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Increases in fecal coliform bacteria resulting from centrifugal dewatering of digested biosolids

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ABSTRACT

In many countries, the classification of biosolids for disposal purposes can be based, in part, on fecal coliform levels, with alternative criteria also available based on the stabilization process used, such as anaerobic digestion. The assumption that these alternative criteria provide equivalent protection may be flawed. This paper demonstrates that fecal coliform levels determined after digestion do not always indicate the bacterial levels after the same biosolids have been dewatered by centrifugation. In samples from mesophilic digestion, half had significant increases in coliform numbers ($P<0.05$) with up to one order of magnitude increase during centrifugation, suggesting coliform regrowth. Thermophilically digested samples had significant increases of several orders of magnitude during dewatering, more likely from reactivation of viable but non-culturable coliforms than from regrowth. In other cases, centrifugation induced coliform regrowth or reactivation upon incubation and storage of dewatered samples, but not digested samples. These 2–3 order of magnitude increases occurred with both 25 and 37 °C incubations. Coliform increases continued for up to 5 days, then gradually declined. However, by day 20 coliform numbers were still 2 orders of magnitude greater than when originally sampled. The magnitude of the increases could be due either to regrowth or reactivation, but the nature of the longer-term increases—also seen in biosolids/soil mixtures—suggests regrowth. Differences in numbers between digested and dewatered samples could not be duplicated with high shear processing in lab-scale devices, with nitrogen purging to remove volatile or gaseous constituents, or with redilution using centrate. They could not be attributed to enumeration methods, to interference of *Bacillus* spp. on apparent coliform counts, or to temperature changes. The increases have practical implications in the use of fecal coliform or alternative criteria to define pathogen content in biosolids.

1. Introduction

In the United States and many other countries, regulations for sludge stabilization include specific criteria for pathogen reduction. If these and other criteria are met, the sludge qualifies as a certain class of biosolids and may be land applied in accordance with this classification. Certain types of restricted land application require a Class B designation, for which the pathogen requirements can be met by any of three alternatives.

One of these alternatives is by monitoring of indicator organisms. Specifically, the fecal coliform density must be less than 2 million per gram of total solids "in the sewage sludge that is used or disposed" (US CFR, 2002). The density

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may be based on either most probable number or colony-forming units. There is no requirement for monitoring of possible regrowth or activation of pathogens or indicator organisms.

More commonly, treatment facilities opt for the alternative of employing a process to significantly reduce pathogens (PSRP). Anaerobic digestion is the most commonly used of the 5 allowed PSRPs, and it qualifies treated biosolids as Class B if the mean cell residence time exceeds 15 days in the mesophilic or thermophilic range 35–55 °C. This means of achieving Class B is often termed as meeting the “time and temperature” requirements.

If the “time and temperature” and the “indicator organism” options are both proper criteria for pathogen reduction, then the attainment of either should mean that the pathogen level has been reduced by approximately the same degree. In other words, it should be a safe assumption that biosolids produced under the “time and temperature” requirement will generally meet the “indicator organism” limits as well.

That the former requirement actually characterizes biosolids directly after digestion, rather than in “the sewage sludge that is used or disposed” would seem to be an unimportant distinction. The few processes that might be used between digestion and use or disposal—such as conditioning and dewatering—would not appear likely to alter organism densities, at least if they are normalized to the solids concentrations.

However, some recent reports have suggested that fecal coliform densities actually increase during or after the centrifugal dewatering of digested biosolids (Iranpour et al., 2002, 2003; Erdal et al., 2003; Hendrickson et al., 2004; Qi et al., 2004; Cooper et al., 2005). This phenomenon, though counter-intuitive, has serious implications for validity of the “time and temperature” criterion for defining Class B biosolids.

Thus, one objective of this research was to determine whether the reported increases in fecal coliform counts are either common or statistically significant. We looked for 2 types of fecal coliform increase: first, significant increases *during* the centrifugation process; and second, fecal coliform increases *subsequent* to dewatering and attributable to this preceding treatment. We defined “significant” in this research as showing a difference at the 95% confidence level or greater.

The second objective was to consider possible hypotheses to explain observed increases in fecal coliform numbers, since both of the above phenomena were ultimately confirmed. Factors that were considered as plausible are discussed below.

1.1. *Hypothesized causes of fecal coliform increases*

Effects of enumeration procedure: We asked first if there might be artifacts in the enumeration procedures. One possibility is a reported discrepancy between the LTB/EC (Standard Methods 9221E.1) and A-1 (Standard Methods 9221E.2) methods for fecal coliform enumeration (Baker et al., 2004). Comparisons indicated that the 2-step heating procedure in the A-1 method fosters the germination of some Gram-positive, aerobic, spore-forming rods (presumably *Bacillus*), leading to false positive results. It was hypothesized that centrifugal dewatering processes might also lead to localized biosolids temperature increases due to the energy-intensive processing that occurs, potentially leading to the same type of false positives.

Another potential artifact could simply be the effect of using a solid-like sample for enumeration. Although the relative bacterial numbers are determined after liquid dilution in any case, it is conceivable that the initial form of the sample could lead to changes in enumeration results. Without hypothesizing a mechanism for this effect, it was nonetheless evaluated.

Effect of shearing: Even though a flocculant polymer is generally added to biosolids prior to dewatering, it is conceivable that some of the aggregated biosolids may be redispersed in the high shear environment of dewatering. When diluted and cultured, the same initial mass of indicator organisms might then provide greater numbers of discrete cells, leading to greater numbers of positive MPN tubes and an apparent increase of bacteria population. One previous study has investigated the analogous possible effect of mixing or blending on coliform counts; Olivieri and Serai, 1988 treated raw wastewater, primary solids, trickling filter humus, or waste activated sludge with a conventional blender or a pilot scale impeller type mixer. No statistically significant increase of total coliforms population was reported after the blending or mixing. Although it is likely that the same finding would extend to digested biosolids, this type of sample was not included in the previous work. Thus, possible effects of intensive shearing on fecal coliform enumeration bears further examination as a possible explanation of effects during centrifugation.

Effects of time and temperature: If MPN increases are observed directly following dewatering, the nature of this phenomenon might be temporary, with no continuance in growth thereafter. However, the observed effects might be ongoing, with the MPN increases continuing over longer time periods. The distinction may suggest a cause for the initial differences in MPN values following dewatering. Of course, the implications regarding subsequent fecal coliform growth or survival are of practical importance, due to possible concerns regarding fecal coliform counts during storage and various disposal or reuse practices.

Chemical factors: Some of the chemicals produced during digestion are toxic or inhibitory to certain bacteria. It has been found that a high volatile fatty acid concentration in anaerobic digesters significantly reduces the pathogen population (Kunte et al., 1997). It is conceivable that the bacterial population would recover if these toxicants and inhibitors were removed as a result of the centrifugal dewatering process. Another possibility is chemical effects of the flocculant polymer used to condition the biosolids prior to dewatering; these polymers are partially biodegradable and thus may provide nutrients or other chemical factors with impacts on bacterial growth (Chang et al., 2001).

2. Procedures

Biosolids samples from five wastewater treatment facilities were sampled. The overall experimental protocol is shown in Fig. 1. Samples collected before dewatering (denoted as digested biosolids) were compared with samples taken after