Improving the assessment of risk from pathogens in biosolids: fecal coliform regrowth, survival, enumeration, and assessment
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ABSTRACT
Reactivation or regrowth of fecal coliform bacteria in biosolids has recently become a concern due to knowledge that Class B materials may fail to meet this criterion after storage or even after land application. In this paper, data show the two types of fecal coliform increases that have been characterized: immediate reappearance of large concentrations directly after dewatering; and the rapid, but less immediate, increases that follow dewatering with some biosolids after dewatering. The latter phenomenon is shown to extend over a time period of days prior to gradual decrease in fecal coliform numbers. Modeling shows that anaerobic or fermentative growth cannot simulate the observed growth, but that a straightforward biokinetic model can duplicate the observed conditions if a doubling time of one hour is assumed, which is supported by literature. Thus regrowth cannot be ruled out as the underlying phenomenon

Key words | biosolids, dewatering, centrifugation, fecal coliform, reactivation, regrowth, sludge

INTRODUCTION
In many countries, the classification of biosolids for disposal purposes can be based, in part, on fecal coliform or E. coli levels. The use of either of these as a surrogate for pathogen content, and the resulting risk, is widely acknowledged as practical, but not necessarily scientific. In fact, the digestion time and temperature criteria appear to have been established as surrogates for fecal coliform levels, so they also may be without a firm scientific foundation. Thus it is important that the fecal coliform and E. coli standards be clearly understood and relatable to the acceptable risks for predetermined biosolids management practices.

An important assumption underlies the way that the fecal coliform standards have been applied to biosolids classification in the United States and elsewhere: although the regulation states that the fecal coliform density must be less than two million per gram of total solids “in the sewage sludge that is used or disposed,” this density is commonly determined after digestion, rather than as “used or disposed.” This assumption might have been assumed to be conservative, because the process of dewatering might be expected to decrease bacterial numbers simply due to its physical intensity.

Recent work has shown this assumption to be seriously flawed: in fact, fecal coliform densities often increase due to the centrifugal dewatering of digested biosolids (Iranpour et al. 2002; Iranpour et al. 2003; Cooper et al. 2005; Erdal et al. 2003; Hendrickson & Denard 2004; Qi et al. 2004; Cooper et al. 2005; Qi et al. 2007). These increases may be of two types:

1) Increases that occur during the dewatering process.
These are exemplified by Figure 1, showing fecal coliform counts before and after centrifugal dewatering of biosolids from a TPAD (thermophilic phased anaerobic digestion) process (all results shown here were obtained using the sampling and enumeration procedures described in the Methods section). In the U.S., the Class B limit for fecal coliform numbers is 2 million per g solids, or 6.3 log units, so these data show that the biosolids would meet this limit.
change in fecal coliform numbers for the four samplings at this site (at the 95% confidence level). The tendency for thermophilic digestion to engender particularly dramatic increases in fecal coliform has been noted by Higgins et al. (2007), although this pattern is not inviolable.

2) Increases that occur after the dewatering process. Figure 3 exemplifies this phenomenon. The same samples characterized in Figure 2, if allowed to incubate at 25°C for 24 hours, show a significant difference in fecal coliform levels, depending on whether the samples were dewatered by centrifuge or not. In all four cases, the fecal coliform levels after dewatering and 24 hours at 25°C clearly exceed levels that would allow classification as Class B biosolids, even though the levels immediately after dewatering would qualify for this category.

Hypotheses regarding mechanisms for this regrowth have been addressed in more detail elsewhere (Qi et al., 2007). Figure 4 shows that the effect is not due to any direct effect of solids concentration, since redilution still results in the same fecal coliform result on a solids basis. That the centrate also exhibits significant increases in fecal coliform shows that this sidestream must be dealt with appropriately, and also contradicts the hypothesis that centrifugation removed inhibitory substances from the solids and concentrates them in the centrate.

The underlying reasons for reactivation or regrowth as shown in Figures 1 and 3 have not been clearly identified. Though it appears that centrifugal dewatering is associated with the phenomenon, the potential for increased pathogen numbers must be regarded as a property of the digested...
biosolids themselves. In other words, low fecal coliform levels after digestion do not establish that pathogens are irreversibly inactivated, regardless of the dewatering process to be utilized (Qi et al. 2007).

It is therefore important to determine why fecal coliform numbers increase and what increase in risk may be associated with this phenomenon. The U.S. EPA established pathogen levels, and indicator organisms levels, on the basis of “operational standards” rather than risk-based calculations (U.S. EPA 1995), so it is difficult to determine impacts on risk that may accrue from higher fecal coliform values when the biosolids are reused, particularly in land application. Although some pathogenic organisms will be measured as fecal coliforms, most are nonpathogenic so that an increase in the indicator organism count may not be correlated to a broad increase in bacterial pathogens.

In this paper, fecal coliform enumerations conducted over time spans of up to one month are presented. These results provide additional clues as to the nature of the observed increases, and an indication of the impact on risk. The intent is to initiate a rational assessment of regrowth/reactivation phenomena and their possible impact on reuse practices.

METHODS

Samples were taken following EPA Method 1680 (US. EPA 2002). Anaerobically digested biosolids, flocculant polymer, dewatered biosolids and supernatants were collected from selected wastewater treatment plants (WWTPs). Names of WWTPs are not included here, as requested by some participants. Consistent with U.S. EPA Method 1680, samples were transported to the laboratory on ice and maintained at 4°C until enumerations, which were conducted within 24 hr of sampling. Some experiments, as indicated, included lab incubation of samples for specified times and temperatures, with enumerations directly thereafter.

All samples were homogenized prior to enumeration. Liquid samples such as centrate and polymer were vortexed. Digested biosolids samples were homogenized by blending at high speed (15 K rpm) for 3 minutes in a conventional kitchen blender. Dewatered biosolids samples were also blended by this method, but diluted to a liquid consistency in the same step by combining 15 g of the dewatered biosolids with 135 mL of distilled water. Blending was conducted in a 4°C room to prevent any temperature increase during the procedure. Samples were then diluted with the dilution water to the desired concentration. Total solids and temperature of samples were measured following Standard Methods for the Examination of Water and Wastewater (1998).

For fecal coliform enumeration, four consecutive dilutions of each sample were tested with the five-tube MPN method for fecal coliform enumeration. A 1 mL volume of each desired dilution was inoculated into 10 mL LTB or A-1 media. Incubation and enumeration were conducted following EPA Method 1680. Further details are given in Qi et al. (2007).

RESULTS

Figures 5 and 6 show fecal coliform enumerations over time spans of up to one month, in digested, dewatered biosolids. Fecal coliform increases of 3–4 orders of magnitude are demonstrated, whereas digested, but non-dewatered biosolids show only slight increases. Over the longer term, the fecal coliform levels gradually decrease.

These increases appear to be very rapid, but they are significantly slower paced than the results shown in Figure 1. For the almost immediate increases illustrated in Figure 1, it would not be possible for the fecal coliform levels to rise so rapidly; Norris & Ribbons (1970) report a
minimum generation (doubling) time for *E. coli* of 60 minutes at 27°C, so the residence time in a centrifuge (about 15 minutes) would not even allow one doubling – although the fecal coliform increase is seen to be 3 or more orders of magnitude.

In Figures 5 and 6, this is not so obvious. Therefore, an effort was made to determine whether the growth trend could be simulated using accepted biokinetic coefficient values. Standard biokinetic equations for substrate utilization and microbial growth were implemented for batch processing, starting with the observed fecal coliform count as converted to a biomass concentration. Time increments were decreased until there was no discernable difference in results, as implemented by spreadsheet. It was assumed that the fecal coliform population represented a fraction of the overall biomass, and this fraction was adjusted so that the maximum fecal coliform counts approximately coincided with the observed peak in counts.

First attempts (not shown) demonstrated that anaerobic, or fermentative growth, cannot provide increases in fecal coliform similar to those shown in Figures 5 and 6. The maximum growth rate under these conditions is too low. Consequently, biokinetic constants for aerobic conditions were assumed, on the basis that biosolids may take up some oxygen or other electron acceptors during or after dewatering. Textbook biokinetic constants for aerobic wastewater treatment were therefore used (Metcalf & Eddy 2003) as shown in Table 1, except for the maximum doubling time (and thus the maximum growth rate) were specifically adjusted within bounds of reported literature values. The conversion of fecal coliform count to mass concentration used a value of $2 \times 10^{12}$ cells/dry g based on reported properties of *E. coli* (Watson 1970).

Figure 7 shows the modeling results. The growth rate is insufficient using typical aerobic parameters, but using a doubling time of 1 hr allows the fecal coliform increase...
to be simulated. The model assumes that most growth is non-enumerated organisms, such as methanogens. The endogenous decay portion of the simulation does not match the data, but this is not surprising because the model greatly simplifies growth dynamics in a mixed culture.

CONCLUSIONS

Data show the two types of fecal coliform increases can occur: immediate reappearance of large concentrations directly after dewatering; and the rapid, but less immediate, increases that follow dewatering with some biosolids after dewatering. The latter phenomenon was shown to extend over a time period of days prior to gradual decrease in fecal coliform numbers. Modeling shows that anaerobic or fermentative growth cannot simulate the observed growth, but that a straightforward biokinetic model can duplicate the observed conditions if a doubling time of one hour is assumed, which is supported by literature. Thus regrowth cannot be ruled out as the underlying phenomenon.

REFERENCES


