Appendix F

Microbial Sampling Considerations

The following are additional critical considerations to be incorporated into any sampling plan or event involving microbial parameters.

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SLUDGE SAMPLING FOR MICROBIAL ANALYSIS

In a sampling plan, collecting a representative sample is a common objective regardless of the target analytes. Much of the information in this document relates to collecting a representative sludge sample is pertinent to collecting samples for microbial analyses. However, specific microbial parameters may require alterations or accommodations in the sampling plan to ensure the collection of representative samples. EPA provides excellent guidance on sludge sampling in general and microbial sampling in particular in *Environmental Regulations and Technology: Control of Pathogens and Vector Attraction in Sewage Sludge* (July 2003).

This Appendix highlights and discusses differences between sampling sludge for microbial analysis and sampling for chemical contaminants. Many of the planning steps for developing a sludge sampling program aimed at microbial sampling are no different than any other sampling effort. In fact, many sampling programs will require sampling for both microbial and chemical constituents. The same sampling plan can accommodate both types of sampling, and in fact the planning processes described in Chapters 3 through 6 are applicable for any type of sampling. The planning elements in these chapters are related to sampling goals, facility description, data quality, and sampling points. In addition, the same sampling SOP can generally be used for collection of microbial samples. Although the planning process is the same, the resulting sampling plans may be different depending on the testing to be performed.

Microbial analysis of sludge generally involves fecal coliform, Salmonella, enteric virus, and helminth ova. The most pronounced differences between microbial and chemical sampling involve preparation of sample containers and sampling equipment and hold times. Although proper cleaning of equipment and containers prior to any sampling event is necessary to prevent sample contamination, microbial analysis requires the extra step of sterilization. This is particularly important when analyzing Class A biosolids, which should have no detectable levels of certain pathogens. While it is always advisable to analyze samples as soon after collection as possible, it is particularly important with microbial samples, which have much shorter hold times than chemical analytes.

Preparation of Sample Containers and Sampling Equipment

Chapter 7 contains guidance on the choice and preparation of sampling containers and sampling equipment that is pertinent to microbial sampling. Sample containers for microbial samples should be made of glass, polycarbonate or polypropylene. Pre-sterilized plastic bags (now widely available) are also suitable sample containers. A non-reactive metal, such as stainless steel, probably works best for collection equipment. Glass and Teflon are also acceptable, but may be too fragile or costly.

The general cleaning procedures described in Chapter 7 are adequate for the initial cleaning of containers or equipment. After the initial cleaning, containers and equipment must be sterilized. Sterilization can be accomplished by one of the following methods:

1) Heat in an oven at 170° C for at least 2 hours.

2) Autoclave at 121° C for a minimum of 30 minutes.

3) Soak in a 10 percent bleach solution for a minimum of one minute. Note: If this option is used, the equipment should be rinsed three times with sterile water prior to use.

Prior to heating or autoclaving sampling equipment, it is advisable to wrap it in aluminum foil or a kraft paper to prevent contamination while being stored or transported. When sterilizing glass, polycarbonate or

polypropylene bottles in an autoclave, their tops should be loosened to prevent breakage or deformation. Depending on cost and convenience, pre-sterilized disposable containers and sampling equipment may be an effective option. Finally, it is recommended that sampling containers and equipment for microbial sampling be dedicated to that purpose.

Preservation and Hold Times:

Industry-wide, there has been much confusion over what constitutes proper preservation and the correct maximum hold times for microbial samples. The best reference for preservation and hold times is the specific analytical method that will be used. To prevent growth or decay of microbial populations, hold times should be as short as possible. However, this can be a challenge. For example, few labs are capable of performing enteric virus and helminth ova assays, and samples frequently need to be shipped to laboratories for these analyses. Since shipping complicates preservation and extends hold times, it is imperative that samplers know and plan for the short hold times associated with microbial samples. Below is a table partially reproduced from *Environmental Regulations and Technology: Control of Pathogens and Vector Attraction in Sewage Sludge* (July 2003), which states maximum hold times using specific preservation methods. It is important to note that maximum holding times and temperatures are method-specific and federal and state regulations should be consulted.

Methods, Preservation, and Maximum Hold Times

Analysis	Method	Preservation	Maximum Hold Time
Enteric Viruses	ASTM(1) Method D 4994-89	–18° C	2 weeks
Fecal coliform	SM(2) Part 9221 E or Part 9222 D	4° C (do not freeze)	24 hours
Salmonella sp.	SM Part 9260D or Kenner and Clark (1974)(3)	4° C (do not freeze)	24 hours
Viable Helminth Ova	Yanko (1987)(4)	4° C (do not freeze)	1 month

1) American Society for Testing and Materials

(2) Standards Methods for the Examination of Water and Wastewater (APHA, 1992)

(3) See Appendix G of *Environmental Regulations and Technology: Control of Pathogens and Vector Attraction in Sewage Sludge* (July 2003)

(4) See Appendix I of *Environmental Regulations and Technology: Control of Pathogens and Vector Attraction in Sewage Sludge* (July 2003)

For bacterial and viral analyses, prompt chilling of samples is required to ensure that samples remain representative. If sample analysis will not begin within two hours of collection, place the sample container in an ice water bath (for a minimum of 30 minutes prior to refrigeration) immediately following sample collection. Laboratories should be contacted in advance to schedule analyses and ensure samples are handled in a timely manner upon receipt.