503 BIOSOLIDS PATHOGENS

Introduction

The land application of biosolids originating from domestic sewage has some obvious public health implications. Since these solids arise primarily from human and animal excreta and food waste, the raw material can contain any of a variety of bacterial, viral and parasitic enteric pathogens. Before solids of this nature are applied to areas in which human or animal contact is probable it is important to either process the solids in such a manner that the numbers of pathogenic microorganisms are reliably reduced to "safe" levels or deny contact with the area of application until such time as deemed "safe". The "safety" of biosolids application requires, among other things, some degree of monitoring for the presence of microbial pathogens. Standards for safe public health levels of these agents are difficult to establish and justify but invariably require that their concentration be low or non-detectable.

The Environmental Protection Agency has promulgated regulations for the disposition of biosolids onto land. These regulations are commonly referred to as the "503'" rule (E.P.A.1993) which include microbiologic criteria. In all cases the solids must receive selected degrees of treatment and most must also meet either fecal coliform or salmonella requirements. Acceptable levels of enteric viruses and parasite ova may also be required depending upon the nature of the solids treatment process. Two classes of sludge are defined, Class A and B. The former can be applied with no restrictions (see Table) and the latter, because of lesser treatment (primarily sludge digestion only), has restrictions placed upon human and animal contact with the application site.

Bacterial pathogens

As stated above there is a great diversity of pathogenic bacteria associated with sewage solids. Monitoring for all of the agents that might be involved is impractical; thus, the use of indicator organisms has been the traditional approach to estimating sanitary quality. Coliform bacteria have been the most used in this regard. Their presence in the environment, particularly the fecal coliforms, is an indication of the presence of fecal matter, animal or human, and thus the possible presence of associated pathogens. The intestinal pathogenic bacteria normally react to environmental phenomena in much the same manner as do the coliforms. The rates of removal of coliforms during biosolids processing should reflect a similar reduction in pathogenic bacteria. In this regard the coliform as indicator has served us well. In the case of "503" Class A biosolids the minimum acceptable fecal coliform level is <1,000 most probable number (MPN) per gram dry weight of solids. In Class B a maximum of a 2,000,000 fecal coliform MPN per gram of dry solids is allowed. As an alternative to the fecal coliform requirement the rule also allows for the direct determination of *Salmonella sp*. and if their concentration is less than 3 salmonella per 4 grams dry weight of sludge the Class A classification will hold.

Viral Pathogens

There are more than 100 enteric animal viruses that can be associated with human feces. Some examples are polio, echo, coxsackie, hepatitis A, norwalk and rota viruses. Their numbers in wastewater and sludges, relative to the coliforms, are reported to be low. Values ranging from <1 to 49,000 plaque forming units (PFU) per 100 mL have been reported in raw sewage (Fatal and Shaub, 1983) and from 500 to 2,800 per 100 mL in raw sludge (Britton, 1980). Because of the low numbers found in biosoids and volummetric limits of virus assay methods some means of concentration which will increase the virus numbers and reduce the sample volume is required. The usual procedure followed in the laboratory is to suspend a portion of the biosolids sample in a buffer-wetting agent solution, "stomach" the mixture, add beef extract, and lower the pH to the isoelectric point of the beef extract protein forming a precipitate. This precipitate, along with any adsorbed viruses, is collected by centrifugation and redissolved in a minimal volume of Na_2HPO_4, to a neutral pH. This concentrate is then assayed for enteric viruses using the plaque assay method on "Buffalo Green monkey Kidney cells" (BGMK).

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Parasites

As in the case of pathogenic bacteria and viruses there are a great number and variety of intestinal parasites that can be found in raw sew age sludge. Some examples are *Taenia* sp.(tape worm), *Ascaris lumbercoides* (round worm) and the protozoa *Giardia lamblia* and *Cryptosporidium parvum.* The cysts of protozoans and the ova of helminths can remain viable for long periods of time outside their host. A difficulty in monitoring for the presence of these cysts and ova is the determination of their viability. The ova or cysts may become non-viable yet their structure remains intact for long periods of time; thus, their presence *per se* does not necessarily signify an inadequate biosolids treatment process. Viability of protozoan cysts is, at present, difficult to determine and available methods unreliable. Because of their high resistance to environmental factors apt to be encountered in sewage processing helminth ova have been selected by the U.S.E.P.A. as indicators of the fate of parasites during biosolids management. In a number of helminth species viability can be determined by the presence of active larvae within the ova.

The presence and viability of helminth ova can be determined by direct light microscopy. The "503" Rule describes a monitoring method that requires the ova be separated from the solids by blending with buffered water containing a surficant, screened, settled and centrifuged in a zinc sulfate gradient. This flotation method concentrates many helminth ova, *Ascaris* in particular. The zinc layer is removed and treated with an acid-alcohol/solvent extraction to remove extraneous proteinaceous material. The aqueous phase of the concentrate is collected and centrifuged. The resultant pellet is resuspended in dilute (0.1 N) sulfuric acid and incubated at 26C to allow any viable ova present to embryonate. Viable ascaris ova (*Ascaris. lumbercoides* var *suum*) are used as a time control. These are incubated in parallel at 26C and when the majority of these have embryo-nated the test sample should be ready for evaluation. (see E.P.A., 1992 for a detailed description of the method). The incubation time can take up to four weeks. Using this method Reimers *et al.* (1981) report over 80 percent recovery of ascaris eggs from "seeded" and "unseeded" sludge. The method is cumbersome but at present there seems to be no viable parasitological substitute procedure available. A major problem associated with the procedure is the long time required, up to one month, before results can be reported. This is not a laboratory problem *per se* but the delay can be a cause of anxiety for the biosolids managers.

Summary of 503 Rule Requirements for Class A* Sludge in Which Microbial Analysis Are Required

ALTERNATIVE	CONDITION	PATHOGEN LIMITS
All	Fecal Coliform or Salmonella	<1,000 MPN / gram Total Solids. < 3 mpn / 4 grams Total Solids
#2	Alkaline Treatment/ Temperature	pH >12 for at least 72 hours. Temperature > 50 C for at least 12 hours. Then air dry sludge to ≥ 50% Total Solids.
#3	Enteric Virus and Helminth Ova prior to pathogen treatment. or If Enteric Virus and/or Helminth Ova prior to pathogen treatment are ≥ 1 PFU or viable ova respectively, test after treatment.	< 1 PFU / 4 grams Total Solids for Virus and < 1 viable Helminth Ova / 4 grams Total Solids for Helminth Ova. < 1 PFU / 4 grams Total Solids for virus and < 1 viable Helminth Ova / 4 grams Total Solids, and document process operating conditions.
#4	Enteric Virus and Helminth Ova after treatment and ready to distribute, etc.	< 1 PFU / 4 grams Total Solids for virus and < 1 viable Helminth Ova / 4 grams Total Solids.

* There are six alternatives that meet Class A requirements. Three of them rely on the treatment process used.

Summary of 503 Rule Requirements for Class B* Sludge in Which Microbial Analysis Are Required

ALTERNATIVE	PATHOGEN LIMIT
#1	Fecal Coliform < 2,000,000 MPN or CFU / gram Total Solids on geometric mean of 7 samples collected over a 2 week period.

* There are 3 alternatives that meet Class B requirements. Two of them rely on the treatment process used.

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