from the lagoon. If it were to be further processed by anaerobic digestion, the likelihood of achieving 38% volatile solids reduction is very low. The additional anaerobic digestion test which requires a long period of batch digestion at temperatures between 30 and 37°C is an appropriate test to determine whether such a sewage sludge has the potential to attract vectors.

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

Under this option, 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) for an additional 30 days. Procedures for this test and the method for calculating additional volatile solids destruction 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). Liquid sludges with greater than 2% solids can be diluted to 2% solids with unchlorinated effluent, and the test can then be run on the diluted sludge. This option should not be used for non-liquid sewage sludge such as dewatered cake or compost.

This option is appropriate for aerobically digested sewage sludges that cannot meet the 38% volatile solids reduction required by Option 1. These include sewage sludges from extended aeration and oxidation ditch processes, 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.

As was suggested for the additional anaerobic digestion test, the additional aerobic digestion test may have application to sewage sludges that have been aerobically treated by other means than classical aerobic digestion.

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

For an aerobically digested sewage sludge with a total solids content equal to or less than 2% which has been processed at a temperature between 10 - 30° C, reduction in vector attraction can also be demonstrated using the SOUR test. The SOUR of the sewage sludge to be used or disposed must be less than or equal to 1.5 mg of oxygen per hour per gram of total sewage sludge solids (dry weight basis) at 20°C (68°F). 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 the total solids primarily to reduce the number of different determinations needed and for consistency with application rates, which are measured in total solids per unit area. Generally, the range in the ratio of volatile solids to total solids in aerobically digested sewage sludges is not large. The 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.

This test is based on the fact that if the aerobically treated 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 it. Frequently, aerobically digested sewage sludges are circulated through the aerobic biological wastewater treatment process 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. The SOUR test should not be used on sewage sludge products such as heat or air-dried sludge or compost. Because of the reduction of microbial populations that occur in these processes, the SOUR results are not accurate and should not be used. SOUR testing on sewage sludges with a total solids content below 0.5% may give inaccurately high results. According to the publication by Farrell, et al. (1996) several investigators indicate such an effect. Farrell, et al. (1996) also note that storage for up to two hours did not cause a significant change in the SOUR measurement. It is therefore suggested that a dilute sewage sludge could be thickened to a solids content less than 2% solids and then tested, provided that the thickening period does not exceed two hours.

The SOUR test requires a poorly defined temperature correction at temperatures differing substantially from 20°C (68°F). SOUR cannot be applied to sewage sludges digested outside the 10-30° C range (50-86°F). The actual temperature of the sewage sludge tested cannot be adjusted because temperature changes can cause short-term instability in the oxygen uptake rate, and this would invalidate the results of the test (Benedict, et al. 1973; Farrell, et al. 1996). Guidance on performing the SOUR test and on sewage sludge-dependent factors are provided in Appendix D.

It should be noted that the limit on the use of the SOUR test for sewage sludges at different solids and temperature levels is due to the lack of research and data on different sewage sludges

9.7 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, but not exclusively, to composted sewage sludge. These processed sewage sludges generally contain substantial amounts of partially decomposed organic bulking agents, in addition to sewage sludge. This option must be used for composted sewage sludge; other options are either not appropriate or have not adequately been investigated for use with compost.

Part 503 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 as long as temperature requirements can be met and the sewage sludge is maintained in an aerobic state for the treatment period, but other methods such as Options 3 and 4 are likely to be easier to meet for this type of sewage sludge.

If composting is used to comply with Class A pathogen requirements, the VAR time-temperature regime must be met along with or after compliance with the pathogen reduction time-temperature regime.

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

Sewage sludge is considered to have undergone adequate vector attraction reduction 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 two hours
- Maintain a pH of at least 11.5 without addition of more alkali for an additional 22 hours

The pH should be measured in a slurry ideally at 25°C. For more information on making a slurry, see Section 10.7. It is acceptable to measure the pH by adjusting for the temperature by using the following calculation:

$$Correction\ Factor = \frac{0.03\ pH\ units\ X\ (Tmeas - 25°C)}{1.0°C}$$

Where T_{meas} = the measured temperature in degrees centigrade

Actual pH = Measured pH +/- the Correction factor

As noted in Section 6.6, the term "alkali" means 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, surviving bacteria become biologically active and the sewage sludge will again putrefy and potentially attract vectors. The more soluble the alkali, the less likely it is that there will be an excess present when a pH of 12 is reached. Consequently, the subsequent drop in pH with time will be more rapid than if a less soluble alkali is used.

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. The requirement of raising the pH to 12 increases the probability that the material will be used before pH drops to a level at which putrefaction can occur.

Raising the pH to 12 and maintaining this pH for two hours and a pH of 11.5 for an additional 22 hours ensures that the pH will stay at adequately high levels to prevent putrefaction before disposal in all but unusual cases. In any event, it is prudent to apply the treated sludge in a timely manner in a thin layer or incorporate it into the soil for the prevention of odors and vector attraction.

More information on alkali addition and measurement of pH are included in Chapter 10.

9.9 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.

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 could attract birds, some mammals, and possibly insects, even if the solids content of the sewage sludge exceeds 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 sewage sludge could equilibrate to a lower solids content and attract vectors. Proper management should be conducted to prevent this from happening.

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

Vector attraction of any sewage sludge is 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 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.

Because sewage sludge meeting vector attraction reduction with this option may contain unstabilized solids, material that absorbs moisture or is rewet may putrefy and attract vectors. Proper storage and use of this material should be considered to prevent potential pathogen growth and vector attraction.

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

Vector attraction reduction can be achieved 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 sewage sludge, which reduces the mobility 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.

The special restriction requiring injection within 8 hours for Class A sewage sludge is needed because these sewage sludges are likely devoid of actively growing bacteria and are thus an ideal medium for growth of pathogenic bacteria (see Section 4.3). If pathogenic bacteria are present (survivors or introduced by contamination), their numbers increase slowly for the first 8 hours after treatment, but after this period their numbers can rapidly increase. This kind of explosive growth is not likely to happen with Class B sludge because high densities of non-pathogenic bacteria are present which suppresses the growth of pathogenic bacteria. In addition, the use of Class B biosolids requires site restrictions which reduce the risk of public exposure to pathogens. Consequently, this special requirement is not needed for Class B biosolids.

9.12 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 six 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 1/100th or less of the mass of soil in the plow layer (approximately the top six inches of soil). 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 that have not already met the vector attraction reduction requirements of the regulation by one of the other options.

The six 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 soaks into the first inch or two of topsoil.

9.13 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.

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

This option applies only to domestic septage applied to agricultural land, forest, a reclamation site, or surface disposal site. 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 6.6.). When this option is used, every container (truckload) must be monitored to demonstrate that it meets the requirement. As noted in Section 6.6, "alkali" refers to a substance that causes an increase in pH.

This vector attraction reduction requirement is slightly less stringent than the alkali addition requirement for sewage sludge. The method is geared toward the practicalities of the use or disposal of domestic septage which is typically treated by lime addition in the domestic septage hauling truck and land applied 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.

If domestic septage is not applied soon after pH adjustment, it is recommended that pH be retested and additional alkali be added to the domestic septage to raise the pH to 12 if necessary. Alternatively, if pH has dropped and the domestic septage begins to putrefy, it is advisable to cover or incorporate the domestic septage to prevent vector attraction

9.15 Number of Samples and Timing

Unlike pathogenic bacteria, volatile solids cannot regenerate once they are destroyed, so samples can be taken at any point along the process. However, since volatile solids are destroyed throughout treatment, it is recommended that samples be taken at the end of processing.

Facilities that use Option 2 or 3 to demonstrate vector attraction reduction must schedule sampling to leave ample time to complete the laboratory tests before sewage sludge is used or disposed. A suggested procedure would be to take several samples at evenly spaced time intervals during the period between the required monitoring dates and calculate running averages comprised of at least four volatile solids results. This has the advantage of not basing the judgement that the process is performing adequately (or inadequately) on one or two measurements that could be erroneous because of experimental error or a poorly chosen sample inadvertently taken during a brief process upset. It also provides an important quality control measure for process operations. Since Part 503 does not specify a sampling program, it is recommended that sewage sludge preparers consult with the regulatory authority regarding sampling schedules.

9.16 References

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10: SAMPLING PROCEDURES AND ANALYTICAL METHODS

10.1 Introduction

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. The purpose of this chapter is to describe procedures for obtaining representative samples and ensuring their quality and integrity. It also summarizes the analytical methods required under Part 503 and directs the reader to other sections of this document that describe some of those methods.

Sampling personnel will benefit also from reading expanded presentations on the subject. The WERF project entitled “An Investigation into Biosolids Sampling and Handling Methods for EPA-Approved Microbial Detection Techniques” provides the most comprehensive data with respect to the Part 503 microbial methods and sewage sludge sampling (WERF 2008). Other useful documents for additional information regarding sampling are “Standard Methods" (APHA, 2020), "Principles of Environmental Sampling" (Keith, 1988), "Sludge Sampling and Analysis Guidance Document" (USEPA, 1993), and ASTM Standard E 300-86, "Standard Practice for Sampling Industrial Chemicals" (ASTM, 1992a) and are highly recommended. 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.

Although extensive sampling is time consuming and facility operators are often under pressure to reduce costs, it is strongly recommended that multiple samples be included in a sampling plan so that the variable quality of sludge can fully be understood. Daily, weekly and seasonal fluctuations that occur in wastewater treatment works and sludge quality make it difficult to adequately represent sludge quality with minimal sampling. Therefore, multiple samples should be taken for any sampling event and samples should be taken over a minimum 2-week period in order to best represent the performance of a sludge treatment process.

Sampling events are intended to reflect the typical 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. For some process changes, long periods of time (as much as 3 months if anaerobic digestion is part of the process train) may be required before steady state operation is reestablished.

10.2 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/or viable helminth ova. Monitoring for these microorganisms presents special problems, primarily caused by the length of time it takes to obtain microbiological test results. This is a function of the time it takes to deliver the samples to a laboratory, have the tests conducted and obtain the results. Microbiological analyses require a substantially longer period than conventional physical and chemical analyses. The approximate time to complete specific microbiological analyses is summarized as follows.

- Fecal coliform (MPN), two-four days
- *Salmonella* sp. (MPN) five to seven days
- Enteric viruses, 14 days
- Viable helminth ova, 28 days

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 to determine whether a process meets or fails to meet a requirement. The Pathogen Equivalency Committee recommends that the monitoring event include at least seven samples taken over a period of approximately 2 weeks (see Section 10.7). Based on the reliability of the treatment process and historic test results, there may be times when a reduction in this monitoring recommendation is justified.

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 generated is unknown. As discussed in Section 4.10, classification of sludge as Class A or B is based on the most recent test results available. Therefore, material can continue to be distributed under its classification as Class A or B until more recent analytical results are available. ***However, it is recommended that material generated during the monitoring event be retained on site until results from the monitoring event are available. This will prevent misclassified sludge from being erroneously distributed.***

For example, consider a facility producing a Class A sludge that is sampled for *Salmonella* sp. analysis every quarter. All historic data have shown the facility to be in compliance with Class A standards including the most recent set of lab analyses from the January monitoring event. Under these results, materials are distributed as Class A products even throughout April when a subsequent monitoring event takes place. This is acceptable because material is still classified under the most recent available lab result. However, suppose the April results show non-compliance with Class A standards. Despite the fact that the preparer complied with regulations, it is possible that some sub-standard material was inadvertently distributed for Class A use.

In order to avoid this situation, it is recommended that the sludge processed during the monitoring event 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). This may take up to 3 weeks in the case

of fecal coliform or *Salmonella* sp. analysis and much longer if sludge is being analyzed for helminth ova or viruses. Contingencies for this type of situation should be discussed with the regulatory authority and included in permit conditions and operational plans. For more discussion on the timing of sampling and distribution, see Section 4.10.

10.3 Comparison of Feed Sludge and Sludge Product Samples

In some instances, it is necessary to compare the quality of the feed sludge to the final treated product to determine vector attraction reduction methods, as well as microbial reduction that occurs during the treatment process. There are many factors that go into ensuring that comparison of feed sludge to treated sludges are accurate (e.g., feed rate, flow rate, solids retention time (SRT), mean cell residence time (MCRT), etc.) All of these factors need to be considered when planning how to properly sample across the treatment process.

Samples taken after the process has reached steady state operation are considered as corresponding. Obtaining samples that correspond can be difficult for sewage sludge treatment processes that treat sludge in fully mixed reactors with long residence times (e.g., anaerobic digestion). For example, as mentioned in Section 11.3, it can take up to 3 months for an anaerobic digester to achieve steady state operation after some substantive change in feed sludge or process condition is made.

Many of the treatment processes that might be considered for demonstrating equivalency to PFRP are either batch or plug flow processes. In theory it is relatively simple to obtain corresponding samples - 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 for 3 months.

Taking multiple samples and appropriately compositing the samples of feed and treated sludge averages out the composition of these sewage sludges and reduces the correspondence problem. It is the regulatory authority's task to determine how many samples should be taken and how much data is necessary to demonstrate reduction of microorganisms in corresponding samples. As indicated in Section 11.6, limitations on the periods of time over which microbiological samples can be collected limit the utility of compositing.

10.4 The Effect of Sludge Processing Additives on Monitoring

Many sewage sludge dewatering and stabilization processes introduce other substances into the sludge. With the exception of large bulky additives such as wood chips, there is no need to modify sampling and analytical procedures. As discussed below, additives such as wood chips can complicate sample preparation and analysis and are best removed prior to analysis.

Lime, ferric chloride, paper pulp, and recycled sludge ash are frequently used to aid in dewatering. Disinfection by alkaline treatment requires the addition of lime or other alkaline materials to increase the temperature of the sewage sludge cake to disinfecting temperature. These materials also reduce the microbial densities by dilution and increase solids content. However, the change in microbial density

caused by dilution may not be substantial. 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.

Polymers are probably the most common additive used during the dewatering process. Polymers that are in a powdered form must be reconstituted with a liquid prior to use. Polymers should not be reconstituted with "treated effluent". Effluents are not treated to the same standards that are required by Part 503 and these materials may contain significant microbial loads. When polymers reconstituted with effluents are combined with treated sewage sludge they can contaminate these materials, as well as concentrate microbes through the dewatering process. This can give an elevated microbial level which can result in the material being out of compliance with the regulatory limits.

Human health can be directly impacted through contact with treated sewage sludge. Therefore, it is imperative that the final product integrity with respect to pathogen levels be considered when handling the material after sludge treatment. This includes the proper timing for pathogen sampling, as required by Part 503, to occur as close to the time of disposal, or application. This requires that the treated sludge, regardless of the mass of other materials added, meet the microbial standards for Class A or Class B biosolids.

For some sludges, particularly those treated by composting (these usually will be Class A biosolids), the amount of additive can be considerable. Nevertheless, the regulation requires that the biosolids meet the standard which means that no correction need be made for dilution. The issues of sampling and analytical procedures for employment are different when considering wood chips or other materials which are often added to sludge as a bulking agent for composting.

Large additives are removed 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 residues from blending. Another reason for removing the wood chips prior to microbial analysis is that any potential exposure concerns when using the compost is relative to the sewage sludge content and not the wood chips.

When analyzing composted materials that contain a considerable amount of wood chips it is necessary to remove these large pieces prior to analysis. This step can be performed using a sterilized sieve and the size of the sieve needed depends on the dimensions of the wood chips, but the same sieve size should be used for each sampling event.

10.5 Collecting Representative Samples

Sludge quality varies depending on the inputs to the wastewater system. In addition, the process is subject to ambient conditions which vary daily as well as seasonally. The goal of a sampling program is to adequately represent the sludge quality overall taking into consideration processing variables. This is completed with frequency of sampling events and the number of samples taken during each sampling event being considered carefully. This section discusses the issue of variability and how sampling frequency and composite sampling can improve the quality of data collected. A sampling plan is recommended for all sampling events to assure representative samples.

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. Cyclic variation can be minimized by sampling on randomly selected days and times-of-day within a given week. For Class B fecal coliform analysis ONLY**,** variability is minimized by taking the geometric mean of analytical results. In the case of Class A biosolids, all samples must meet the fecal coliform or *Salmonella* sp. numerical limit.

Seasonal Variability

For some sewage sludge treatment processes, performance is poorer during certain parts of the year due to seasonal variations in temperature, sun radiation, 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 at intervals of 2 or 3 months, 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. It may also be beneficial to initially conduct sampling more frequently than the required minimum (e.g., on a quarterly basis) in order to determine the range of sludge quality. Process criteria of PSRPs and PFRPs should be discussed by the facility with the regulatory authority and specific requirements should be included in permit conditions.

Composite Sampling

Composite sampling, or the combination of several grab samples to better represent a large quantity of sludge, is frequently practiced in wastewater treatment. Composites may consist of grab samples taken over time (typically for continuous flow processes) or from random locations in a vessel or pile (typically for batch processes). Since the purpose of composite sampling is to provide representation of a large quantity of sludge, certain factors should be considered: the number and distribution of grab samples, the locations from where the samples are taken, and the process of combining the grab samples.

The following is an example of a sampling procedure for compositing a continuous flow process. A small stream of wastewater or sludge is drawn off at a rate proportional to the flow of the mainstream 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-averaged sample over the period of time the sample is drawn. The sample is chilled during the period it is being collected to prevent chemical/microbiological change until it can be brought back to the laboratory for analysis.

Composite sampling from stockpiled solid material involves taking multiple grab samples from a range of locations in the stockpile. Samples should be taken from different interior sections of the pile which may represent material produced in different time periods. Grab samples should all be of the same size so that the composite is an equal representation of all grab samples. The grab samples should be mixed thoroughly, and a subsample pulled from the mixture. In the case of monitoring a lagoon when dredging the sludge for land application after a number of years, the lagoon can be cordoned into seven areas and a composite sample can be taken from each section or as described in Section 10.9.

Composite sampling is useful for any type of sampling, but the protocol must be modified when microbial analyses are intended. Samples must be taken over a shorter period of time so that microbial populations do not undergo significant changes during the sampling event. For example, a composite time-average sample can be obtained by combining a series of small samples taken 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 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). In addition to limiting the sampling period, sterile equipment must be used when taking grab samples or compositing the samples for microbiological analysis to prevent introducing pathogenic bacteria.

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.

Requirements for Sampling Equipment and Containers

Sampling containers may be made of glass or plastic that does not contain a plasticizer (Teflon, polypropylene, and polyethylene are acceptable). Pre-sterilized bags are especially useful for thick sewage sludges and free-flowing solids. Stainless steel containers are acceptable, but steel or zinc coated steel vessels are not appropriate. In addition to providing guidance on appropriate containers for specific analyses, analytical laboratories will typically provide sample containers. Sampling containers used for microbiological analyses must be sterile. Sampling tools that come in contact with the sample should be constructed of stainless steel, which is easily cleaned and sterilized. Tools made of wood are difficult to sterilize because of porosity and should not be used.

Equipment

The sampling equipment used is primarily dependent on the type of material being sampled. For relatively high solids content biosolids, a hand trowel or scoop may be adequate, whereas, collecting stratified samples from a lagoon requires more sophisticated and specialized equipment. Automated sampling equipment that is commonly used for wastewater should not be used since it can cause solids separation during sampling and it is difficult to clean, resulting in cross contamination. Sampling equipment should be constructed of non-corrosive materials such as stainless steel, Teflon, or glass that can be thoroughly cleaned and sterilized. Sampling equipment should be dedicated for this task and should not be used for other applications. Equipment should be cleaned well with detergent and a nylon scrub brush after each use and stored in a dedicated location. The types of sampling equipment and their applications are presented in Table 10.1. The use of this equipment is discussed in greater detail in Sections 10.6 and 10.7.

Table 10.1: Equipment used for Collecting Sewage Sludge Samples

Equipment	Application
Composite Liquid Waste Sampler (Coliwasa)	The Coliwasa is a device employed to sample free-flowing sewage sludges contained in drums, shallow tanks, pits, and similar containers. It is especially useful for sampling wastes that consist of several immiscible liquid phases. The Coliwasa consists of a glass, plastic, or metal tube equipped with an end closure that can be opened and closed while the tube is submerged in the material to be sampled.
Weighted Bottle	This sampling device consists of a glass or plastic bottle, sinker, stopper, and a line that is used to lower, raise, and open the bottle. The weighted bottle is used for sampling free flowing sewage sludges and is particularly useful for obtaining samples at different depths in a lagoon. A weighted bottle with line is built to the specifications in ASTM Method D270 and E300.
Dipper	The dipper consists of a glass or plastic beaker clamped to the end of a two- or three-piece telescoping aluminum or fiberglass pole that serves as the handle. A dipper is used for obtaining samples of free-flowing sewage sludge that are difficult to access. Dippers are not available commercially and must be fabricated.
Sampling Thief	A thief consists of two slotted concentric tubes, usually made of stainless steel or brass. The outer tube has a conical pointed tip that permits the sampler to penetrate the material being sampled. The inner tube is rotated to open and close the sampler. A thief is used to sample high solids content materials such as composted and heat dried biosolids for which particle diameter is less than one-third the width of the slots. Thief samplers are available from laboratory supply companies.
Trier	A trier consists of a tube cut in half lengthwise with a sharpened tip that allows the sampler to cut into sticky materials such as dewatered cake and lime stabilized biosolids. A trier is used to sample moist or sticky solids with a particle diameter less than one-half the diameter of the trier. Triers 61 to 100 cm long and 1.27 to 2.54 cm in diameter are available from laboratory supply companies
Auger	An auger consists of sharpened spiral blades attached to a hard metal central shaft. An auger can be used to obtain samples through a cross section of a biosolids stockpile. Augers are available at hardware and laboratory supply stores.
Scoops and Shovels	Scoops are used to collect samples from sewage sludge or biosolids stockpiles, shallow containers, and conveyor belts. Stainless steel or disposable plastic scoops are available at laboratory supply houses. Due to the difficulty of sterilizing shovels and other large sampling equipment, this type of equipment should only be used for accessing the center of stockpiles and should not be used for collecting the actual sample.

Sterilization

The containers and tools used for sampling must be sterilized if the biosolids product is to be analyzed for Class A and Class B microbiological parameters. Alternatively, pre-sterilized, disposable scoops, and other sampling devices can be purchased, alleviating the need to sterilize sampling equipment. After the samples are collected, the sampling equipment should be cleaned well with soap and water and put away

until the next sampling event. Equipment should be dedicated to sampling and not used for other activities. Only equipment that touches the actual sample must be sterilized. Equipment such as shovels or heavy equipment used to access a sludge pile interior does not need to be sterilized, but should be clean, as long as another sterile sample collection device (such as a hand trowel) is used to access and collect the actual sample. Sterilization is not required when collecting samples of sewage sludge to be used in vector attraction reduction tests, but all equipment must be clean.

Either steam or a sterilizing solution such as sodium hypochlorite (household bleach) should be used for sterilizing equipment. If bleach is used, equipment must be rinsed thoroughly to prevent residual bleach from influencing the microbial population in the sample. Equipment should be thoroughly washed with water, soap, and a brush prior to sterilization. If an autoclave or large pressure cooker is available, enclose the sampling tool in a kraft paper bag and place the bag in the autoclave. A minimum of 30 minutes at a temperature of 121^{o}C is required for sterilization. The kraft paper bag keeps the sampling device from becoming contaminated in the field. A steam cleaner can also be used to sterilize sampling equipment. Place the equipment in a heat resistant plastic bucket and direct steam onto the equipment for a minimum of 10 minutes. When done, place the sterilized equipment in a kraft paper bag. Many facilities do not have an autoclave or steam cleaning equipment and will need to purchase presterilized collection bottles or bags. In these situations, it is acceptable to use a sterilizing solution to sterilize some equipment (e.g., collection spoons or shovels). This protocol should not be used for sample bottles due to the large potential for disinfectant residual to affect the microbial population. A 10% household bleach solution (1-part bleach, 9-parts water) is readily available and works well. However, bleach is corrosive and may also affect the microbial population of a sample and does need to be adequately removed from the equipment prior to sample collection. Make up the 10% solution in a clean plastic bucket. Immerse each piece of clean equipment in the solution for a minimum contact time of a minute. Rinse the equipment in another bucket containing sterile or boiled water. Let the equipment air dry for a few minutes or dry with sterile paper or cloth towels. After drying, place the equipment in a paper bag. Sterile plastic bags obtained from a scientific equipment supplier can also be used for short-term sterile equipment storage.

10.6 Laboratory Selection

A very important, but often overlooked component of pathogen and vector attraction monitoring is selecting an appropriate analytical laboratory. The analysis of sewage sludge or biosolids for indicator and pathogenic organisms is more complex than water analysis. Solid samples such as biosolids are prepared differently than water samples and typically contain a much higher background microbial population than water contains. Biosolids products such as compost can be very heterogeneous, requiring special sample preparation procedures. It is therefore important to use a laboratory that has developed an expertise through the routine analysis of biosolids products.

To ensure that a laboratory has adequate experience with biosolids analyses, the following information should be obtained and reviewed:

- For how long has the laboratory been analyzing biosolids for the specified parameters?
- Approximately how many biosolids samples does the laboratory analyze per week or month?
- For how many wastewater treatment facilities is the laboratory conducting the specified analyses?
- A list of references.
- Does the laboratory have a separate and distinct microbiology lab?
- Does the laboratory have microbiologists on staff? Request and review their resumes.
- Who will perform the analyses?
- Is the laboratory familiar with the analytical procedures including sample preparation, holding times, and QA/QC protocols?

A laboratory tour and reference check are also recommended. A good laboratory should be responsive, providing requested technical information in a timely manner. It is the biosolids generator's responsibility to provide accurate analytical results. Consequently, the selection of an appropriate laboratory is an important component of developing a biosolids monitoring plan.

10.7 Safety Precautions

Sewage sludge that is 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, 2017) in Sections 1060 and 1090.

Individuals performing sampling (usually employees of wastewater treatment works) should receive training in the microbiological hazards of sewage sludge and in safety precautions to take when sampling. Laboratory personnel should be aware that the outside of every sample container is probably contaminated with microorganisms, some of which may be pathogens. Personal hygiene and laboratory cleanliness are also extremely important. Several safety practices that should be standard procedures during sample collection and analysis are:

- Gloves should be worn when handling or sampling untreated sewage sludge or treated biosolids.
- Personnel taking the samples should clean sample containers, gloves, and hands before delivering the samples to others.
- Hands should be washed frequently and always before activities that involve hand-to-mouth contact.

- 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.
- Mouth pipetting should be forbidden.

Employees involved in sample collection (or any other activity where they are exposed to wastewater or sewage sludge) should review their immunization history. At a minimum, employees should be immunized against tetanus. However, employees should consider immunization for other diseases, particularly hepatitis A and B. Employees should also consider having a blood sample analyzed to determine if they still have active antibodies for the various immunizations they received as children.

Personnel analyzing sewage sludge or biosolids samples should receive training in awareness and safety concerning biohazards. Because microbiological laboratories have safety programs, this subject is not covered in depth in this document. A facility's sampling plan should include a section on microbiological hazards and appropriate safety practices or, alternatively, refer the reader to another document where this information is presented.

10.8 Sampling Frequency and Number of Samples Collected

The primary objective of biosolids monitoring is to assure that all biosolids produced meet the regulatory requirements related to land application. It is not feasible to sample and analyze every load of biosolids leaving a facility, nor is it necessary. However, a sampling plan does need to adequately account for the variability of the biosolids. The plan should entail collecting a sufficient number of samples at an adequate frequency. The minimum sampling frequency and number of samples to be analyzed as listed in 40 CFR Part 503 are listed in table 10.2.

Table 10.2 Testing frequency required by Part 503.

Frequency of Monitoring for Land Application and Surface Disposal	
Amount of Biosolids[1] (metric tons dry solids per 365-day period)	Minimum 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 biosolids applied to the land, or the amount of sewage sludge received by a person who prepares biosolids that is sold or given away in a bag or other container for application to the land (dry weight basis), or the amount of biosolids (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)

The number of samples which must be analyzed for compliance with Class A microbiological parameters is not specified, however, it is strongly recommended that multiple samples per sampling event be analyzed. The number of samples taken must be sufficient to adequately represent biosolids quality. It must be noted that for Class A biosolids, analytical results are not averaged; ***every sample analyzed must meet the Class A requirements.***

To meet Class B Alternative 1 requirements, seven samples must be taken, and the geometric mean of results must meet the 2.0×10^6 MPN/CFU fecal coliform per dry gram limit (see Chapter 6). It is recommended that the samples be taken over a two-week period to adequately represent variability in the sewage sludge.

The actual sampling and analysis protocols are typically developed by the facility and reported to the regulatory authority which can require a more stringent sampling and analysis protocol than what is stipulated in Part 503. In some cases, the regulatory authority may initially require a more stringent monitoring schedule that may be relaxed once product consistency is established. There may also be requirements that analysis be conducted by certified laboratories. The biosolids preparer should carefully consider the treatment process, analytical variability, end-use and other factors when determining the frequency and number of samples to be analyzed. Collecting samples more frequently or analyzing more samples will help to ensure the product meets the regulatory criteria and that pathogen and vector attraction reduction goals have been met.

It is also recommended that additional sampling be conducted for heterogeneous biosolids products. A single grab sample may adequately represent the sewage sludge in a digester that is being mixed but might not adequately represent tons of compost product stored in several stockpiles. Likewise, a facility that conducts a single annual analysis should consider more frequent monitoring, particularly if the analytical results from the annual analysis are near the regulatory limit. It is a facility's responsibility, and in the facility's best interest, to develop a monitoring plan that assures product quality.

10.9 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 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 sewage sludge 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 10.7.

Filling Containers

Liquid sewage sludge samples are usually transferred into sterile wide mouth bottles or flexible plastic containers. Sewage sludge can generate gases, and pressure may build up in the container. Consequently,

the bottle or container is generally not filled completely allowing at least 1 inch of headspace. If the sewage 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 microorganisms in the sewage sludge. Conversely, if the sewage 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, sample bottles for the anaerobic digestion test must be filled to the top. Bottles should have closures that can pop off or made of flexible plastic that 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 sewage sludges of solids contents below about 3%.

Collecting Liquid Sewage Sludge Samples

Liquid sewage should be sampled downstream of a pump that serves to mix the sewage sludge thoroughly. The sample is taken through 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 sewage sludge that is not liquid because fibrous deposits can 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.

The collection of a representative sample often requires the use of time compositing procedures. 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. If the sample is being analyzed for bacteria, viruses, or vector attraction reduction, the composite should be prepared within an hour of collecting the first individual grab sample. Holding times are listed in 40 CFR Part 136 for the specific method that is used for each sample. These holding times must be observed for testing to be compliant with the regulations. A longer holding time might allow microbiological changes to occur in the first sample taken.

Sampling Sewage Sludge in Tanks

The purpose of the sampling is to determine the properties of the entire mass of the sewage sludge. This requires that the tank be well-mixed, otherwise many subsamples must be taken throughout the tank and composited. Large tanks, even if they are well-mixed, have the potential for developing gradients in composition. An enclosed tank such as an anaerobic digester must be sampled through pipelines entering the digester. A minimum of three taps on a side wall of the enclosed tank is recommended. The sample

tap pipe should project several feet into the tank. Precautions must be taken to minimize contamination from sample collection lines. When a sample is taken, enough material must be withdrawn to thoroughly flush the line before the sample is collected. This helps flush any contaminants in the sample line and assure that a representative sample is collected from the tank. The sample line should be back-flushed with water after the sample is withdrawn to clean out residual sewage sludge and prevent microbial growth. Sampling should be conducted 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 to a rigid tube lowered to the desired level in the tank. A weighted sampling bottle may also be used to sample the liquid at the desired depth in the tank (see ASTM E30086, Par. 21, in ASTM [1992a]).

10.10 Sampling Thick Sewage Sludges

If sewage sludges are above 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 sewage sludge stored in lagoons may be very difficult depending on the objectives of sampling and the nature of the sewage sludge in the lagoon. The thickened sewage 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 the nonuniformity of the solids deposit into account. 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 sewage 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 sewage sludge layer may be useful. Weighted bottle samplers (ASTM, 1992a) that can be opened at a given depth can be used to collect samples at a desired depth. Samples at three depths should be taken and composited. Most likely the sewage sludge will be as much as twice as high in solids content at the bottom of the sewage 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 for microbiological and VAR parameters because there is no practical way of separately removing layers of sewage sludge from a lagoon. Determining whether there are gradients with length and width makes more sense because sewage sludge could be removed selectively from part of a lagoon, leaving behind the unacceptable material.

Sewage sludges from dewatering equipment such as belt filter presses and centrifuges can have a solids content up to 35% and even higher following some conditioning methods. High solids content sewage sludges are easy to sample if they are on moving conveyors, as described in Section 9.5. However, if the sewage sludge is stored in piles, obtaining a representative sample requires more planning and a greater overall effort. As a result of different environmental conditions between the pile surface and interior, a gradient will develop over time in the sewage sludge storage pile. The sampling methodology used needs to address this potential gradient between the pile surface and interior. Sampling devices such as augers (a deeply threaded screw) are used on high solids cakes (ASTM, 1992a) to collect a cross sectional sample.

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. Alternatively, a shovel can be used to collect subsamples for compositing from the pile interior. The pile should be sampled in proportion to its mass; more samples are taken where the pile is deeper.

10.11 Sampling Dry Sewage Sludge

For purposes of this discussion, "dry" sewage sludge contains as much as 60% water. This includes heat dried and composted sewage sludge, and sewage sludge 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 sewage sludge is best sampled when it is 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.

Collecting a representative sample from a stockpile containing a dried sewage sludge poses a greater challenge than collecting the sample from a conveyor. The settling and classification of the material and the different environments between the pile edge and interior must be considered. When a material comprised of discrete particles is formed into a pile, classification occurs. If the particles are homogeneous in size and composition a representative sample can be easily obtained (assuming the sample is collected within 24 hours of pile construction). However, if the particles are of a different size or composition an unequal distribution of the particles may result during settling. For example, a composted sewage sludge may be heterogeneous with respect to particle composition even when oversized bulking agents have been removed. It is important that the edges and interior of such piles are properly weighted as part of the sample collection procedure. A sampling grid that prevents bias, such as that presented in ASTM E300-86, Item 31.4 (ASTM, 1992a), should be used.

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

Stockpile sampling is made more difficult by the constant evolution of the characteristics of stored material. Immediately after a sewage sludge stockpile is constructed, physical, chemical, and biological changes begin to occur within and on the surface of the stockpile. Within a period as short as 24 hours, the characteristics of the surface and outer part of the pile can differ substantially from that of the pile interior. The outer part of a pile tends to remain at or near ambient temperature, loses moisture through evaporation, and volatilizes some compounds such as ammonia. In contrast, pile interiors retain heat (achieving temperatures that can be 40^{o}C greater than the pile surface) and lose little moisture or chemical compounds through evaporation and volatilization. As a result, the level of microbial growth and activity within the pile and on the pile surface will differ. The potential for growth of fecal coliform bacteria in mesophilic regions of the pile is of particular concern. If a sewage sludge stockpile is more

than one day old the sample should be collected from a pile cross section. This is especially important when there is a large temperature gradient between the pile surface and interior.

10.12 Temperature, pH, and Oxygenation Control for Microbial Tests

Table 10.2 summarizes allowed microbial methods along with the maximum holding times and temperatures for sewage sludge samples for microbial analyses. All samples should be cooled to appropriate temperatures immediately after they are collected to minimize changes in indicator organism and pathogen populations. 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 takes much longer to cool than a quart bottle. Use of bottles no larger than a quart is recommended, particularly if the sewage sludge being sampled is from a process operated at above ambient temperature. Granular solids and thick sewage sludges take a long time to cool, so use of containers smaller than one quart is advised in these situations. For rapid cooling, place the sample container in a slurry of water and ice. Placing the sample container in a cooler containing 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. The presence or absence of oxygen is not a serious concern for the microbiological tests if the samples are promptly cooled.

Table 10.2. Analytical Methods Required Under Part 503

Analysis	Methodology	Maximum Holding Time[3]/Temperature
Enteric Viruses	American Society for Testing and Materials Method D 4994-89 (ASTM, 1992b)[1](Appendix F of this document)	-18°C (0°F); up to 2 weeks
Fecal Coliform	EPA Method 1680, EPA Method 1681 (Appendix E of this document) **Preferred methods by US EPA** Standard Methods Part 9221 C E (APHA, 2006) or Part 9222 D (APHA, 1997) [2]	4°C (39.2°F) (do not freeze); 8 hours with the exception of Class A composted, and Class B Aerobically or Anaerobically digested materials may have a 24 hr maximum hold time as listed in 40 CFR part 136.
Salmonella sp. Bacteria	EPA 1682 (Appendix E of this document)	4°C (39.2°F) (do not freeze); 8 hours
Viable Helminth Ova	Yanko (1987) (see Appendix G of this document)	4°C (39.2°F) (do not freeze); 1 month

Analysis	Methodology	Maximum Holding Time[3]/Temperature
Specific Oxygen Uptake Rate (SOUR)	Standard Methods Part 2710B (APHA,1992)	20°C (sewage sludge must be digested in the 10-30°C range); 2 hours
Total, Fixed, and Volatile Solids	Standard Methods Part 2540G (APHA,1992)	NA
Percent Volatile Solids Reduction	Appendix C of this document	NA

[1]Appendix H of this document presents a detailed discussion of this method.

[2]Method SM-9221D, the membrane filtration procedure is also allowable for Class B biosolids. See Appendix F of this document for recommended sample preparation procedures and a discussion of the reporting of results.

[3]Time between sampling and actual analysis, including shipping time

EPA conducted a hold time study for analysis of fecal coliform and *Salmonella* using EPA Methods 1680, 1681, and 1682 (USEPA, 2014 and 2006). The results of that study determined that a holding time longer than 8 hours could result in significantly lower numbers of fecal coliforms and *Salmonella* in the final biosolid material, except for composted Class A, and aerobically or anaerobically digested Class B products. **The results of this study have been used to determine the hold times that are listed in 40 CFR Part 136, and as such they supersede any other contradicting hold times that are present in any other approved method. The sample should never be frozen.**

Proper planning and coordination with the courier service and analytical laboratory are essential if bacterial analyses are to be conducted within the proper hold time of sample collection. The laboratory needs to be notified several days in advance so they can be prepared to initiate the analysis within several hours of receiving the sample. If they are not notified, the laboratory may not be adequately prepared, and another day may lapse before the samples are analyzed. Careful coordination with the laboratory needs to ensure that the tests for fecal coliform and *Salmonella* start no longer than 8 hours after time of collection, unless allowed a longer hold time to 24 as permitted in 40 CFR Part 136. The holding times for enteric virus and viable helminth ova are much longer and as such are typically not a problem for coordinating with the lab.

Follow-up with the laboratory is important to determine the actual sample holding time and temperature of the sample when it was received. This information can be used to improve the overall sample collection and transfer procedure. Feedback received from the lab regarding sample condition and holding times may also provide an explanation for erroneous or unexpected test results.

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 can conduct bacterial analyses, so time

between sample collection and analysis can routinely exceed the hold times. Fortunately, viruses are not harmed by freezing. Typically, virology laboratories store samples at -70°C (-94°F) before analysis. Samples can be frozen in a -18°C (0°F) freezer and stored for up to 2 weeks without harm. Samples should be frozen, packed in dry ice, and shipped overnight to the analytical laboratory.

Viable helminth ova are only slightly affected by temperatures below 35°C (95°F), provided chemicals such as lime, chlorine, or ammonia are not 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 not well understood.

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 provides 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.

Depending on whether the sewage sludge is from an aerobic process or anaerobic digestion, the presence or lack of oxygen will determine which vector attraction reduction test is appropriate and therefore how the sample should be handled. For aerobic sewage sludges, a lack of oxygen 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. With samples taken for SOUR analysis, it has been the experience of some investigators that if the test is not run almost immediately after collection (within about 15 minutes), erroneous results are obtained. The additional aerobic digestion test is more "forgiving" (because it is a long-term test and shocked bacteria can revive); up to 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.

No pH adjustment is to be made for any of the vector attraction reduction tests. 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 9.7).

pH Adjustment and Sewage Sludges

For addition of alkali to sewage sludges, the pH requirement is part of both the PSRP process description (see Section 6.3) and the requirement of a vector attraction option (see Section 9.7). Monitoring is required from 1 to 12 times a year (see Table 3.4 in Chapter 3), and the process must meet the prescribed operating conditions throughout the year.

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 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 USEPA, 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 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 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 composite samples collected for pH measurement must be reduced in size for the pH measurement. The alkaline-treated sludge may be discharged from the mixing devices in the form of irregular balls that can be up to 5 to 7.6 cm (2 or 3 inches) in diameter. If the discharged biosolids are ball shaped and the alkali has not penetrated the entire ball, one or both of these goals is not met for the material inside the ball. The entire ball 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 the balls.

Vector Attraction Reduction Tests

Testing samples for vector attraction reduction is different from testing for microbes in that it is not necessary to use sterilized equipment. There is no concern for microbial contamination with these samples. There are, however, some important points to consider for VAR sampling. When sampling to

measure for volatile solids reduction it is important to keep the aerobic samples aerobic and to prevent the anaerobic samples from coming into contact with air, which is done by filling the bottle completely with no air space once capped. Subsamples for the anaerobic tests can be collected into individual bottles at the sampling location. As noted previously, 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 acceptable sample size reduction procedure is to flush a large sterile 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. Analytical laboratories generally can perform this size reduction procedure.

10.13 Packaging and Shipment

Proper packaging and shipment are important to ensure that the samples arrive in good condition (proper temperature, no spillage) within the specified time frame.

Sealing and Labeling Sample Containers

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. A permanent label should be affixed to each sample container. Sterile collection bottles or bags that are used and shipped out on ice also should be placed in individual sealable bags to avoid contamination with the ice that is used during shipping. Never place a bottle directly into ice, this will cause contamination of the sample inside the bottle. At a minimum the following information should be provided on each sample container:

- Type of analysis to be performed (e.g., *Salmonella* sp., fecal coliform bacteria, enteric virus, or viable helminth ova)
- Sample identification code (if used) or a brief description of the sample (that distinguishes it from other samples) if no sample code system is used
- Sample number (if more than one sample was collected at the same point on the same day)

Other information may include:

- Facility name, address and telephone number
- Date and time the sample was taken
- Facility contact person

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
- Requested preprocessing (subsampling, compositing, particle size reduction)
- Requested analyses
- Sample code number for each sample (if used)
- Signatures of the persons involved in the chain of possession

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 forms can be obtained from the laboratory and should be used even if the laboratory is on-site and part of the treatment facility.

Shipment Container

A soundly constructed and insulated shipment box is essential to provide the proper environment for preserving the sample at the required temperature and to ensure the sample arrives intact. Small plastic cased coolers are ideal for sample shipping. It is recommended that the outside of the shipment container be labeled with the following information:

- The complete address of the receiving laboratory (including the name of the person responsible for receiving the samples and the telephone number)
- Appropriate shipping label that conforms to the courier's standards
- Number of samples included (e.g., "This cooler contains 10 samples")
- 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. Additional packing material (bubble wrap, Styrofoam peanuts, balled-up newspaper) should be placed in the shipping container to fill in empty space to prevent sample containers from moving and potentially breaking or spilling during shipping. It is recommended also that the courier be contacted to determine if there are any special shipping requirements for these types of samples.

Adherence to Holding and Shipment Times

Adherence to sample preservation and holding time limits described in Section 10.11 is critical. Samples that are not processed within the specified time and under the proper conditions can yield erroneous results and are out of regulatory compliance. Make sure the analytical laboratory reports the date and time when the samples arrived, and total holding time (period from when the sample was collected to the initiation of analysis). This information will be valuable for improving future sample events and maintaining quality control.

10.14 Documentation

Sampling Plan

It is recommended that all procedures used in sample collection, preparation and shipment be described in a sampling plan. At a minimum, a sampling plan should provide the following information:

- Sample collection locations
- Volume of sample to be collected
- Sample compositing procedures
- Days and times of collection
- Required equipment
- Instructions for labeling samples and ensuring chain of custody
- 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 (USEPA, 1980).

Sampling Log

All information pertinent to a sampling event should be recorded in a bound log book, preferably with consecutively numbered pages. At a minimum, the following information should be recorded in the log book.

- Purpose of sampling event
- Date and time of sample collection
- Location where samples were collected
- Grab or composite sample (for composite samples, the location, number, and volume of subsamples should be included)
- Name of the person collecting the sample(s)
- Type of sewage sludge
- Number and volume of the sample taken
- Description of sampling point
- Date and time samples were shipped

10.15 Analytical Methods

40 CFR Part 503.8(b) and 40 CFR Part 136 specify methods that must be used when analyzing for enteric viruses; fecal coliform; *Salmonella* sp.; viable helminth ova; specific oxygen uptake rate; and total, fixed and volatile solids. Table 10.2 lists the required methods.

Table 10.2 Analytical methods required by 40 CFR Part 503, and 40 CFR Part 136

Method	Appendix in this document
Calculating volatile solids reduction	Appendix B
Conducting additional digestion and specific oxygen uptake rate (SOUR) tests	Appendix C
Determination of residence time in digesters	Appendix D
Sample preparation and analytical methods -- fecal coliform and *Salmonella* sp.	Appendix E
Analytical method -- enteroviruses in sewage sludge	Appendix F
Analytical method -- viable helminth ova	Appendix G

10.16 Quality Assurance

Quality assurance involves establishing a sampling plan and implementing 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 description of quality assurance is found in Standard Methods and is strongly 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 Part 503 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 suggested is recommended for the microbiological tests required by Part 503. Part 9020B-4, Analytical Quality Control Procedures state that precision be initially established by running multiple duplicates. Additional duplicates (5% of total samples) should be run during testing to determine whether precision is being maintained.

Spiking and recovery tests are an important part of quality assurance. EPA methods 1680, 1681, and 1682 list the spiking tests that should be used for the bacterial indicator tests. Yanko (1987) found that spiking is useful for the viable helminth ova test with. With any of the EPA methods listed above, the density of the measured pathogens should be at levels that are relevant to Part 503. For example, for viable helminth ova, samples should be spiked to density levels of approximately 100 per gram.

For both commercial and in-house laboratories, quality assurance procedures should be incorporated into the sampling and analytical methods and assessed routinely. Communication with the analytical personnel is an important part of developing a good sampling and analysis protocol. The sewage sludge preparer should review quality assurance data along with analysis results to ensure that laboratory performance is acceptable.

Vector Attraction Reduction Tests

It is not possible to test for accuracy for any of the vector attraction reduction tests because standard sewage sludges with consistent qualities do not exist. Standard Methods give guidance on precision and bias. However, for some of the vector attraction reduction options, this information was not available or was approximate. Section 10.7 provides guidance on the number of samples to take. The procedures for three of the vector attraction options developed for Part 503 (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.

10.17 References

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12: APPENDIX A

The text in Appendix A has been taken from the previously published document "Control of Pathogens and Vector Attraction in Sewage Sludge" (July 2003, EPA 625-R-92-013). Page numbers will be inconsistent with the previous text.

Subpart D of the Part 503 Regulation

[Code of Federal Regulations]

[Title 40, Volume 21, Parts 425 to 699]
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TITLE 40 - PROTECTION OF ENVIRONMENT

CHAPTER I - ENVIRONMENTAL PROTECTION AGENCY (Continued)

PART 503 - STANDARDS FOR THE USE OR DISPOSAL OF SEWAGE SLUDGE-Table of Contents

Subpart D-Pathogens and Vector Attraction Reduction

Sec. 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.

Sec. 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.

Sec. 503.32 Pathogens.

(a) Sewage sludge-Class A. (1) The requirement in Sec. 503.32(a)(2) and the requirements in either Sec. 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 Sec. 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 Sec. 503.33, except the vector attraction reduction requirements in Sec. 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 Sec. 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.

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

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).

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

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 Sec. 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 Sec. 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 Sec. 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 Sec. 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 Sec. 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 Sec. 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 Sec. 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 Sec. 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 Sec. 503.32(b)(5) shall be met when sewage sludge that meets the Class B pathogen requirements in Sec. 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 Sec. 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 Sec. 503.32 (b)(5)(i) through (b)(5)(iv) shall be met.

Sec. 503.33 Vector attraction reduction.

(a)(1) One of the vector attraction reduction requirements in Sec. 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 Sec. 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 Sec. 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 Sec. 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 Sec. 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 Sec. 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 Sec. 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 Sec. 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.

13: APPENDIX B

The text in Appendix B has been taken from the previously published document "Control of Pathogens and Vector Attraction in Sewage Sludge" (July 2003, EPA 625-R-92-013). Page numbers will be inconsistent with the previous text.

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 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 com- parison is between the sewage sludge entering the digester and the sewage sludge leaving the digester. The remain- der of the discussion is limited to this circumstance, ex- cept 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.

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.

Method Full Mass Balance

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 B-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,

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

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

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

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

Nomenclature is given in Table B-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

Table B-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	$\overline{\quad}$	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	41.42	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.

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.

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 deterrnine 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 wiIl 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 volatiie 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:

$$M_f = M_b + M_d + (\text{loss of total solids}) \tag{25}$$

$$M_f \cdot VS_f = M_b \cdot VS_b + M_d \cdot VS_d + (\text{loss of volatile solids}) \quad (26)$$

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,

$$FVSR = \frac{VS_f - VS_b}{VS_f - (VS_f \cdot VS_b)} \quad (31)$$

This is the form of the Van Kleek 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/m3	0.75
2	8 m^3	50 kg/m3	0.82
3	13 m^3	60 kg/m3	0.80
4	10 m^3	55 kg/m3	0.77

(33)

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 concentrations. 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 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.

References

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Water Pollution Control Federation. 1968. Manual of Practice No. 16, Anaerobic Sludge Digestion. Washington, DC.

14: APPENDIX C

The text in Appendix C has been taken from the previously published document "Control of Pathogens and Vector Attraction in Sewage Sludge" (July 2003, EPA 625-R-92-013). Page numbers will be inconsistent with the previous text.

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 9 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 watersealed 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 holdup 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 contents 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 B.

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:

$$FVSR(m.b.) = \frac{y_f(1-x_b)-y_b(1-x_f)}{y_f(1-x_b-y_b)} \quad (1a)$$

$$FFSR(m.b.) = \frac{x_f(1-y_b)-x_b(1-y_f)}{x_f(1-x_b-y_b)} \quad (1b)$$

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:

$$FVSR(m.b.) = (y_f - y_b)/y_f\,(1-y_b) \quad (2)$$

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 reduc-

tions 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:

$$(SOUR)_{T1} / (SOUR)_{T2} = \theta^{(T1-T2)} \quad (3)$$

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 coeffficient 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:

$$SOUR_{20} = SOUR_T \times \theta^{(20-T)} \qquad (4)$$

where

θ = 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 rnixing 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 probably 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 $\leq$ 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 9). These options are feasible for many, but not all, digested sludges. For example, sludges from extended aeration treatment works that are aerobically di-

gested 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 vectors. 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, 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 solitis 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 labora-

tory 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 contents 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 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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15: APPENDIX D

The text in Appendix D has been taken from the previously published document "Control of Pathogens and Vector Attraction in Sewage Sludge" (July 2003, EPA 625-R-92-013). Page numbers will be inconsistent with the previous text.

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:

$$\theta = V/q \qquad (1)$$

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:

$$\theta_n = \frac{\Sigma(\delta s \times \theta)}{\Sigma(\delta s)} \qquad (2)$$

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:

$$\theta_n = \frac{VC_v}{qC_q} \qquad (3)$$

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 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:

$$\theta_n = \frac{\text{mass of solids in the digester}}{\text{mass flow rate of solids leaving}} \qquad (4)$$

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
- Sludge is introduced in daily batches of volume (V_i) and solids concentration (C_i)
- Vessel contains a "heel" of liquid sludge (V_f) at the beginning of the digestion step
- 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:

$$\theta_n = \frac{\Sigma v_i C_i \text{ x time that batch i remains in the reactor}}{\Sigma v_i C_i}$$

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 m3 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.

Then $\theta_n = \frac{(10\cdot12\cdot10+10\cdot12\cdot9+...+10\cdot12\cdot1)}{(10\cdot12+10\cdot12+...\ 10\cdot12)}$

$$\theta_n = \frac{10\cdot12\cdot55}{10\cdot12\cdot10} = 5.5 \text{ days}$$

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 = 30m^3, and V_d = 130m^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

$$\theta_n = \frac{(10\cdot10\cdot12+10\cdot15\cdot11+10\cdot20\cdot10+... \ ...+10\cdot10\cdot3+10\cdot15\cdot2+10\cdot20\cdot1)}{(10\cdot10+10\cdot15+10\cdot20+... \ +10\cdot10+10\cdot15+10\cdot20}$$

θ_n = 11,950/1,900 = 6.29d

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

$$\theta_n = \frac{100x60}{5 \times 60} = 20d$$

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.

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16: APPENDIX E

Method 1680: Fecal coliforms in Sewage Sludge (Biosolids) by Multiple-Tube Fermentation using Lauryl Tryptose Broth (LTB) and EC Medium

Method 1681: Fecal Coliforms in Sewage Sludge (Biosolids) by Multiple Tube Fermentation using A-1 Medium | July 2006

Method 1682: *Salmonella* in Sewage Sludge (Biosolids) by Modified Semisolid Rappaport-Vassiliadis (MSRV) Medium

17: APPENDIX F

The text in Appendix F has been taken from the previously published document "Control of Pathogens and Vector Attraction in Sewage Sludge" (July 2003, EPA 625-R-92-013). Page numbers will be inconsistent with the previous text.

Method for the Recovery and Assay of Total Culturable Viruses from Sludge

1. Introduction

1.1. Scope

This chapter describes the method that must be followed to produce Class A sludge when virus monitoring under 40 CFR Part 503 is required. The method is designed to demonstrate that sludges meet the requirement that human enteric viruses (i.e., viruses that are transmitted via the fecal-oral route) are less than one plaque-forming unit (PFU) per 4 g of total dry solids.

1.2. Significance

More than 100 different species of pathogenic human enteric viruses may be present in raw sludge. The presence of these viruses can cause hepatitis, gastroenteritis and numerous other diseases. Hepatitis A virus and noroviruses are the primary human viral pathogens of concern, but standard methods for their isolation and detection have not been developed. The method[1] detailed in this chapter detects total culturable viruses, which primarily include the human enteroviruses (e.g., polioviruses, coxsackieviruses, echoviruses) and reoviruses.

1.3. Safety

The sludges to be monitored may contain pathogenic human enteric viruses. Laboratories performing virus analyses are responsible for establishing an adequate safety plan and must decontaminate and dispose of wastes according to their safety plan and all applicable regulations. Aseptic techniques and sterile materials and apparatus must be used throughout the method.

2. Sample Collection

For each batch of sludge that must be tested for viruses, prepare a composite sample by collecting ten representative samples of 100 mL each (1,000 mL total) from different locations of a sludge pile or at different times from batch or continuous flow processes. Combine and mix thoroughly all representative samples for a composite. Batch samples that cannot be assayed within 24 hours of collection must be frozen at -70°C; otherwise, they should be held at 4°C until processed. If representative samples must be frozen before they can be combined, then thaw, combine and mix them thoroughly just prior to assay. Then remove a 50 mL portion from each composite sample for solids determination as described in section 3. 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.

Freeze/thawing biosolids may result in some virus loss.

3. Determination of Total Dry Solids[2]

3.1. 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.

3.2. Place the pan and its contents into an oven maintained at 103-105°C for at least one hour.

3.3. Cool the sample to room temperature in a desiccator and weigh again.

3.4. Repeat the drying (1 h each), cooling and weighing steps until the loss in weight is no more than 4% of the previous weight.

3.5. Calculate the fraction of total dry solids (T) using the formula:

$$T = \frac{(A - C)}{(B - C)}$$

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).

4. Total Culturable Virus Recovery from Sludge

4.1. Introduction

Total culturable viruses 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

[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 Biohazard Assessment Research Branch, National Exposure Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio, USA 45268.

bind free virus to solids. Virus is then eluted from the solids and concentrated prior to assay.

4.2. Conditioning of Suspended Solids

Conditioning of sludge binds unadsorbed total culturable viruses present in the liquid matrix to the sludge solids.

Each analyzed composite sample (from the portion remaining after solids determination) must have an initial total dry solids content of at least 16 g. This amount is needed for positive controls and for storage of a portion of the sample at -70°C as a backup in case of procedural mistakes or sample cytotoxicity.

4.2.1 Preparation

(a) Apparatus and Materials

(a.1) Refrigerated centrifuge capable of attaining 10,000 ×*g* and screw-capped centrifuge bottles with 100 to 1000 mL capacity.

Each bottle must be rated for the relevant centrifugal force.

(a.2) A pH meter with an accuracy of at least 0.1 pH unit, equipped with a combination-type electrode.

(a.3) Magnetic stirrer and stir bars.

(b) Media and Reagents

Analytical Reagent or ACS grade chemicals (unless specified otherwise) and deionized, distilled water (dH_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.

(b.1) Hydrochloric acid (HCl) — 1 and 5 M.

Mix 10 or 50 mL of concentrated HCl with 90 or 50 mL of dH_2O, respectively.

(b.2) Aluminum chloride ($AlCl_3 \cdot 6H_2O$) — 0.05 M.

Dissolve 12.07 g of aluminum chloride in a final volume of 1000 mL of dH_2O. Autoclave at 121°C for 15 minutes.

(b.3) Sodium hydroxide (NaOH) — 1 and 5 M.

Dissolve 4 or 20 g of sodium hydroxide in a final volume of 100 mL of dH_2O, respectively.

(b.4) Beef extract (Difco Product No. 0115-17-3 or equivalent).

Prepare buffered 10% beef extract by dissolving 10 g beef extract, 1.34 g $Na_2HPO_4 \cdot 7H_2O$ and 0.12 g citric acid in 100 mL of dH_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) for virus elution. This beef extract tends to elute cytotoxic materials from sludges.

(b.5) HOCl — 0.1%

Add 19 mL of household bleach (Clorox, The Clorox Co., or equivalent) to 981 mL of dH_2O and adjust the pH of the solution to 6-7 with 1 M HCl.

(b.6) Thiosulfate — 2% and 0.02%

Prepare a stock solution of 2% thiosulfate by dissolving 20 g of thiosulfate in a total of 1 liter of dH_20. Sterilize the solution by autoclaving at 121°C for 15 minutes. Prepare a working solution of 0.02% thiosulfate just prior to use by mixing 1 mL of 2% thiosulfate with 99 mL of sterile dH_2O.

4.2.2 Conditioning Procedure

Figure 1 gives a flow diagram for the procedure to condition suspended solids.

(a) Calculate the amount of sample to condition.

Use a graduated cylinder to measure the volume. If the volumes needed are not multiples of 100 mL (100, 200, 300 mL, etc.), add sterile water 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.

(a.1) Calculate the amount needed to measure the endogenous total culturable virus in a composite sludge sample using the formula:

$$Xts = \frac{12}{T}$$

where Xts equals the milliliters of sample required to obtain 12 g of total solids and T equals the fraction of total dry solids (from section 3).[3]

(a.2) Calculate the amount needed for a recovery control for each sludge composite from the formula:

$$Xpc = \frac{4}{T}$$

where Xpc equals the milliliters of sample required to obtain 4 g of total solids.

Add 400 plaque forming units (PFU) of a Sabin poliovirus stock to the recovery control sample. Use a virus stock that has been filtered through a 0.2 µm filter (see Section 4.3.1) prior to assay to remove clumped virus particles.

(a.3) Place 30 mL of 10% buffered beef extract and 70 mL of dH_2O into a 250 mL beaker with stir bar to serve as a negative process control.

(a.4) Freeze any remaining composite sample at -70°C for backup purposes.

[3]This formula is based upon the assumption that the density of the liquid in sludge is 1 g/mL. If the fraction of total dry solids is too low (e.g., less than 0.02), then the volume of sludge collected must be increased.

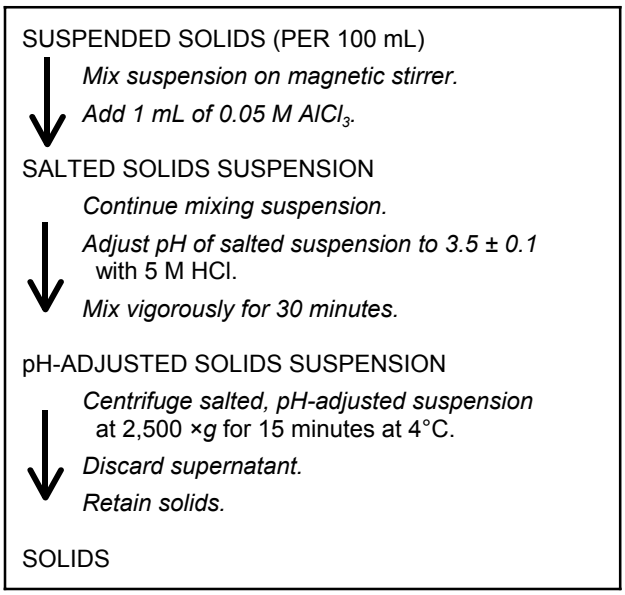

Figure 1. Flow diagram of method for conditioning suspended solids

(b) Perform the following steps on each 100 mL aliquot from steps 4.2.2a.1 to 4.2.2a.3.

(b.1) Place the beaker on a magnetic stirrer, cover loosely with aluminum foil, and stir at a speed sufficient to develop 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.

(b.2) 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 an electrode, clean it by moving up and down gently in the mixing aliquot.

After adjusting the pH of each sample, rinse the electrode with dH_2O and sterilize it with 0.1% HOCl for five minutes. Neutralize the HOCl by submerging the electrode in sterile 0.02% thiosulfate for one to five minutes.

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.

(b.3) Pour the conditioned aliquot into a centrifuge bottle and centrifuge at 2,500 ×*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. If a large enough centrifuge bottle is available, the test sample aliquots may be combined into a single bottle at this step. If there is more than one recovery control aliquot, they may also be combined into another centrifuge bottle.

(b.4) Decant the supernatant into a beaker and discard. Replace the cap onto the centrifuge bottle. Elute the solids by following the procedure described in section 4.3.

4.3. Elution of Viruses from Solids

4.3.1 Apparatus and Materials

In this and following sections only apparatus and materials which have not been described in previous sections are listed.

(a) Membrane filter apparatus for sterilization — 47 mm diameter Swinnex filter holder and 60 mL slip-tip syringe (Millipore Corp. Product No. SX00 047 00 and Becton Dickinson Product No. 1627 or equivalent).

(b) Disc filters, 47 mm diameter — 3.0, 0.45, and 0.2 µm pore size filters (Mentec America, Filterite Div., Duo- Fine series, Product No. 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 0.2 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.

4.3.2 Elution Procedure

A flow diagram of the virus elution procedure is given in Figure 2.

(a) Place a stir bar and 100 mL of buffered 10% beef extract into the centrifuge bottle containing the solids (from section 4.2.2b.4).

If the test and control samples are divided into more than one centrifuge bottles, the solids should be combined at this step.

Place the centrifuge bottle on a magnetic stirrer, and stir at a speed sufficient to develop a vortex for 30 min at room temperature.

To minimize foaming (which may inactivate viruses), do not mix faster than necessary to develop vortex.

(b) Remove the stir bar from each bottle with a long sterile forceps or a magnet retriever and centrifuge the solids-eluate mixture at 10,000 ×*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.

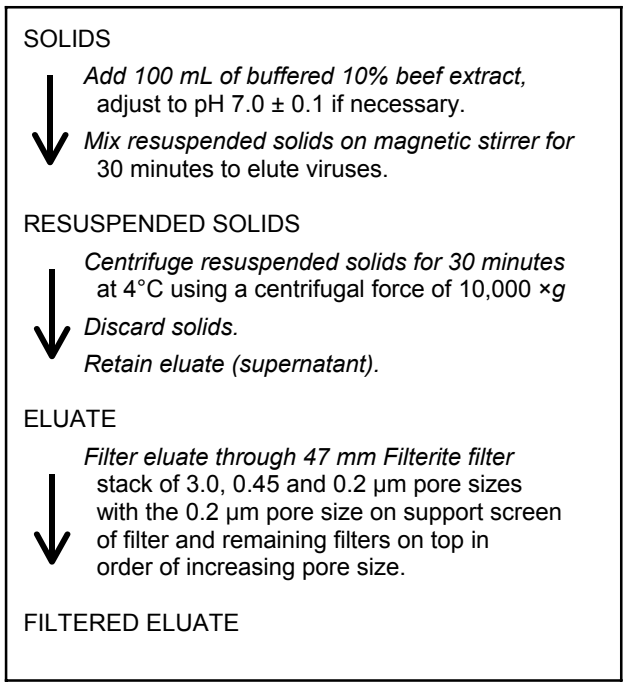

Figure 2. Flow diagram of method for elution of virus from solids.

Centrifugation at 10,000 ×g is normally required to clarify the sludge samples sufficiently to force the resulting supernatant through the filter stacks.

(c) Place a filter holder that contains filter stacks (from section 4.3.1b) onto a 250 mL Erlenmeyer receiving flask. Load 50 mL syringes with the supernatants from step 4.3.2c. Place the tip of the syringe into the filter holder and force the supernatant through the filter stacks into 250 mL receiving flasks.

Prior to use, pass 15 mL of 3% beef extract through each filter holder to minimize non-specific adsorption of viruses. Prepare 3% beef extract by mixing 4.5 mL of 10% beef extract and 10.5 mL of dH_2O. 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 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. This procedure may be repeated as often as necessary to filter the entire volume of supernatant. Disassemble each filter holder and examine the bottom 0.2 µm filters to be certain they have not ruptured. If a bottom filter has ruptured, repeat the step with new filter holders and filter stacks.

Proceed immediately to section 4.4.

4.4. 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 total culturable viruses in the eluate. The step significantly reduces costs associated with labor and materials.

Floc formation capacity of the beef extract reagent must be pretested. Because some beef extract lots may not produce sufficient floc, each new lot must be pretested to determine virus recovery. This may be performed by spiking 100 mL of dH_2O with a known amount of poliovirus in the presence of a 47 mm nitrocellulose filter. This sample should be conditioned using section 4.2 above to bind virus to the filter. Virus should then be eluted from the filter using the procedure in section 4.3, and concentrated and assayed using the following procedures. Any lot of beef extract not giving a overall recovery of at least 50% should not be used.

4.4.1 Media and Reagents

In this and following sections only media and reagents which have not been described in previous sections are listed.

(a) Sodium phosphate, dibasic ($Na_2HPO_4 \cdot 7H_2O$) — 0.15 M.

Dissolve 40.2 g of sodium phosphate in a final volume of 1000 mL. Autoclave at 121°C for 15 minutes.

4.4.2 Virus Concentration Procedure

A flow diagram for the virus concentration procedure is given in Figure 3.

(a) Pour the filtered eluates from the test sample, recovery control and negative process control from section 4.3.2d into graduated cylinders, and record their volumes. Transfer the samples into separate 600 mL beakers and cover them loosely with aluminum foil.

(b) For every 3 mL of beef extract eluate, add 7 mL of dH_2O to the 600 mL beakers. Add stir bars to each 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.

(c) Record the total volume of the diluted eluates. Place the beakers onto a magnetic stirrer, cover loosely with aluminum foil, and stir at a speed sufficient to develop vortex.

To minimize foaming (which may inactivate viruses), do not mix faster than necessary to develop vortex.

(d) For each diluted, filtered beef extract, insert a sterile combination-type pH electrode and then add 1 M HCl slowly until the pH of the 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. Sterilize the electrode by treating it with 0.1% HOCl for five minutes. Neutralize the HOCl by treating the electrode with 0.02% sterile thiosulfate for one to five minutes.

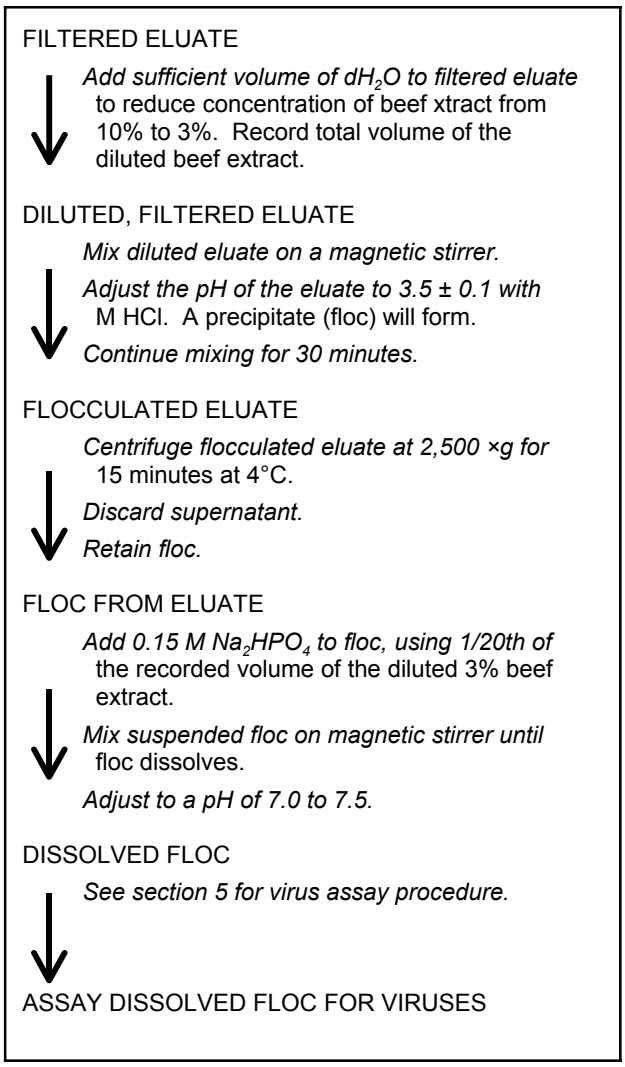

Figure 3. Flow diagram of method for concentration of viruses from beef extract eluate.

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.

(e) Pour the contents of each beaker into 1,000 mL centrifuge bottles. Centrifuge the precipitated beef extract suspensions at 2,500 ×*g* for 15 minutes at 4°C. Pour off and discard the supernatants.

To prevent the transfer of the stir bar into a centrifuge bottle, hold another stir bar or magnet against bottom of the beaker when decanting contents.

(f) Place stir bars into the centrifuge bottles that contains the precipitates. To each, add a volume of 0.15 M $Na_2HPO_4 \cdot 7H_2O$ equal to exactly 1/20 of the volume recorded in step 4.4.2c. If the precipitate from a sample is in more than one bottle, divide the 1/20th volume equally among the centrifuge bottles containing that sample. Place the bottles onto a magnetic stirrer, and stir slowly until the precipitates have dissolved completely.

Support the bottles as necessary to prevent toppling. Avoid foaming which may inactivate or aerosolize viruses. The precipitates may be partially dissipated with sterile spatulas before or during the stirring procedure.

(g) Measure the pH of the dissolved precipitates.

If the pH is above or below 7.0-7.5, adjust to that range with either 1 M HCl or 1 M NaOH.

(h) Freeze exactly one half of the dissolved precipitate test sample (but not the positive and negative controls) at -70°C. This sample will be held as a backup to use should the sample prove to be cytotoxic. Record the remaining test sample volume (this volume represents 6 g of total dry solids). Refrigerate the remaining samples immediately at 4°C until assayed in accordance with the instructions given in section 5 below.

If the virus assay cannot be undertaken within 24 hours, store the remaining samples at -70°C.

5. Assay for Plaque-forming Viruses[4]

5.1. Introduction

This section outlines procedures for the detection of viruses in sludge by use of the plaque assay system. 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, whose number is proportional to the amount of infectious virus particles inoculated.

The detection methodology presented in this section is geared towards 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.

5.2. Plaque Assay Procedure

5.2.1 Apparatus and materials.

(a) Waterbath set at 50 ± 1°C.

Used for maintaining the agar temperature (see section 5.2.2j).

5.2.2 Media and Reagents.

(a) ELAH — 0.65% lactalbumin hydrolysate in Earle's base.

Dissolve 6.5 g of tissue culture, highly soluble grade lactalbumin hydrolysate (Gibco BRL Product No. 11800 or

[4]Modified from EPA/600/4-84/013(R11), March 1988 Revision

equivalent) in 1 L of Earle's base (Gibco BRL Product No. 14015 or equivalent) prewarmed to 50-60°C. Sterilize ELAH through a 0.22 μm filter stack and store for up to two months at 4°C.

(b) Wash medium — Add 1 mL of penicillin-streptomycin stock (see section 6.4.2e.1 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.

(c) HEPES — 1 M (Sigma Chemical 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 dH_2O. Sterilize by autoclaving at 121°C for 15 min.

(d) 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 dH_2O. Sterilize by filtration through a 0.22 μm filter.

(e) 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 dH_2O. Sterilize by autoclaving at 121°C for 15 min.

(f) Neutral red solution — 0.333%, 100 mL volume (GIBCO BRL Product No. 630-5330 or equivalent).

Procure one 100 mL bottle.

Some neutral red solutions are cytotoxic. All new solutions should be tested prior to their use for assaying sludge samples. Testing may be performed by assaying a stock of poliovirus with known titer using this plaque assay procedure.

(g) 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.

(h) 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.

(h.1) Place a three inch stir bar into a one liter flask. Add the contents of a 1 liter packet of Medium 199 into the flask. Add 355 mL of dH_2O. Rinse medium packet with three washes of 20 mL each of dH_2O and add the washes to the flask.

Note that the amount of dH_2O is 5% less than desired final volume of medium.

(h.2) Mix on a magnetic stirrer until the medium is completely dissolved. Filter the reagent under pressure through a filter stack (see section 6.2.6).

Test each lot of medium to confirm sterility before the lot is used (see section 6.5). Each batch may be stored for two months at 4°C.

(i) 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 section 5.2.2j.

(i.1) Add 79 mL of Medium 199 (2X concentration) and 4 mL of serum to a 250 mL flask.

(i.2) Add the following to the flask in the order listed, with swirling 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 6.4.2e for a description of antibiotic stocks), 0.1 mL of tetracycline stock, and 0.04 mL of fungizone stock.

(i.3) Place flask with overlay medium in waterbath set at 36 ± 1°C.

(j) Preparation of overlay agar for plaque assay.

(j.1) Add 3 g of agar (Sigma Chemical Product No. A-9915 or equivalent) and 100 mL of dH_2O to a 250 mL flask. Melt by sterilizing the agar solution in an autoclave at 121°C for 15 min.

(j.2) Cool the agar to 50°C in waterbath set at 50 ± 1°C.

(k) Preparation of agar overlay medium.

(k.1) Add 2 mL of 10% skim milk to overlay medium prepared in section 5.2.2i.

(k.2) 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 min.

5.2.3 Procedure for Inoculating Test Samples.

Section 6.6 provides the procedures for the preparation of cell cultures used for the virus assay in this section.

BGM cell cultures used for virus assay are generally found to be at their most sensitive level between the third and sixth days after initiation. Those older than seven days or which are not 100% confluent should not be used.

(a) 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 one to four hours before cultures are to be inoculated and carefully decanted so as not to disturb the cell monolayer.

(b) Replace discarded medium with an equal volume of wash medium (from section 5.2.2b) on the day the cultures are to be inoculated.

Table 1. 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 Number of Cells
1 oz glass bottle[1]	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

[1]Size is given in oz only when it is commercially designated in that unit.

To reduce shock to cells, prewarm the wash 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 wash medium to the side of cell culture test vessel opposite the cell monolayer.

(c) 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 monolayers are to be inoculated.

(d) Decant and discard the wash medium from cell culture test vessels.

Do not disturb the cell monolayer.

(e) Inoculate BGM cultures with the test sample and positive and negative process control samples from section 4.4.2h. Divide each sample onto a sufficient number of BGM cultures to ensure that the inoculum volume is no greater than 1 mL for each 40 cm^2 of surface area. Use Table 1 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.

If the samples are frozen, thaw them rapidly by placing them in warm water. Samples should be shaken during the thawing process and removed from the warm water as soon as the last ice crystals have dissolved.

(e.1) Inoculate BGM cultures with the entire negative process control sample using an inoculum volume per vessel that is appropriate for the vessel size used.

(e.2) Inoculate two BGM cultures with an appropriate volume of 0.15 M $Na_2HPO_4 \cdot 7H_2O$ preadjusted to pH 7.0-7.5 and seeded with 20-40 PFU of poliovirus. These cultures will serve as a culture sensitivity control.

(e.3) 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 4.4.2h. Seed 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 5.2.5b).

(e.4) Inoculate BGM cultures with the entire recovery control sample using an inoculum volume per vessel that is appropriate for the vessel size used.

(e.5) 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 using an inoculum volume per vessel that is appropriate for the vessel size used. Inoculation of the entire volume is necessary to demonstrate a virus density level of less than 1 PFU per 4 g total dry solids.

(f) 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.

(g) Incubate the inoculated cell cultures at room temperature for 80 min to permit viruses to adsorb onto and infect cells and then proceed immediately to section 5.2.4.

It may be necessary to rock the vessels every 15-20 min during the 80 min incubation to prevent cell death in the middle of the vessels from dehydration.

5.2.4 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 5.2.5b.

(a) 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 1).

The preparation of the overlay agar and the agar overlay medium must be made far enough in advance so that they will be at the right temperature for mixing at the end of the 80 min 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.

(b) 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 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 min.

(c) After 30 min, invert the cell culture test vessels and incubate them covered in the dark at 36.5 ± 1°C.

5.2.5 Plaque Counting Technique.

(a) Count, mark and record plaques in cell culture test vessels on days one, two, three, four 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.

(b) Determine if samples are cytotoxic by macroscopic examination of the appearance of the cell culture monolayer (compare negative, positive and recovery controls from section 5.2.3e with seeded and unseeded test samples) after one to four days of incubation at 36.5 ± 1°C. Samples show cytotoxicity if cell death is observed on test and recovery control samples prior to its development on positive controls. 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. 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 (section 5.2.3e). If samples are cytotoxic, do not proceed to the next steps. Re-assay a small amount of the remaining sample using 1:2, 1:4 and 1:8 dilutions. Then re-assay the remaining sample as specified in section 5.2.3 using the dilution which removes cytotoxicity and the specified number of flasks times the reciprocal of the dilution.

A small amount of sample may be tested for cytotoxicity prior to a full assay.

(c) Examine cell culture test vessels as in step 5.2.5a on days six, eight, twelve and sixteen.

If no new plaques appear at 16 days, proceed with step 5.2.6; otherwise continue to count, mark and record plaques every two days until no new plaques appear between counts and then proceed with step 5.2.6.

Inoculated cultures should always be compared to uninoculated control cultures so that the deterioration of the cell monolayers is not recorded as plaques. If experience shows that cultures start to deteriorate prior to 16 days, a second layer of agar can be added after 7 days as described in section 5.2.4.

If negative process controls develop plaques or if positive controls fail to develop plaques, stop all assays until the source of the problem is corrected.

Samples giving plaque counts that are greater than 2 plaques per cm^2 should be diluted and replated.

5.2.6 Virus Plaque Confirmation Procedure

The presence of virus in plaques must be confirmed for all plaques obtained from sludge samples. Where more than ten plaques are observed, it is allowable to confirm at least ten well-separated plaques per sample or 10% of the plaques in a sample, whichever is greater. Flasks may be discarded after samples are taken for plaque confirmation.

(a) Apparatus, Materials and Reagents

(a.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 end of the tip until the tip bends to an approximate angle of 45°. Place the pipettes into a 4 liter beaker covered with aluminum foil and sterilize by autoclaving or by dry heat.

(a.2) 16 x 150 mm cell culture tubes containing BGM cells.

See section 6.6 for the preparation of cell culture tubes.

(a.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).

(a.4) Freezer vial, screw-capped (with rubber insert) or cryogenic vial — 0.5-1 dram capacity.

(b) Procedure for obtaining viruses from plaque.

In addition to plaques from sludge samples, perform the procedure on at least three negative regions of negative process control flasks and at least three plaques from positive control flasks.

(b.1) 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.2) 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 insure that virus-cell-agar plug enters the pipette.

(b.3) Remove the pipette from the plaque bottle and tightly replace the cap or stopper.

(c) Procedure for inoculating cell cultures with agar plugs from negative control samples and from plaques.

(c.1) Prepare plaque conformation maintenance medium by adding 5 mL of serum and 5 mL of dH_2O per 90 mL of wash medium (section 5.2.2b) on day samples are to be tested.

(c.2) Pour the spent medium from cell culture tubes and discard the medium. Replace the discarded medium with 2

mL of the plaque conformation 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 on cell monolayer.

To prevent disturbing cells with the force of the liquid against the cell monolayer, add the maintenance medium to the side of 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.

(c.3) Remove the cap from a cell culture tube and place the tip of a Pasteur pipette containing the agar plug from section 5.2.6b.3 into the maintenance medium in the cell culture tube. Force the 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.

(c.4) Place the cell culture tubes in the drum used with the tissue culture roller apparatus. 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 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 enteric virus 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.

If tubes receiving agar plugs from negative controls develop CPE or tubes receiving agar plugs from positive controls fail to develop CPE, stop all assays until the source of the failure is identified and corrected.

Tubes developing CPE may be stored in a -70°C freezer for additional optional tests (e.g., the Lim Benyesh-Melnick identification procedure.[5]

(c.5) Determine the fraction of confirmed plaques (C) for each sludge sample tested. Calculate "C" by dividing the number of tubes inoculated with agar plugs from plaques that developed CPE by the total number of tubes inoculated (i.e., if CPE was obtained from 17 of 20 plaques, C = 0.85).

5.2.7 Calculation of virus titer.

If more than one composite sample was assayed, average the titer of all composite samples and report the average titer and the standard deviation for each lot of sludge tested.

(a) If the entire remaining portion of a test sample was inoculated onto BGM cultures as described in section 5.2.3e.5, calculate the virus titer (V) in PFU per 4 g of total dry solids according to the formula:

$$V = 0.8 \times P \times C$$

where P is the total number of plaques in all test vessels for that sample and C equals the fraction of confirmed plaques.

(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; see section 5.2.5c), calculate the virus titer (V) in PFU per 4 g total dry solids with the formula:

$$V = 0.8 \times \frac{P}{I} \times D \times S \times C$$

where P is the total number of plaques in all test vessels for dilution series, I is the volume (in mL) of the dilution inoculated, D is reciprocal of the dilution made on the inoculum before plating, S is the volume of the remaining portion of the test sample (as recorded in section 5.2.3e.5) and C is the fraction of confirmed plaques.

5.2.8 Calculate the percent of virus recovery (R) using the formula:

$$R \quad \frac{P}{400} \times 100$$

where P is the total number of plaques on all test vessels inoculated with the recovery control.

6. Cell Culture Preparation and Maintenance[6]

6.1. Introduction

This section outlines procedures and media for culturing the Buffalo Green monkey (BGM) kidney 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 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 Biohazard Assessment Research Branch, National Exposure Research Laboratory, U. S. Environmental Protection Agency, Cincinnati, Ohio, USA 45268. Although BGM

[5]For more information see EPA/600/4-84/013(R12), May 1988 Revision

[6]Modified from EPA/600/4-84/013(R9), January 1987 Revision

cells will not detect all enteric viruses that may be present in sludges, the use of this cell line alone is sufficient to meet the requirements of 40 CFR Part 503.

6.2. Apparatus and Materials

6.2.1 Glassware, Pyrex (Corning Product No. 1395 or equivalent).

Storage vessels must be equipped with airtight closures.

6.2.2 Autoclavable inner-braided tubing with metal quick-connect connectors or with screw clamps for connecting tubing to equipment to be used under pressure.

Quick-connect connectors can be used only after equipment has been properly adapted.

6.2.3 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 manufacturer.

6.2.4 Dispensing pressure vessel — 5 or 20 liter capacity (Millipore Corp. Product No. XX67 00P 05 and XX67 00P 20 or equivalent).

6.2.5 Disc filter holders — 142 mm or 293 mm diameter (Millipore Corp. Product No. YY30 142 36 and YY30 293 16 or equivalent).

Use only pressure type filter holders.

6.2.6 Sterilizing filter stacks — 0.22 µm pore size (Millipore Corp. Product No. GSWP 142 50 and GSWP 293 25 or equivalent). Fiberglass prefilters (Millipore Corp. Product No. 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.

6.2.7 Positively-charged cartridge filter — 10 inch (Zeta plus TSM, Cuno 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).

6.2.8 Culture capsule filter (Gelman Sciences Product No. 12140 or equivalent).

6.2.9 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.

6.2.10 Screw caps, black with rubber liners (Brockway Product No. 24-414 for 6 oz bottles[7] or equivalent).

Caps for larger culture bottles usually supplied with bottles.

6.2.11 Roller apparatus (Bellco Product No. 7730 or equivalent).

6.2.12 Incubator capable of maintaining the temperature of cell cultures at 36.5 ± 1°C.

6.2.13 Waterbath, equipped with circulating device to assure even heating at 36.5 ± 1°C.

6.2.14 Light microscope, with conventional light source, equipped with lenses to provide 40X, 100X, and 400X total magnification.

6.2.15 Inverted light microscope equipped with lenses to provide 40X, 100X, and 400X total magnification.

6.2.16 Cornwall syringe pipettors, 2, 5 and 10 mL sizes (Curtin Matheson Scientific Product No. 221-861, 221-879, and 221-887 or equivalent).

6.2.17 Brewer-type pipetting machine (Curtin Matheson Scientific Product No. 138-107 or equivalent).

6.2.18 Phase contrast counting chamber (hemocytometer) (Curtin Matheson Scientific Product No. 158-501 or equivalent).

6.2.19 Conical centrifuge tubes, sizes 50 mL and 250 mL.

6.2.20 Rack for tissue culture tubes (Bellco Product No. 2028 or equivalent).

6.2.21 Bottles, aspirator-type with tubing outlet, size 2,000 mL.

Bottles for use with pipetting machine.

6.2.22 Storage vials, size 2 mL.

Vials must withstand temperatures to -70°C.

6.3. Media and Reagents

6.3.1 Sterile fetal calf, gammagobulin-free newborn calf or iron-supplemented calf serum, certified free of

[7]Size is given in oz only when it is commercially designated in that unit.

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 should be heat-inactivated at 56°C for 30 min and stored at 4°C for short term use.

6.3.2 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).

6.3.3 Sodium (tetra) ethylenediamine tetraacetate powder (EDTA), technical grade, (Fisher Scientific Product No. S657-500 or equivalent).

6.3.4 Thioglycollate medium (Difco Laboratories Product No. 0257-01-9 or equivalent).

6.3.5 Fungizone (amphotericin B, Sigma Chemical Product No. A-9528 or equivalent), Penicillin G (Sigma Chemical Product No. P-3032 or equivalent), dihydrostreptomycin sulfate (ICN Biomedicals Product No. 100556 or equivalent), and tetracycline (ICN Biomedicals Product No. 103011 or equivalent).

Use antibiotics of at least tissue culture grade.

6.3.6 Eagle's minimum essential medium (MEM) with Hanks' salts and L-glutamine, without sodium bicarbonate (GIBCO BRL Product No. 410-1200 or equivalent).

6.3.7 Leibovitz's L-15 medium with L-glutamine (GIBCO BRL Product No. 430-1300 or equivalent).

6.3.8 Trypan blue (Sigma Chemical Product No. T-6146 or equivalent).

Note: This chemical is on the EPA list of proven or suspected carcinogens.

6.3.9 Dimethyl sulfoxide (DMSO; Sigma Chemical Product No. D-2650 or equivalent).

6.3.10 Mycoplasma testing kit (Irvine Scientific Product No. T500-000 or equivalent).

6.4. Preparation of Cell Culture Media

6.4.1 General Principles

(a) Equipment care — Carefully wash and sterilize equipment used for preparing media before each use.

(b) Disinfection of work area — Thoroughly disinfect surfaces on which the medium preparation equipment is to be placed. Many commercial disinfectants do not adequately kill total culturable viruses. To ensure thorough disinfection, disinfect all surfaces and spills with either a solution of 0.5% (5 g per liter) iodine in 70% ethanol or 0.1% HOCl.

(c) Aseptic technique — Use aseptic technique when preparing and handling media or medium components.

(d) 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.

(e) 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.

(f) Sterility test — Test each lot of medium and medium components to confirm sterility as described in section 6.5 before the lot is used for cell culture.

(g) Storage of media and medium components — Store media and medium components in clear airtight containers at 4°C or -20°C as appropriate.

(h) 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.

6.4.2 Media Preparation Recipes

(a) 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 dH_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.

(b) Procedure for the preparation of EDTA-trypsin.

The procedure described is for the preparation of 10 liters 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 four months. The amount of reagent prepared should be based on projected usage over a four-month period.

(b.1) Add 30 g of trypsin (1:250) or 25 g of trypsin (1:300) and two liters of dH_2O to a six liter flask containing a three inch stir bar. Place the flask onto a magnetic stirrer and mix the trypsin solution rapidly for a minimum of one hour.

Trypsin remains cloudy.

(b.2) Add four liters of dH_2O and a three-inch stir bar into 20 liter 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.

(b.3) Add four more liters of dH_2O to carboy.

Continue mixing until all chemicals are completely dissolved.

(b.4) Add the two liters of trypsin from step 6.4.2b.1 to the prepared solution in step 6.4.2b.3 and mix for a minimum of one hour. Adjust the pH of the EDTA-trypsin reagent to 7.5 - 7.7.

(b.5) 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 (section 6.2.7) can be used in line with the culture capsule sterilizing filter (section 6.2.8) as an alternative to a filter stack (section 6.2.6).

(c) Procedure for the preparation of MEM/L-15 medium.

The procedure described is for preparation of 10 liters of MEM/L-15 medium.

(c.1) Place a three inch stir bar and four liters of dH_2O into 20 liter carboy.

(c.2) Place the carboy onto a magnetic stirrer. Stir at a speed sufficient to develop a vortex and then add the contents of a five liter packet of L-15 medium to the carboy. Rinse the medium packet with three washes of 200 mL each of dH_2O and add the rinses to the carboy.

(c.3) Mix until the medium is evenly dispersed.

L-15 medium may appear cloudy as it need not be totally dissolved before proceeding to step 6.4.2c.4.

(c.4) Add three liters of dH_2O to the carboy and the contents of a five liter packet of MEM medium to the carboy. Rinse the MEM medium packet with three washes of 200 mL each of dH_2O and add the rinses to the carboy. Add 800 mL of dH_2O and 7.5 g of $NaHCO_3$ and continue mixing for an additional 60 min.

(c.5) 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 liter bottle) and store in tightly stoppered or capped containers at 4°C.

Medium may be stored for periods of up to two months.

(d) 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. As 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.

(d.1) Add 0.5 g of trypan blue to 100 mL of dH_2O in a 250 mL flask. Swirl the flask until the trypan blue is completely dissolved.

(d.2) Sterilize the solution by autoclaving at 121°C for 15 minutes and store in a screw-capped container at room temperature.

(e) 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.

(e.1) 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 6.4.2e.1.1 may not correspond to the concentrations obtained from other lots or from a different source.

(e.1.1) Add appropriate amounts of penicillin G and dihydrostreptomycin sulfate to a 250 mL flask containing 100 mL of dH_2O. Mix the contents of the flasks on magnetic stirrer until the antibiotics are dissolved.

For penicillin supplied at 1435 units per mg, add 7 g of the antibiotic.

For streptomycin supplied at 740 mg per g, add 14 g of the antibiotic.

(e.1.2) Sterilize the antibiotics by filtration through 0.22 µm membrane filters and dispense in 10 mL volumes into screw-capped containers.

(e.2) 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 dH_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.

(e.3) 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.

6.5. 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. BGM cell lines should be monitored every six months for mycoplasma contamination according to test kit instructions. Cells that are contaminated should be discarded.

6.5.1 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 seven 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.

6.5.2 Visual Evaluation of Media for Microbial Contaminants. Incubate media at 36.5 ± 1°C for at least one 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.

6.6. 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.

6.6.1 Vessels and Media for Cell Growth

(a) 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.

(b) 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 dH_2O, respectively).

6.6.2 General Procedure for Cell Passage

Pass stock BGM cell cultures at approximately seven day intervals using growth medium.

(a) 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.

(b) Add to the cell cultures a volume of warm EDTA-trypsin reagent equal to 40% of the volume of medium replaced.

See Table 2 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.

(c) 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 five min).

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.

(d) 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.

(e) Centrifuge cell suspension at 1,000 ×*g* for 10 min to pellet cells. Pour off and discard the supernatant.

Do not exceed this speed as cells may be damaged or destroyed.

TABLE 2. Guide for Preparation of BGM Stock Cultures

Vessel Type	EDTA-Trypsin Volume (mL)[1]	Media Volume (mL)[2]	Total No. Cells to Plate per Vessel
16 oz glass flat bottles[3]	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

[1]The volume required to remove cells from vessels.
[2]Serum requirements: growth medium contains 10% serum; maintenance medium contains 2-5% serum. Antibiotic requirements: penicillin-streptomycin stock solution, 1.0 mL/ liter; tetracycline stock solution, 0.5 mL/liter; fungizone stock solution, 0.2 mL/liter.
[3]Size is given in oz only when it is commercially designated in that unit.

(f) Suspend the pelleted cells in growth medium (see section 6.6.1b) and perform a viable count on the cell suspension according to procedures in section 6.7.

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.

(g) 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 section 6.7 and the cell and volume parameters given in Table 2 for stock cultures and in Table 3 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 two hundred 25 cm^2 cell culture flasks weekly from a low-level passage of the line would require the preparation of six roller bottles (surface area 690 cm^2 each): two to prepare the six roller bottles and four to prepare the 25 cm^2 flasks.

(h) Except during handling operations, maintain BGM cells at 36.5 ± 1°C in airtight cell culture vessels.

6.6.3 Procedure for Changing Medium on Cultured Cells — Cell monolayers normally become 95 to 100% confluent three to four 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% to 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.

6.7. 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.

6.7.1 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 × 10^5 and 1.5 × 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.

6.7.2 Disperse cells by repeated pipetting.

Avoid introducing air bubbles into the suspension, because air bubbles may interfere with subsequent filling of the hemocytometer chambers.

6.7.3 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 one min to allow the trypan blue to penetrate the cell membranes of nonviable cells.

Do not under or over fill the chambers.

6.7.4 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.

Table 3. Guide for Preparation of Virus Assay Cell Cultures

Vessel Type	Volume of Medium (mL)[1]	Final Cell Count per Bottle
1 oz glass bottle[2]	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 × 150 mm tubes	2	4.0×10^4

[1]Serum requirements: growth medium contains 10% serum; maintenance medium contains 2-5% serum. Antibiotic requirements: penicillin-streptomycin stock solution, 1.0 mL/liter; tetracycline stock solution, 0.5 mL/liter; fungizone stock solution, 0.2 mL/liter.
[2]Size is given in oz only when it is commercially designated in that unit.

6.7.5 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 4000, and where necessary, multiplying the resulting product by the reciprocal of the dilution.

6.8. 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.

6.8.1 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.

(a) Prepare cell storage medium by adding 10 mL of DMSO to 90 mL of growth medium (see section 6.6.1b). Sterilize cell storage medium by passage through an 0.22 µm sterilizing filter.

Collect sterilized medium in 250 mL flask containing a stir bar.

(b) Harvest BGM cells from cell culture vessels as directed in section 6.6.2. Count the cells according to the procedure in section 6.7 and resuspend them in the cell storage medium at a concentration of 1 × 10^6 cells per mL.

(c) Place the flask containing suspended cells on a magnetic stirrer and slowly mix for 30 min. Dispense 1 mL volumes of cell suspension into 2 mL vials.

6.8.2 Procedure for Freezing Cells

The freezing procedure requires slow cooling of the cells with the optimum rate of -1°C per min. 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.

(a) Place the vials in a rack and place the rack in refrigerator at 4°C for 30 min, in a -20°C freezer for 30 min, 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.

(b) Rapidly transfer vials into boxes or other containers for long-term storage.

To prevent substantial loss of cells during storage, temperature of cells should be kept constant after -70°C has been achieved.

6.8.3 Procedure for Thawing Cells

Cells must be thawed rapidly to decrease loss in cell viability.

(a) 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% iodine in 70% ethanol.

(b) 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 3). Use two vials of cells for 6 oz bottles and one vial for 25 cm^2 flasks.

(c) Incubate BGM cells at 36.5 ± 1°C. After 18 to 24 h 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 section 6.6.

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18: APPENDIX G

The text in Appendix G has been taken from the previously published document "Control of Pathogens and Vector Attraction in Sewage Sludge" (July 2003, EPA 625-R-92-013). Page numbers will be inconsistent with the previous text.

Test Method for Detecting, Enumerating, and Determining the Viability of *Ascaris* Ova in Sludge

1.0 Scope

1.1 This test method describes the detection, enumeration, and determination of viability of *Ascaris* ova in water, wastewater, sludge, and compost. These pathogenic intestinal helminths occur in domestic animals and humans. The environment may become contaminated through direct deposit of human or animal feces or through sewage and wastewater discharges to receiving waters. Ingestion of water containing infective *Ascaris* ova may cause disease.

1.2 This test method is for wastewater, sludge, and compost. It is the user's responsibility to ensure the validity of this test method for untested matrices.

1.3 This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use. For specific hazard statements, see section 9.

2.0 Referenced Documents

2.1 ASTM Standards:

- D 1129 Terminology Relating to Water[1]
- D 1193 Specification for Reagent Water[2]
- D 2777 Practice for Determination of Precision and Bias of Applicable Methods of committee D-19 on Water[3]

3.0 Terminology

(Definitions and Descriptions of Terms must be approved by the Definitions Advisor.)

3.1 Definitions - For definitions of terms used in this test method, refer to Terminology D 1129.

3.2 Descriptions of Terms Specific to This Standard:

3.2.1 The normal nematode life cycle consists of the egg, 4 larval stages and an adult. The larvae are similar in appearance to the adults; that is, they are typically worm-like in appearance.

3.2.2 Molting (*ecdysis*) of the outer layer *(cuticle*) takes place after each larval stage. Molting consists of 2 distinct processes, the deposition of the new cuticle and the shedding of the old one or exsheathment. The cuticle appears to be produced continuously, even throughout adult life.

3.2.3 A molted cuticle that still encapsulates a larva is called a *sheath*.

3.2.4 Ascarid egg shells are commonly comprised of layers. The outer tanned, bumpy layer is referred to as the *mammillated* layer and is useful in identifying *Ascaris* eggs. The mammillated layer is sometimes absent. Eggs that do not possess the mammillated layer are referred to as *decorticated* eggs.

3.2.5 A potentially infective *Ascaris* egg contains a third stage larva[4] encased in the sheaths of the first and second larval stages.

4.0 Summary of Test Method

4.1 This method is used to concentrate pathogenic *Ascaris* ova from wastewater, sludge, and compost. Samples are processed by blending with buffered water containing a surfactant. The blend is screened to remove large particulates. The solids in the screened portion are allowed to settle out and the supernatant is decanted. The sediment is subjected to density gradient centrifugation using magnesium sulfate (specific gravity 1.20). This flotation procedure yields a layer likely

[1]Annual Book of ASTM Standards, Vol 11.01.
[2]Annual Book of ASTM Standards, Vol 11.01.
[3]Annual Book of ASTM Standards, Vol 11.01.

[4]P.L. Geenen, J. Bresciani, J. Boes, A. Pedersen, L. Eriksen, H.P. Fagerholm, and P. Nansen (1999)The morphogenesis of Ascaris suum to the infective third-stage larvae within the egg, J. Parasitology 85(4):616-622.

to contain *Ascaris* and some other parasitic ova, if present, in the sample. Small particulates are removed by a second screening on a small mesh size screen.[5] The resulting concentrate is incubated at 26EC until control *Ascaris* eggs are fully embryonated. The concentrate is then microscopically examined for the categories of *Ascaris* ova on a Sedgwick-Rafter counting chamber.

5.0 Significance and Use

5.1 This test method is useful for providing a quantitative indication of the level of *Ascaris* ova contamination of wastewater, sludge, and compost.

5.2 This test method will not identify the species of *Ascaris* detected nor the host of origin.

5.3 This method may be useful in evaluating the effectiveness of treatment.

6.0 Interferences

6.1 Freezing of samples will interfere with the buoyant density of *Ascaris* ova and decrease the recovery of ova.

7.0 Apparatus

7.1 A good light microscope equipped with brightfield, and preferably with phase contrast and/or differential contrast optics including objectives ranging in power from 10X to 45X.

7.2 Sedgwick-Rafter cell.

7.3 Pyrex beakers, 2 L. Coat with organosilane.

7.4 Erlenmeyer flask, 500 mL. Coat with organosilane.

7.4 A centrifuge that can sustain forces of at least 660 X G with the rotors listed below.

7.4.1 A swinging bucket rotor to hold 100 or 250 ml centrifuge glass or plastic conical bottles.

7.4.2 A swinging bucket rotor to hold 15 ml conical glass or plastic centrifuge tubes.

7.5 Tyler sieves.

7.5.1 20 or 50 mesh.

7.5.2 400 mesh, stainless steel, 5 inch in diameter.

7.5.3 A large plastic funnel to support the sieve. Coat with organosilane.

7.6 Teflon spatula.

7.7 Incubator set at 26EC.

7.8 Large test tube rack to accommodate 100 or 250 mL centrifuge bottles.

7.9 Small test tube rack to accommodate 15 mL conical centrifuge tubes.

7.10 Centrifuge bottles, 100 or 250 mL. Coat with organosilane.

7.11 Conical centrifuge tubes, 15 mL. Coat with organosilane.

7.12 Pasteur pipettes. Coat with organosilane.

7.13 Vacuum aspiration apparatus.

7.13.1 Vacuum source.

7.13.2 Vacuum flask, 2 L or larger.

7.13.3 Stopper to fit vacuum flask, fitted with a glass or metal tubing as a connector for 1/4 inch tygon tubing.

7.14 Spray bottles (16 fl oz.) (2).

7.14.1 Label one “Water”.

7.14.2 Label one “1% 7X”.

8.0 Reagents and Materials

8.1 Purity of Reagents — Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents shall conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society[6]. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination.

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[5]This method is based on a protocol published by Bowman, D.D., M.D. Little, and R.S. Reimers (2003) Precision and accuracy of an assay for detecting *Ascaris* eggs in various biosolid matrices. Water Research **37**(9):2063-2072.

[6]Reagent Chemicals, American Chemical Specifications, American Chemical Society, Washington, D.C. For suggestions on testing of Reagents not listed by the American Chemical Society, see Analar Standards for Laboratory Chemicals, BHD Ltd., Poole, Dorset, U.K. and the United States Pharmacopeia and National Formulary, U.S. Pharmaceutical Convention, Inc. (USPC).

8.2 Purity of Water — Unless otherwise indicated, references to water shall be understood to mean reagent water conforming to Specification D 1193, Type I.

8.3 Preparation of Reagents — Prepare reagents in accordance with Practice E200.

8.3.1 Phosphate-buffered water (1 L = 34.0 g KH_2PO_4, pH adjusted to 7.2 ± 0.5 with 1 N NaOH).

8.3.2 1% (v/v) 7X ("ICN" laboratory detergent) (1 L = 999 mL phosphate-buffered water, 1 mL 7X "ICN", Adjust pH to 7.2 ± 0.1 with 1N NaOH).

8.3.3 Magnesium sulfate, sp. gr. 1.20. (1 L = 215.2 g $MgSO_4$, check specific gravity with a hydrometer; adjust as necessary to reach 1.20).

8.3.4 Organosilane. For coating glassware. Coat all glassware according to manufacturer's instructions.

8.3.5 Fresh *Ascaris* ova for positive control, purified from *Ascaris* infected pig fecal material.

9.0 Precautions

9.1 When handling *Ascaris* ova and biosolids, personal protective measures must be employed to prevent infection. Prevention of infection in humans is a matter of good personal hygiene. Wear a laboratory coat at all times in the laboratory. In addition, latex or nitrile gloves and splash protection safety glasses should always be worn in the laboratory. Mouth pipetting is strictly forbidden. Contaminated pipettes are never laid down on the bench top but are immediately placed in a pipette discard container which has disinfectant in it. Contaminated equipment is separated as it is used into containers for disposable materials and containers for re-cycling. After these containers which are always autoclave pans, are full, they are autoclaved for 30 minutes at 121EC and 15 pounds/in^2. Contaminated glassware is never washed until after it has been autoclaved. Eating, drinking, and smoking in the laboratory is not permitted. Likewise, refrigerators are not to be used for storing lunches or other items for human consumption. If infective *Ascaris* ova are ingested they may cause disease.

10.0 Sampling

10.1 Collect 1 liter of compost, wastewater, or sludge in accordance with Practice D 1066, Specification D 1192, and Practices D 3370, as applicable.

10.2 Place the sample container(s) on wet ice or around chemical ice and ship back to the laboratory for analysis within 24 hours of collection.

10.3 Store the samples in the laboratory refrigerated at 2 to 5EC. Do not freeze the samples during transport or storage.

11.0 Preparation of Apparatus

11.1 Test the centrifuge with a tachometer to make sure the revolution's per minute correlate with the speed gauge.

11.2 Calibrate the incubator temperature with a NIST traceable thermometer.

11.3 Microscope.

11.3.1 Clean the microscope optics.

11.3.2 Adjust the condenser on the microscope, so Köhler illumination is established.

12.0 Procedure

12.1 The percentage moisture of the sample is determined by analyzing a separate portion of the sample, so the final calculation of ova per gram dry weight can be determined. The concentration of ova in liquid sludge samples may be expressed as ova per unit volume.

12.2 Initial preparation:

12.2.1 Dry or thick samples: Weigh about 300 g (estimated dry weight) and place in about 500 ml water in a beaker and let soak overnight at 4 - 10EC. Transfer to blender and blend at high for one minute. Divide sample into four beakers.

12.2.2 Liquid samples: Measure 1,000 ml or more (estimated to contain at least 50 g dry solids) of liquid sample. Place one half of sample in blender. Add about 200 mL water. Blend at high speed for one minute transfer to a beaker. Repeat for other half of sample.

12.3 Pour the homogenized sample into a 1000 mL tall form beaker and using a wash bottle, thoroughly rinse blender container into beaker. Add 1% 7X to reach 900 ml final volume.

12.4 Allow sample to settle four hours or overnight at 4 - 10EC. Stir occasionally with a wooden applicator, as needed to ensure that material floating on the surface settles. Additional 1% 7X may be added, and the mixture stirred if necessary.

12.5 After settling, vacuum aspirate supernatant to just above the layer of solids. Transfer sediment to blender and add water to 500 ml, blend again for one minute at high speed.

12.6 Transfer to beaker, rinsing blender and add 1% 7X to reach 900 ml. Allow to settle for two hours at 4 - 10EC, vacuum aspirate supernatant to just above the layer of solids.

12.7 Add 300 ml 1% 7X and stir for five minutes on a magnetic stirrer.

12.8 Strain homogenized sample through a 20 or 50 mesh sieve placed in a funnel over a tall beaker. Wash sample through sieve with a spray of 1% 7X from a spray bottle.

12.9 Add 1% 7X to 900 mL final volume and allow to settle for two hours at 4 - 10EC.

12.10 Vacuum aspirate supernatant to just above layer of solids. Mix sediment and distribute equally to 50 mL graduated conical centrifuge tubes. Thoroughly wash any sediment from beaker into tubes using water from a wash bottle. Bring volume in tubes up to 50 ml with water.

12.11 Centrifuge for 10 minutes at 1000 X G. Vacuum aspirate supernatant from each tube down to just above the level of sediment. (The packed sediment in each tube should not exceed 5 mL. If it exceeds this volume, add water and distribute the sediment evenly among additional tubes, repeat centrifugation, and vacuum aspirate supernatant.)

12.12 Add 10 to 15 mL of $MgSO_4$ solution (specific gravity 1.20) to each tube and mix for 15 to 20 seconds on a vortex mixer. (Use capped tubes to avoid splashing of mixture from the tube.)

12.13 Add additional $MgSO_4$ solution (specific gravity 1.20) to each tube to bring volume to 50 mL. Centrifuge for five to ten minutes at 800 to 1000 X g. DO NOT USE BRAKE.

12.14 Allow the centrifuge to coast to a stop without the brake. Pour the top 25 to 35 mL of supernatant from each tube through a 400 mesh sieve supported in a funnel over a tall beaker.

12.15 Using a water spray bottle, wash excessive flotation fluid and fine particles through sieve.

12.16 Rinse sediment collected on the sieve into a 100 mL beaker by directing the stream of water from the wash bottle onto the upper surface of the sieve.

12.17 After thoroughly washing the sediment from the sieve, transfer the suspension to the required number of 15 mL centrifuge tubes, taking care to rinse the beaker into the tubes. Usually one beaker makes one tube.

12.18 Centrifuge the tubes for three minutes at 800 X G, then discard the supernatant.

12.19 If more than one tube has been used for the sample, transfer the sediment to a single tube, fill with water, and repeat centrifugation.

12.20 Aspirate the supernatant above the solids.

12.21 Resuspend the solids in 4 mL 0.1 N H_2SO_4 and pour into a 20-mL polyethylene scintillation vial or equivalent with loose caps.

12.22 Before incubating the vials, mark the liquid level in each vial with a felt tip pen. Incubate the vials, along with control vials containing *Ascaris* ova mixed with 4 mL 0.1 N H_2SO_4, at 26EC for three to four weeks. Every day or so, check the liquid level in each vial. Add reagent grade water up to the initial liquid level line as needed to compensate for evaporation. After 18 days, suspend, by inversion and sample small aliquots of the control cultures once every 2 - 3 days. When the majority of the control *Ascaris* ova are fully embryonated, samples are ready to be examined.

12.23 Examine the concentrates microscopically using a Sedgwick-Rafter cell to enumerate the detected ova. Classify the ova as either unembryonated, embryonated to the first, second, or third larval stage. In some embryonated *Ascaris* ova the larva may be observed to move. See Figure 1 for examples of various *Ascaris* egg categories.

13.0 Calculation

13.1 Calculate % total solids using the % moisture result:

$$\% \text{ Total solids} = 100\% - \% \text{ moisture}$$

13.2 Calculate catagories of ova/g dry weight in the following manner:

$$\text{Ova/g dry wt} = \frac{(NO) \times (CV) \times (FV)}{(SP) \times (TS)}$$

Where:

NO = no. ova
CV = chamber volume(= 1 mL)
FV = final volume in mL
SP = sample processed in mL or g
TS = % total solids

14.0 Report

14.1 Report the results as the total number of *Ascaris* ova, number of unembryonated *Ascaris* ova, number of 1st, 2nd, or 3rd stage larva; reported as number of *Ascaris* ova and number of various larval *Ascaris* ova per g dry weight.

15.0 Keywords

Ascaris, ova, embryonation, viability assay, helminth.

Notice

The PEC was consulted in a recent (1998-1999) pilot study by Lyonnaise des Eaux concerning the use of a microscope in making helminth ova counts for different types of sludge. Solids and debris present in the sludge being viewed with the microscope were found to impair ones ability to count. Dilution of raw sludge and digested sludge, however, with phosphate-buffered water prior to analyzing them significantly improved the number of ova that could be counted. Raw sludges were diluted by a factor of 20 and digested sludges by a factor of 5. QA/QC procedures were followed to validate this procedure. The PEC should be consulted for more details.

[revised May 15, 2003]

Figure A1.1. *Ascaris* ovum: potentially non-fertile, note bumpy mammilated outer layer.

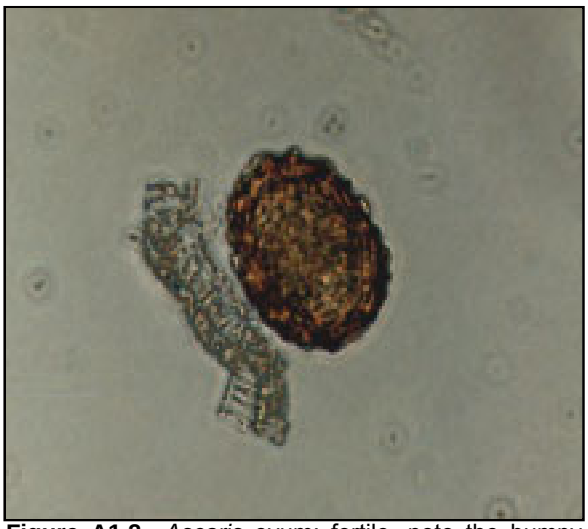

Figure A1.2. *Ascaris* ovum: fertile, note the bumpy outer mammilated layer.

Figure A1.3. *Ascaris* ovum: decorticated, unembryonated. Note the outer mammilated layer is gone

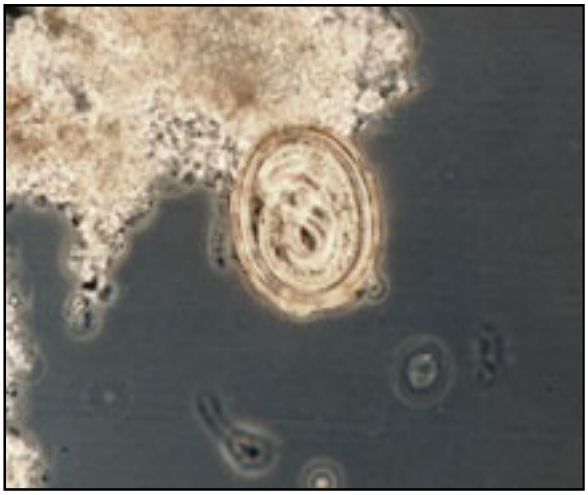

Figure A1.4. *Ascaris* ovum: decorticated and embryonated.

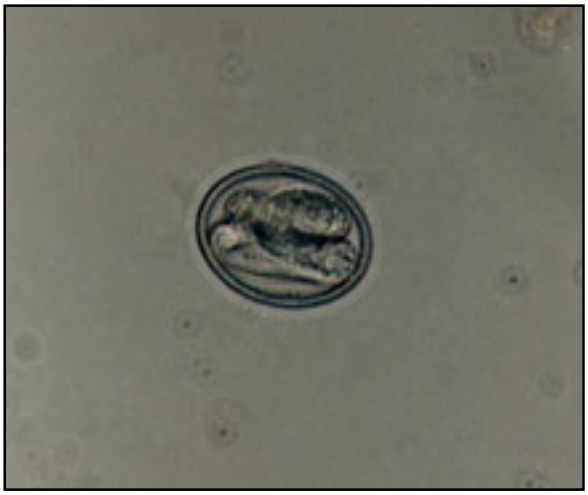

Figure A1.5. *Ascaris* ovum: decorticated, embryonated.

Figure A1.6. *Ascaris* ovum with second stage larva; note the first stage larval sheath at the anterior end of the worm

19: APPENDIX H

The text in Appendix H has been taken from the previously published document "Control of Pathogens and Vector Attraction in Sewage Sludge" (July 2003, EPA 625-R-92-013). Page numbers will be inconsistent with the previous text.

The Biosolids Composting Process

Introduction

Composting is the biological decomposition of organic matter under controlled aerobic conditions. The objectives of composting are to reduce pathogens to below detectable levels, degrade volatile solids, and produce a usable product. Pathogen reduction is a function of time and temperature. Composted biosolids is one way to meet 40 CFR Part 503 pathogen (and vector attraction) reduction requirements. Composted biosolids can meet either a "Process to Significantly Reduce Pathogens" (PSRP/Class B) or a "Process to Further Reduce Pathogens" (PFRP/Class A) standard, depending upon the operating conditions maintained at the facility. Process and operational considerations must be taken into account when a facility desires to meet the pathogen and vector attraction requirements of 40 CFR 503. The 40 CFR Part 503 regulations require composted biosolids applied to the land to meet specific pollutant limits, site restrictions, management practices, and pathogen and vector attraction reduction processes, depending upon whether they: 1) are applied to agricultural land, forest, a public contact site, or a reclamation site; 2) are sold or given away in a bag or other container; or 3) are applied to a lawn or home garden. Discussions provided here are presented in summary form; it is recommended that the facility seek additional details in developing a compost operation.

Composting Process Description

The addition of a bulking agent to sewage sludge provides optimum conditions for the composting process, which usually lasts 3 to 4 weeks. A bulking agent acts as a source of carbon for the biological process, increases porosity, and reduces the moisture level. The composting process has several phases, including the active phase, the curing phase, and the drying phase.

Active phase. During the active or stabilization phase, the sewage sludge/bulking agent mix is aerated and the sewage sludge is decomposed due to accelerated biological activity. The biological process involved in composting can raise the temperature up to 60°C or more. At these high temperatures, all of the disease-causing pathogens are destroyed. Windrow systems must meet this condition by achieving 55°C for a minimum of 15 consecutive days during which time the windrow is turned five times. The critical requirement is that the material in the core of the compost pile be maintained at the required temperatures (55°C) for the required time (3 days). Therefore, the first phase typically lasts 21 days. Aeration is accomplished in one of two ways: 1) by mechanically turning the mixture so that the sewage sludge is exposed to oxygen in the air; or 2) by using blowers to either force or pull air through the mixture.

Curing phase. After the active phase, the resulting material is cured for an additional 30 days to 180 days. At this time, additional decomposition, stabilization, pathogen destruction, and degassing takes place. Composting is considered complete when the temperature of the compost returns to ambient levels. Depending upon the extent of biodegradation during the active phase and the ultimate application of the finished product, the curing phase may not be carried out as a separate process.

Drying phase. After curing, some operations add another step called the drying phase which can vary from days to months. This stage is necessary if the material is to be screened to either recover the unused bulking agent for recycling or for an additional finished product. If the product is to be marketable, the final compost should be 50% to 60% solids.

There are two main process configurations for the composting process:

Unconfined composting. This process is conducted in long piles (windrows) or in static piles. Operations using unconfined composting methods may provide oxygen to the compost by turning the piles by hand or machine or by using air blowers which may be operated in either a positive (blowing) or negative (suction) mode. For windrows without blower aeration, it is typical to turn the windrow two or three times a week, using a front-end loader. Properly operating aerated static piles do not require turning.

Confined (in-vessel) composting. This process is carried out within an enclosed container, which minimizes odors and process time by providing better control over the process variables. Although in-vessel composting has been effective for small operations, typically these operations are proprietary and therefore will not be described any further in this fact sheet.

Operational Considerations

The key process variables for successful composting are the moisture content and carbon to nitrogen (C:N) ratio of the biosolids/bulking agent mixture, and temperature and aeration of the compost pile. Other process parameters such as volatile solids content, pH, mixing and the materials used in the compost also affect the process.

Bioso/ids/Bu/king Agent Mixture Moisture Content. Moisture control is an important factor for effective composting. Water content must be controlled for effective stabilization, pathogen inactivation, odor control and finished compost quality (Benedict, 1988). The optimum moisture content of the mix is between 40% and 60%. At less than 40% water, the material is too fluid, has reduced porosity and has the potential for producing septic conditions and odors; above 60% solids, the lack of moisture may slow down the rate of decomposition. Since typical dewatered sewage sludge or biosolids are often in the range of 15% to 20% solids for vacuum filtered sewage sludge or biosolids and 20% to 35% solids for belt press or filter pressed sewage sludge or biosolids, the addition of drier materials (bulking agents) is usually essential.

Biosolids/Bulking Agent Mixture Carbon to Nitrogen Ratio. Microorganisms need carbon for growth and nitrogen for protein synthesis. For efficient composting, the carbon to nitrogen (C:N) ratio of the biosolids/bulking agent mixture should be in the range of 25:1 to 35:1

Oxygen /eve/s. For optimum aerobic biological activity, air within the pile should have oxygen levels of between 5% and 15%. Lower levels of oxygen will create odors and reduce the efficiency of the composting. Excessive aeration will cool the pile, slow the composting process, and will not provide the desired pathogen and vector attraction reduction.

Conventional windrows obtain necessary oxygen through the natural draft and ventilation induced from the hot, moist air produced during active composting and from the periodic windrow turning. Where blowers are used for aeration, it is typical to provide at least one blower per pile.

Biosolids/Bulking Agent Mixture Volatile Solids Con tent. The volatile solids content of the biosolids/bulking agent mix should be greater than 50% for successful composting (EPA, 1985). This parameter is an indicator of the energy available for biological activity and therefore compostability.

Bioso/ids/Bu/king Agent Mixture pH. The pH of the biosolids/bulking agent mix should be in the range of 6 to 9 for efficient composting (EPA, 1985). Higher pH mixtures may result if lime stabilized biosolids are used. They can be composted; however, it may take longer for the composting process to achieve the temperatures needed to reduce pathogens.

Biosolids and Bulking Agent Mixing. Uniform mixing is necessary in order to assure that moisture concentration is constant through the pile and that air can flow throughout.

Type of Biosolids. The type of biosolids used may have an effect on the composting process. Composting can be accomplished with unstabilized biosolids, as well as anaerobically and aerobically digested biosolids. Raw sludge has a greater potential to cause odors because they have more energy available and will, therefore, degrade more readily. This may cause the compost pile to achieve higher temperatures faster unless sufficient oxygen is provided and may also cause odors (EPA, 1985).

Material for Bulking Agents. Materials such as wood chips, sawdust and recycled compost are usually added as "bulking agents" or "amendments" to the compost mixture to provide an additional source of carbon and to control the moisture content of the mixture. Other common bulking agents used by facilities around the country include wood waste, leaves, brush, manure, grass, straw, and paper (Goldstein, 1994). Because of their cost, wood chips are often screened out from the matured compost, for reuse. Although sawdust is frequently used for in-vessel composting, coarser materials such as wood chips, wood shavings, and ground-up wood are often preferred because they permit better air penetration and are easier to remove. Recycled compost is often used as a bulking agent in windrows, especially if bulking agents must be purchased. However, its use is limited because the porosity decreases as the recycle ages (EPA, 1989). The amount of biosolids and bulking agent which must be combined to make a successful compost is based on a mass balance process considering the moisture contents, C:N ratio, and volatile solids content.

Compost Pile Size. In general, assuming adequate aeration, the larger the pile the better. A larger pile has less surface area per cubic yard of contents and therefore retains more of the heat that is generated and is less influenced by ambient conditions. In addition, less cover and base material (recycled compost, wood chips, etc.) is needed as well as the overall land requirements for the compost operation. Larger piles tend to retain moisture longer. The surface area to volume ratio has an effect on the temperature of the pile. Assuming other factors are constant (e.g., moisture, composition, aeration), larger piles (with their lower surface area to volume ratio), retain more heat than smaller piles. Ambient temperatures have a significant impact on composting operations (Benedict, 1988).

A typical aerated static pile for a large operation would be triangularly shaped in cross section about 3 meters(m) high by 4.5 to 7.5 m wide (15 to 25 feet) at the base by 12 to 15 m long (39 to 50 feet) (Haug, 1980). One survey study indicates that extended aerated static pile (where piles are formed on the side of older piles) heights were typically 12 to 13 feet high. Minimum depths of base and cover materials (recycled compost, wood chips, etc.) were 12 and 18 inches, respectively (Benedict, 1988).

In windrow composting, the compost mix is stacked in long parallel rows. In cross section, windrows may range from rectangular to trapezoidal to triangular, depending

upon the material and the turning equipment. Atypical trapezoidal windrow might be 1.2 m (4 feet) high by 4.0 m (13 feet) at its base and 1.0 m (3 feet) across the top (Haug, 1980).

Monitoring and Sampling of the Compost Pile

Unless the entire composting mass is subject to the pathogen reduction temperatures, organisms may survive and repopulate the mass once the piles or windrows are cooled. Therefore it is crucial that temperatures be attained throughout the entire pile. For aerated static piles or in-vessel systems using static procedures such as tunnels or silos, temperature monitoring should represent points throughout the pile, including areas which typically are the coolest. In aerated static piles this is usually the toes of the pile (Figure 1). Temperatures should be taken at many locations and at various depths to be assured that the core of the pile maintains the required temperature. Records of the temperature, date, and time should be maintained and reviewed on an ongoing basis. Microbial analysis should at a minimum be taken in a matter to represent the entire compost pile. Operational parameters such as moisture, oxygen as well as the others should be monitored at a frequency necessary to assure that the compost operation is operating within acceptable ranges.

For composting, vector attraction reduction (VAR) is achieved through the degradation of volatile solids. The extent to which the volatile solids are degraded is often referred to as compost stability. Stabilization requires sufficient time for the putrescible organic compounds and for other potential food sources for vectors to decompose. Under this vector attraction reduction option, the Part 503 requires that biosolids be maintained under aerobic conditions for at least 14 days, during which time temperatures are over 40°C (104°F), and the average temperature is over 45°C (113°F) (503.33(b)(5). These criteria are based on studies which have shown that most of the highly putrescible compounds are decomposed during the first 14 days of composting and that significant stability is achieved at mesophilic (<45°C) temperatures.

Recommendations for Specific Technologies

Aerated static pile – Aerated static piles should be covered with an insulation layer of sufficient thickness to ensure that temperatures throughout the pile, including the pile surface, reach 55°C. It is recommended that the insulation layer be at least 1 foot thick. Screened compost is a more effective insulation than unscreened compost or wood chips. Screened compost also provides more odor control than the other two materials.

Air flow rate and the configuration of an aeration system are other factors which affect temperature. Air flow must be sufficient to supply oxygen to the pile, but excessive aeration removes heat and moisture from the composting material. The configuration of an aeration system is also important. Aeration piping too close to pile edges may result in uneven temperatures in the pile and excessive cooling at the pile toes. If holes in the perforated piping are too large or not distributed properly, portions of the pile may receive too much air and be too cool as a result.

Windrows – Compliance with the pathogen reduction requirements for windrows depends on proper windrow size and configuration. If windrows are too small, the high surface area to volume ratio will result in excessive heat loss from the pile sides. Turning must ensure that all material

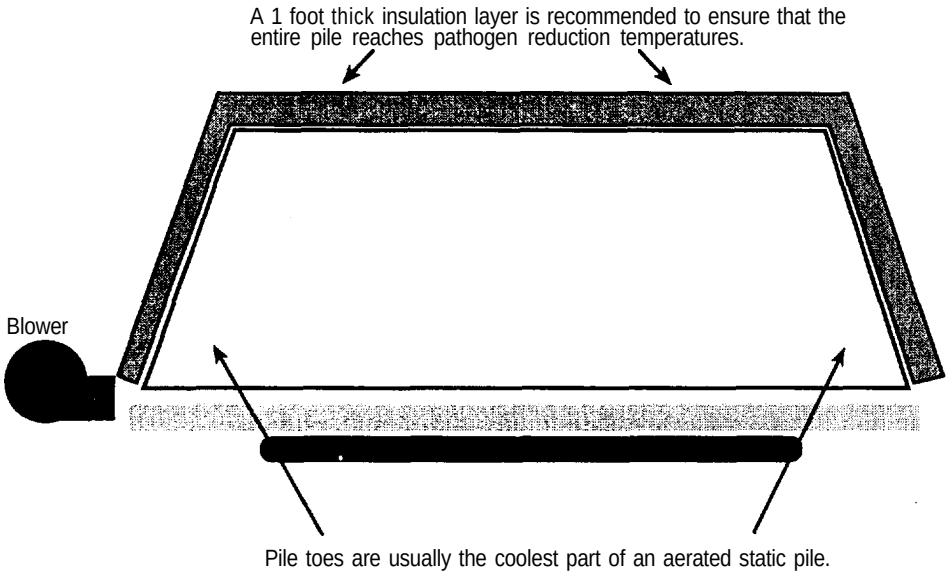

Figure 1a. Aerated static pile.

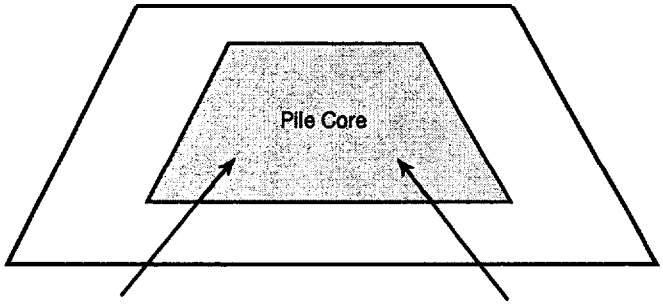

Figure 1b. Windrow.

in a windrow be introduced into the pile core and raised to pathogen reduction temperatures. This is most easily achieved with a windrow turning machine.

In-Vessel systems- It is difficult to provide guidance for these systems as there are numerous types with varying configurations. Two key factors that apply to all in-vessel systems are aeration and available carbon. As with aerated static piles, the air flow configuration and rate can affect the distribution of aeration to different parts of a composting mass and the temperature profile of a pile. Many in-vessel systems use sawdust as an amendment. This may not provide sufficient energy if the volatile solids in the biosolids are low.

Requirements for Class A/Class B Compost

For class A biosolids, aerated static pile, conventional windrow and in-vessel composting methods must meet the PFRP requirements, including the following temperature/ time requirements:

- Aerated static piles and in-vessel systems must be maintained at a minimum operating temperature of 55°C (131°F) for at least 3 days; and
- Windrow piles must be maintained at a minimum operating temperature of 55°C (131°F) for 15 days or longer. The piles must be turned five times during this period.

For class B biosolids, aerated static pile, conventional windrow and in-vessel composting methods must meet the PSRP requirements, including the following temperature/ time requirements:

- The compost pile must be maintained at a minimum of 40°C for at least five days; and
- During the five-day period, the temperature must rise above 55°C for at least four hours to ensure pathogen destruction. This is usually done near the end of the active composting phase in order to prevent inactivating the organic destroying bacteria.

To meet 40 CFR Part 503 vector attraction reduction requirements using the "aerobic process" alternative, composting operations must ensure that the process lasts for 14 days or longer at a temperature greater than 40°C. In addition, the average temperature must be higher than *45°C.*

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20: APPENDIX I

The text in Appendix I has been taken from the previously published document "Control of Pathogens and Vector Attraction in Sewage Sludge" (July 2003, EPA 625-R-92-013). Page numbers will be inconsistent with the previous text.

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.

21: APPENDIX J

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

OFFICE OF WATER

MEMORANDUM

SUBJECT: Land Application Requirements for Class A Exceptional Quality Treated Sewage Sludge

FROM: David P. Ross
Assistant Administrator

DAVID ROSS

Digitally signed by DAVID ROSS
Date: 2020.11.05 18:56:07 -05'00'

TO: Regional Administrators
Regions 1–10

The Environmental Protection Agency's (EPA) Biosolids Program has received several requests for clarification on Class A Exceptional Quality (EQ) treated sewage sludge land application requirements. The purpose of this memorandum is to provide EPA's interpretation of 40 CFR § 503.10, specifically §§ 503.10(e), (f), and (g), regarding whether certain land application requirements apply to entities, including a treatment works or a soil blender, that derive material from Class A EQ sewage sludge.[1]

EPA regulations set out treatment standards for different classes of sewage sludge and different management and land application requirements, depending on the class of sewage sludge or material derived from that sludge. Class A "Exceptional Quality" or "EQ" sludge is treated sewage sludge that meets the pollutant concentrations in § 503.13(b)(3), the Class A pathogen requirements in § 503.32(a) and one of the vector attraction reduction requirements in §§ 503.33(b)(1) through (b)(8). As such, Class A EQ sewage sludge meets the most stringent pollutant, pathogen, and vector attraction reduction requirements under EPA's regulations. Class A and Class B sewage sludge meet less stringent requirements than Class A EQ.

The Standards for the Use or Disposal of Sewage Sludge are set out in 40 CFR Part 503. Subpart B (40 CFR § 503.10) provides requirements for land application of sewage sludge, including when these requirements apply; and management practices, monitoring, recordkeeping and reporting requirements. This memorandum clarifies the land application applicability provisions found at 40 CFR §§ 503.10(e), (f), and (g). Section 503.10(e) provides that seven of the nine land application requirements apply when Class A EQ sewage sludge is produced and then distributed or sold in a bag or other container. Section 503.10(f) provides that seven of the nine land application requirements apply when a Class A EQ material is produced and then distributed or sold in a bag or other container. Section 503.10(g) provides

[1] There are similar provisions for bulk sewage sludge (as opposed to in a bag or other container) at 40 CFR §§ 503.10(b), (c), and (d); however, Class A EQ sewage sludge is rarely distributed as bulk material. That said, this interpretation would equally apply to those provisions if such a scenario arose.

that none of the nine land application requirements apply when a material *is derived from* Class A EQ sewage sludge and then that material is distributed or sold in a bag or other container.

Under EPA's biosolids regulations (40 CFR §§ 503.10(e), (f), and (g)), any preparer of sewage sludge (including a treatment works or a soil blender) that (1) produces Class A EQ sewage sludge, (2) derives a material from that Class A EQ sewage sludge, and (3) sells or gives that material away in a bag or other container is exempt from all land application requirements (*i.e.*, it benefits from the exclusions under 40 CFR § 503.10(g)), even if that preparer began the process with non-Class A EQ sewage sludge. Note that such a preparer would remain subject to 40 CFR § 503.10(e) or § 503.10(f) for the initial Class A EQ sewage sludge or material derived from non-Class A EQ sewage sludge (*i.e.*, the preparer would have to demonstrate that the initial sludge or material meets Class A EQ standards).

This interpretation is reasonable, fair, and protective of human health and the environment. It ensures that any preparer, *e.g.*, a treatment works or a soil blender, that derives a material from Class A EQ sewage sludge is subject to the same regulatory requirements. This interpretation is reasonable because it focuses on the quality of the sewage sludge and/or material derived from sewage sludge, rather than on the actor who is managing or treating the material to ensure that Class A EQ quality is achieved. This interpretation avoids creating inequities between treatment works and private contractors (*e.g.*, soil blenders) that are taking the same action – deriving a material from Class A EQ sewage sludge and then selling or giving away that material in a bag or other container. This interpretation is also protective of human health and the environment as such protections depend not on who is taking the actions but on what actions are being taken – treating sewage sludge to Class A EQ standards before deriving a material from it, and then selling or giving away that material in a bag or other container.

If you have any questions, please contact Elizabeth Resek at (202) 566-1228 or Resek.Elizabeth@epa.gov.

cc: Water Management Division Directors, Regions 1-10

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