



## **Reactivation and Regrowth of Fecal Coliforms in Anaerobically Digested Biosolids**

### **Technical Practice Update**

Prepared by **Biosolids Pathogens** Task Force of the **Water Environment Federation**

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# **Reactivation and Regrowth of Fecal Coliforms in Anaerobically Digested Biosolids**

## **EXECUTIVE SUMMARY**

Recently published reports indicate that some wastewater treatment plants using anaerobic digestion and certain dewatering processes have experienced increases in fecal coliform concentrations immediately after dewatering and/or conveyance. The mechanisms behind the phenomena of fecal coliform reactivation and regrowth are still being studied and researchers are trying to understand why this has been observed at some facilities but not at others using similar processes. This document presents a summary of the current body of knowledge on this issue and a preview of ongoing research. It gives an overview of related regulatory issues for biosolids stabilization and testing protocols for fecal coliforms. Recommendations for communication strategies on this issue are provided. Finally, a description of currently known methodologies to address reactivation/regrowth phenomena is presented.

## **INTRODUCTION**

Recent reports indicate that some wastewater treatment agencies using anaerobic digestion and centrifuges for dewatering have experienced increases in fecal coliform concentrations immediately after centrifugation and/or solids

conveyance. However, other municipal facilities using similar treatment and dewatering processes reportedly have not observed such increases. A Water Environment Research Foundation (WERF) report titled *Examination of Reactivation and Regrowth of Fecal Coliforms in Centrifuge Dewatered, Anaerobically Digested Sludges* (2006a) summarizes research data on this topic and provides recommendations for further research needed to more fully understand the phenomena and its causes and implications to the wastewater profession. It is the purpose of this Technical Practice Update (TPU) to provide information to the wastewater profession on this issue.

The Water Environment Federation's (WEF's) working definition for a TPU is a document that "provides a summary of the state of knowledge regarding the practical implications of an emerging issue". A TPU is developed using standard review procedures established by WEF's Technical Practice Committee. The goal of this particular effort is to provide information for the wastewater profession on the current state of knowledge regarding reactivation and potential regrowth of fecal coliforms in anaerobically digested biosolids. A TPU is not a research document, guidance document, or summary of standard practices. A TPU is simply a document that frames the current issue and the profession's body of knowledge at this point in time. It will provide information about what other practitioners are doing to address the fecal coliform reactivation/regrowth issue with currently available information. The intent of this document is to serve as a resource from which users can draw sound and accurate information on the

current state of knowledge until future standard practices regarding the issue are more fully developed. Users can draw upon this information to better understand the issue, determine how it may affect their operations, and make more informed decisions on how to proceed to manage their own biosolids programs in as responsible a manner as possible while continuing to protect the health and welfare of their employees, the public, and the environment.

## **BACKGROUND AND LITERATURE REVIEW**

This section provides background information regarding the observation of fecal coliform reactivation and/or regrowth in anaerobic digested biosolids and summarizes published literature on the subject. It should be noted that, for the purpose of this review, *reactivation* is defined as an increase in fecal coliforms or *Escherichia coli* (*E. coli*) density in the biosolids collected immediately after centrifugation or other dewatering process compared with the feed to the dewatering equipment. *Regrowth* is defined as an additional increase in fecal coliforms or *E. coli* density during further storage of biosolids over a period of hours or days. High-solids centrifuges can be defined as centrifuges producing biosolids in the range of 25 to 35% cake solids, whereas low-solids centrifuges produce biosolids in the range of 20 to 25% cake solids.

In 2001, Donald Hendrickson was one of the first to report on fecal coliform reactivation and regrowth in an internal report to a municipal utility

(Hendrickson, 2006). In August of 2001, the largest known installation of a phased temperature anaerobic digestion process at a 227 000-m<sup>3</sup>/d (60-mgd) wastewater treatment plant was placed into operation. The facility reportedly met the time and temperature requirement for Class A biosolids. Samples collected after the digestion process, which included thermophilic/anaerobic followed by mesophilic/anaerobic digestion, indicated that there was no detectable level of fecal coliform bacteria in the treated biosolids. However, subsequent testing of the biosolids following dewatering by high-solids centrifugation revealed high levels of fecal coliform bacteria, above both Class A and Class B requirements. Fecal coliform measurement was conducted using standard culturing methods.

Later in August 2001, numerous samples were collected aseptically, stored on ice, and sent to a microbiological laboratory for testing. The results of this testing are discussed in detail in Hendrickson et al. (2004), which indicated that regrowth/reactivation of fecal coliforms was occurring following high-solids centrifugation. This work showed that the increase of fecal coliform densities was not a result of contamination of the centrifuge. The increase in numbers of fecal coliforms was unlikely to have been caused by cross-contamination (samples were collected aseptically immediately downstream of the dewatering device). In a separate test, high-solids centrifuges were disinfected using a sodium hypochlorite solution before addition of digested solids. Immediately after disinfection, samples of digested biosolids were taken downstream of the centrifuge to be analyzed. Fecal reactivation was observed in all of the samples.

Also, in Hendrickson's work, a very small continuous feed of 15 ppm (v/v) hypochlorite in digested solids downstream of the high-solids centrifuge destroyed the majority of microorganisms and produced Class B biosolids. This is of considerable interest because this small dose was not expected to significantly reduce the number of fecal coliforms.

Since Hendrickson's work, several publications have also reported similar results. Erdal et al. (2003) reported that reactivation and regrowth occurred from a mesophilic digested solids after both low-solids and high-solids centrifugation. However, after belt filter press (BFP) dewatering, no reactivation occurred, and moderate regrowth occurred after one day of storage. Screw conveyance of biosolids was also found to increase the reactivation and regrowth. Erdal et al. (2003; 2004) found that a low-dose lime application to the biosolids controlled reactivation and regrowth, especially when the pH was increased to higher than 8.5. They considered low dosages in the range of 1 to 9% lime, based on dry weight bases, where lime was added before screw conveyance.

Iranpour et al. (2002; 2003) reported that reactivation and regrowth occurred after high-solids centrifugation of thermophilically digested biosolids. Before dewatering, the biosolids met Class A requirements of less than 1000 CFU/g dry solids of fecal coliforms; after dewatering, increases were measured in fecal coliform density. Iranpour et al. (2002) observed that laboratory centrifugation of the liquid solids did not result in reactivation or regrowth, suggesting that the *g* force alone is not the primary factor causing reactivation

and regrowth. Insulating postdigestion processes to maintain temperatures during dewatering and handling eliminated reactivation and regrowth (Iranpour et al., 2003).

Qi et al. (2004) reported on four mesophilic digestion processes. At one plant that uses BFPs, low-solids centrifuges, and high-solids centrifuges, reactivation did not occur after BFP dewatering, but an increase was measured after low-solids and high-solids centrifugation. At the second plant, they sampled eight days during a one-month period and, for seven of the days, an increase in fecal coliform density was measured in biosolids immediately after dewatering compared with feed to the centrifuge. For three of those days, the increase was statistically significant. Eight samples were also collected at a third plant, and none of the samples had greater fecal coliform density in the centrifuge cake compared with the feed. At the fourth plant, they reported that statistically significant increases in fecal coliform density occurred for both low-solids and high-solids centrifuges from two different mesophilically digested biosolids. In addition to this reactivation, significant regrowth also occurred. The researchers reported that the increase was not likely a result of false positives, in that the presence of *Bacillus* sp. did not interfere with enumeration results. Shearing of the liquid digested product in the laboratory, in this case using a kitchen blender, did not result in reactivation.

A recently published WERF study (2006a) evaluated reactivation and regrowth of fecal coliforms in anaerobic systems. The study sampled seven

different plants with centrifuge dewatering multiple times. The sites included three mesophilic digestion plants, two thermophilic digestion plants, and two temperature phased anaerobic digestion (TPAD) plants that use thermophilic followed by mesophilic digestion. Four of the seven plants experienced reactivation and/or regrowth following centrifugation. One of the plants that did not show reactivation/regrowth operates thermophilic reactors in series, which appeared to fully destroy the fecal coliforms and *E. coli*, with no reactivation and regrowth. The second plant that did not show reactivation and regrowth uses a TPAD process. However this plant has a high industrial flow and, therefore, has low densities of fecal coliforms and *E. coli* entering the digestion process. The third plant that did not show reactivation and regrowth uses a conventional mesophilic digestion process.

The principal investigators of the WERF project hypothesized that the fecal coliform bacteria entered a viable but nonculturable state during digestion. This meant that the bacteria would not grow on standard culturing media; therefore, standard culturing methods would underestimate the actual viable concentration of indicator organisms. However, the bacteria would still be viable and could be reactivated. Once reactivated, additional growth of these reactivated cells would contribute to added numbers of organisms measured.

To examine reactivation and the viable but nonculturable hypothesis, a series of experiments were performed in which filter-sterilized dewatering centrate obtained from a facility showing reactivation during centrifuge

dewatering was mixed with undewatered liquid digester effluent that previously had low or nondetectable fecal coliform counts. The raw sludges before and after thickening contained  $10^5$  fecal coliforms/g dry solids and, after the thermophilic digester, the density were 10 fecal coliforms/g dry solids. When the filter-sterilized centrate was added to the thermophilic digester sample, fecal coliforms increased by at least two orders of magnitude and addition of the centrate plus cationic polymer resulted in an increase of at least four orders of magnitude. Because the centrate was filter-sterilized before addition to the thermophilic digester sample rather than through fecal coliform reseeded, this rapid increase in fecal coliforms was likely caused by induction by a signaling substance present and released into the centrate during the dewatering step. The induction suggested that the mechanical dewatering step released a chemical signal that subsequently resulted in fecal coliforms becoming culturable.

Using quantitative polymerase chain reaction (PCR), the researchers found that a large population of nonculturable *E. coli* could exist in samples after digestion, and this nonculturable population was greater in thermophilic samples. After centrifugation, these nonculturable *E. coli* were “reactivated”, which made them culturable. This would explain the immediate increase in *E. coli* or fecal coliform density measured after high-solids centrifugation. Furthermore, after reactivation, the conditions in the dewatered biosolids were able to support additional growth of the bacteria, leading to an additional increase in the fecal coliforms and *E. coli* densities during storage for several days. Additional WERF-

sponsored research on this phenomenon is underway. Further details about the objectives of a second phase and an update on recent results can be found at the end of this TPU.

A study by Jolis (2006) examined eight different *liquid* Class A biosolids from pilot- and full-scale processes and reported that two of the eight samples exhibited growth during storage at 35 °C. However, storage of all samples at either 20 or 50 °C did not show regrowth. It should be noted that the samples were not dewatered and the analysis was performed on the liquid digested biosolids.

Cheung et al. (2003) examined a plant with mesophilic digestion and high-solids centrifuge dewatering and found that, after dewatering, the *E. coli* density increased by up to 2.17 log<sub>10</sub> units compared with the centrifuge feed. They also reported that several laboratory homogenization techniques did not improve the recovery of *E. coli*; although, an increase in the homogenization time of one of the homogenization devices (stomacher) from 2 to 4 minutes increased the recovery by approximately 0.5 log<sub>10</sub>.

Monteleone et al. (2004) examined five plants with mesophilic anaerobic digestion. Three of the mesophilic anaerobic digesters were followed by secondary digestion and two were preceded by pasteurization. Four of the plants used centrifuge dewatering and one used BFP dewatering. All four sites using centrifuge dewatering measured an increase in *E. coli* density in the biosolids compared with the centrifuge feed and two were statistically significant. The

increases were reported as 63, 74, 394, and 3452%, indicating that reactivation occurred. The plant with BFP dewatering had a 44% decrease in *E. coli* density in the biosolids compared with the feed. Centrifugation alone does not seem to increase *E. coli* because a 40% decrease in *E. coli* was observed when dewatering raw sludges from one of the studied plants. No regrowth measurements were made as part of this study. Similar to Iranpour et al. (2002), centrifuging the samples with a laboratory centrifuge at various *g* values did not cause reactivation.

A study by Lebuhn et al. (2005) reported that higher densities of *E. coli* were enumerated using quantitative PCR compared with standard culturing methods for a process treating cattle manure that used anaerobic mesophilic, thermophilic, and mesophilic reactors in series. Similar to the Jolis (2006) study, the Lebuhn et al. (2005) study did not include dewatered samples to determine if reactivation and/or regrowth could occur. They also attributed the difference to the possible presence of undegraded DNA or nonculturable cells that are not enumerated by culturing techniques.

In summary, the body of knowledge to date from a limited number of plants seems to indicate that some plants with a *combination of anaerobic digestion and centrifuge dewatering* may be experiencing fecal coliform reactivation and/or regrowth. For utilities that use BFPs for dewatering of anaerobically digested biosolids, fecal coliform reactivation/regrowth was not observed in the small number of plants reviewed. However, it is important to note

that the literature to date suggests that anaerobic digestion processes—whether mesophilic, thermophilic, or TPAD—rather than destroying fecal coliforms, may convert them to a viable but nonculturable state, whereby they can be reactivated by centrifuge dewatering but not BFP dewatering. Because the mechanism for reactivation is unclear, it is conceivable that the viable but nonculturable fecal coliforms in the BFP biosolids could also subsequently be reactivated at some point. Thus, low counts in the BFP cake versus elevated counts in the centrifuged cake may not be relevant. Because fecal coliforms are indicator organisms, the “reduction” of which during digestion is assumed to coincide with a reduction to acceptable levels of pathogens, it raises the question as to whether there is really an issue or simply an analytical artifact. Thus, evaluating the direct relationship between fecal coliform regrowth/reactivation and actual pathogenic activity in biosolids is needed.

Several methods for mitigation of regrowth were investigated to meet Class A and Class B biosolids regulations, including low-dose chlorination, low-dose lime addition, and biosolids storage to achieve Class B biosolids and thermophilic reactors in series and insulating postdigestion processes to attain Class A biosolids. Additional research is needed to better understand this issue and the conditions that are likely to support fecal coliform reactivation and/or regrowth and methods for mitigation of this problem. Finally, the research to date has focused only on pathogen-indicating organisms, which does not necessarily represent pathogenic activity.

## **REGULATORY FRAMEWORK FOR CLASS A AND CLASS B BIOSOLIDS**

**Standards for the Use and Disposal of Sewage Sludge.** As indicated above, reactivation and regrowth phenomena are important research areas. Additional research is needed to better understand this issue and the conditions that are likely to support reactivation or regrowth. It may help to review some of the regulations for Class A and Class B biosolids to add context to the reactivation phenomenon.

In section 405 of the Clean Water Act, the U.S. Congress set forth a comprehensive program designed to reduce potential health and environmental risks and maximize the recycling of sewage sludge. Section 405(d)(2)(A) of the Act required the first round of regulation to be based on available information on the toxicity, persistence, concentration, mobility, or potential for exposure of toxic pollutants in sewage sludge. The U.S. Environmental Protection Agency (U.S. EPA) published the Round One standards (40 CFR 503) (U.S. EPA, 1993), establishing requirements for the final use or disposal of sewage sludge when it is (1) applied to the land; (2) placed in a surface disposal site, including sewage sludge-only landfills; or (3) incinerated.

Biosolids applied to land must meet operational standards to control disease-causing organisms (i.e., pathogens) and reduce the attraction of vectors (e.g., insects, rodents, and birds) in biosolids.

The Part 503 rule categorizes biosolids as Class A or Class B, depending on the level of pathogenic or indicator organisms in the material, and describes specific processes to reduce pathogens to these levels.

Class A biosolids must meet one of the following bacteria limitations:

- (1) Fecal coliform density is less than 1000 CFU/g cake solids (dry weight).
- (2) *Salmonella* sp. bacteria density is less than 3 CFU/4 g cake solids (dry weight).

To achieve Class A certification, biosolids must undergo heating, composting, digestion, or increased pH that reduces pathogens to below detectable levels. Some treatment processes change the composition of the biosolids to a pellet or granular substance. Class A biosolids can be land applied, unlike Class B biosolids, without pathogen-related restrictions at the site.

The alternatives for achieving Class B biosolids are

- Use of a process defined by the regulations as a process to significantly reduce pathogens (PSRP), such as aerobic digestion, air drying, composting, or lime stabilization.
- Demonstrating through testing that the product meets bacterial limits based on fecal coliforms. Those limits include achieving a geometric mean

fecal coliform density of less than 2 000 000 CFU/g cake solids (dry weight) based on seven grab samples collected.

As indicated, Class B biosolids undergo less stringent treatment and contain small but compliant amounts of bacteria. Class B requirements ensure that pathogens in biosolids are reduced and include certain restrictions for crop harvesting, grazing animals, and public contact for all forms of Class B biosolids. Site use, harvesting, and grazing restrictions for Class B biosolids can be found in 40 CFR Part 503.32. During site restriction periods, this material can receive further treatment when exposed to the natural environment where the sun's UV radiation, heat, wind, and soil microbes naturally stabilize the biosolids.

The reactivation issue is important from a regulatory standpoint because Class B anaerobic digestion is designed to produce a fecal coliform level of 2 million CFU or fewer or PSRP is to be followed. If the fecal coliform level is more than 2 million CFU, then there is less certainty regarding whether pathogen reductions are also being achieved during treatment.

**Vector Attraction Reduction.** Vectors, which most likely include insects, rodents, and birds, can transmit pathogens to humans and other hosts physically through contact or biologically by playing a specific role in the life cycle of the pathogen. Reducing the attractiveness or the availability for significant contact of biosolids to vectors reduces the potential for transmitting diseases from

pathogens in biosolids.

Section 503.33 contains 12 options for demonstrating reduced vector attraction for biosolids. The following is a summary of some of the options for Class A and Class B vector attraction reduction:

- Meet 38% reduction in volatile solids content;
- Demonstrate vector attraction reduction with additional anaerobic or aerobic digestion in a bench-scale unit;
- Meet specific oxygen-uptake rate for aerobically digested biosolids;
- Use aerobic processes at higher than 40 °C for 14 days or longer;
- Add alkali under specific conditions;
- Dry biosolids with no unstabilized solids to at least 75% solids or biosolids with unstabilized solids to at least 90% solids; and
- Inject biosolids beneath the soil surface or incorporate biosolids to the soil within 6 hours of application.

### **National Research Council and National Academy of Sciences Report**

**Recommendations.** U.S. EPA commissioned the National Research Council (NRC) of the National Academy of Sciences to review the scientific basis of the regulations governing the land application of sewage sludge. The NRC searched for evidence of human health effects related to biosolids exposure and the technical methods and approaches used by U.S. EPA to establish its human-

health-based chemical and pathogen standards for biosolids. An NRC report (NAS, 2002) concluded that, although there is no documented scientific evidence that the Part 503 regulations have failed to protect public health, further scientific work is needed to reduce persistent concerns about exposure to sewage sludge.

### **The U.S. Environmental Protection Agency and the Use of Biosolids.**

According to a U.S. EPA fact sheet (2006), the WERF study on reactivation and regrowth raises the possibility that, in some site-specific situations, reactivation following anaerobic digestion and high-solids centrifuge dewatering may be occurring. U.S. EPA cannot assess how widespread this occurrence is because of the small sample size in the WERF study. The study did not identify all of the conditions that caused dewatered biosolids from some plants to have reactivation while others did not.

These are important areas of research and warrant further study. A follow-up study by WERF is looking at both how the treatment processes and bacteria test methods could be contributing to an increase in the measured level of fecal coliforms following high-solids centrifuge dewatering. U.S. EPA will continue to evaluate this phenomenon in partnership with WERF through additional WERF-sponsored research projects and other research efforts. The results of these efforts should help develop a better understanding of this phenomenon.

When warranted by adequate data, U.S. EPA may provide updated technical guidance or propose appropriate amendments to Part 503

requirements relative to pathogen-reduction technologies and pathogen bacteria or pathogen indicator monitoring techniques. The Agency continues to believe that the pathogen requirements and operational standards of Subpart D of the 40 CFR Part 503 regulations are protective of public health and continues to support biosolids management in full compliance with the regulations. U.S. EPA supports the reuse of wastewater and biosolids as viable options available to communities and asserts that those choices are local decisions subject to state and federal regulations.

## **CONSIDERATIONS FOR ADDITIONAL TESTING AND COMMUNICATION STRATEGIES**

Fecal coliform testing typically is not required if biosolids are treated in one of the five U.S. EPA-approved PSRP processes or an equivalent process designed to significantly reduce pathogens. However, some states may require pathogen testing coupled with operational controls to demonstrate compliance with state or local requirements. Agencies may wish to demonstrate additional stewardship with self-imposed standards that augment regulatory mandatory requirements. Public concerns over the presence of pathogens in biosolids may warrant additional monitoring and enhancement processes to ensure environmentally sound and sustainable biosolids-management programs. Additional monitoring is an opportunity to show continual improvement and provide quality-monitoring

programs to enhance biosolids products.

Agencies may also want to share the information from the WERF regrowth/reactivation study with their citizen constituents and other interested parties. It may take considerable effort (presentation and discussion) to share this information and what utilities and the wastewater profession are doing as a result. However, this will be worthwhile as it will inform people as to the complex issues that environmental professionals and their served communities sometimes have to address.

Communications experts in the water-quality field developed *Survival Guide: Public Communications for Water Professionals* (WEF, 2002) to help biosolids managers and others overcome challenges and seize opportunities to develop positive relationships with customers, community leaders, interest groups, the media, and other individuals and organizations that may play a role in the success of an agency's biosolids program. As water professionals, biosolids managers can help shape public perceptions about biosolids through effective communication. Biosolids managers should also refer to *Background Information Regarding WERF Study* (WEF, 2006a) and *WEF's Q&As Regarding the WERF Report* (WEF, 2006b), which were developed to specifically address the recent and preliminary WERF study.

Biosolids managers may also want to seek the assistance of their agency's public information professional or outside public relations counsel before engaging with the media or public. It is important to develop a

communications plan and message points specific to the agency's operations. A spokesperson should be trained to deliver messages and provide responses to questions that the public or media might ask.

## **FECAL COLIFORM TESTING PROTOCOLS**

Fecal coliforms are a group of bacteria that are defined by their ability to use lactose for growth when incubated at elevated temperatures. As a group, fecal coliforms are typically found in high densities within the gastrointestinal tract and fecal material of warm-blooded mammals. Consequently, these organisms are always found in municipal wastewater and process residuals and, when found in surface water, are an indicator of fecal pollution. With respect to biosolids, fecal coliforms are also used as indicators or measure of process effectiveness in reducing microbial densities.

*Escherichia coli* are members of the fecal coliform group of organisms. Moreover, it typically is the predominant coliform organism found in municipal wastewater and biosolids samples. Therefore, fecal coliform monitoring of biosolids includes measurement of *E. coli*.

Under the current federal regulations (40 CFR 503), for biosolids that are treated by anaerobic digestion with a mean cell residence time of 15 days at temperatures between 35 and 55 °C or with a mean cell residence time of 60 days at 20 °C, fecal coliform densities do not need to be monitored before land

application (Class B only). However, state or local regulations may require fecal coliform monitoring before land application. Anaerobic digestion systems that do not meet time and temperature limits under the federal regulation may demonstrate compliance by monitoring fecal coliform densities in biosolids. The minimum frequency of monitoring is based on the total amount of biosolids produced by a facility.

- Facilities that produce up to 290 metric tons annually must monitor once each year;
- Facilities which produce 290 to 1500 metric tons annually must monitor once each quarter (four times per year);
- Facilities producing 1500 to 15 000 metric tons must monitor once every 60 days (six times per year); and
- Facilities producing 15 000 or more metric tons must monitor monthly (12 times per year).

In accordance with current U.S. regulations, a monitoring event includes collection of a minimum of seven grab samples. Ideally, these samples should be collected over a two-week time frame to allow for process variability. The geometric mean of the fecal coliform densities of the samples should then be representative of process conditions. To determine if reactivation of fecal coliforms may be occurring, biosolids samples must be collected following the

digestion process and immediately before dewatering and then immediately following dewatering. Similar to compliance monitoring, multiple samples from each location (before and after dewatering) should be taken over time. These samples should be temporally related with respect to operation of the dewatering process. Storage, handling, and site conditions can affect die-off and growth of fecal coliforms. Regrowth of bacteria in treated biosolids is dependant on numerous factors, including the availability of nutrients, pH, and moisture content, among others. Therefore, a comprehensive sampling and analysis plan is needed to accurately determine regrowth and die-off of fecal coliforms and will not be addressed here.

Whenever possible, samples should be collected from process transfer points as the biosolids are moved to the dewatering process and then again as the solids are conveyed to storage following dewatering. All samples for fecal coliform analysis should be assayed within two hours of sample collection. If assay procedures will not begin within this timeframe, immediately following collection, samples should be cooled to <10 °C by immersing the sample vessels in an ice water bath for a minimum of 30 minutes before transferring them to a refrigerator (approximately 4 °C). In no case should samples be held for more than 24 hours before being assayed.

In accordance with U.S. regulations (40 CFR 503.8), biosolids must be assayed for fecal coliforms using a multiple tube fermentation method, 9221E, or a membrane filter method, 9222D, as described in the *Standard Methods for the*

*Examination of Water and Wastewater* (APHA et al., 1992). Additional guidance on preparing biosolids for these assays is provided in *Environmental Regulations and Technology: Control of Pathogens and Vector Attraction in Sewage Sludge* (U.S. EPA, 1992). Also, U.S. EPA has recently published two multiple tube fermentation methods, 1680 and 1681 (U.S. EPA, 2005a; 2005b) in accordance with *Guidelines Establishing Test Procedures for the Analysis of Pollutants* (2005). These methods are based on method 9221E of *Standard Methods* (APHA et al., 1992) and have been validated through a multilaboratory testing procedure.

Following fecal coliform analysis, geometric means and standard deviations of the samples from a given location should be calculated. The results of means obtained from samples before and after dewatering should be compared to determine if reactivation is evident. For the purpose of this document, reactivation is evident when the geometric mean of the fecal coliform density of biosolids immediately following dewatering is significantly greater than the geometric mean of the fecal coliform density of biosolids before dewatering. Standard deviations observed for each group of assays should be equivalent. If they are not, compare the log transform of each individual fecal coliform value to the geometric mean value and discard those fecal coliform values that are more than three standard deviations above or below the mean and recalculate the results. Additional sampling may be required to obtain a series of fecal coliform values from each sample location that will yield comparable standard deviations.

## **POTENTIAL FECAL COLIFORM REDUCTION TREATMENT METHODOLOGIES FOR AGENCIES DESIRING STEWARDSHIP BEYOND REGULATORY COMPLIANCE**

**Digestion Flow Hydrodynamics.** Digesters are a vital line of defense for reduction of pathogens in biosolids to acceptable levels, providing contact time at or above the temperature required for disinfection. The Part 503 regulations are clear on the point that the Class A time–temperature relationship for pathogen reduction is applicable to every molecule. The easiest method for meeting this requirement is a batch operation, but batch operations are problematic in solids-processing trains where continuous flow is the standard. The alternative is to characterize the flow hydrodynamics of the digester as a means of ensuring that contact times are adequate.

Digesters are complex vessels characterized by factors such as

- The configuration of the vessel;
- The arrangement of inlet and outlet structures for entrance of feed and removal of effluent;
- The grit deposits and scum layer, which reduce the effective volume of the vessel and alter mixing patterns; and

- The type of mixing systems, which can vary from gas to draft tube to hydraulic/pump systems.

As a result, digesters have complex flow patterns that may cause flow-through characteristics different from those assumed in design. The difference will typically result from short-circuiting, which causes a reduction in the contact time for the adequate disinfection of some fraction of the feed to the digester.

To minimize short-circuiting, measures have been developed to characterize and enhance digester flow hydrodynamics in the absence of batch processing. Tracer tests can be used to characterize the time required for a given percentage (say 5 or 10%) of the feed to pass through the vessel into the effluent. This metric can then be used to confirm the adequacy of flow hydrodynamics in the vessel.

For a short-term defense against short-circuiting, it is suggested that the preventive maintenance of the digester mixing, heating, and gas-handling systems should be up to date. Lithium tracer testing or computational fluid dynamics modeling can be conducted to benchmark the extent of short-circuiting in a digester. A capital improvement program with short- and long-term elements may be warranted to maximize digestion system performance for pathogen reduction.

**Low Lime Dosing and Other Alkaline Additives.** A practical approach to controlling fecal coliform regrowth is addition of alkaline materials (e.g., quick lime, lime kiln dust, cement kiln dust, and coal combustion products) to biosolids. Standard industry practices already recognize processes using alkaline amendments to achieve federal Class A and Class B pathogen reduction standards. Control of fecal coliform regrowth is a modification of these practices.

*Mechanisms for Fecal Coliform Inactivation.* Control of indicator organism regrowth in biosolids using alkaline amendments is understood to involve several mechanisms. Alkaline amendments increase the pH of biosolids and result in a corresponding release of un-ionized ammonia that is toxic to bacteria, viruses, and pathogenic worms. Some alkaline amendments, notably quick lime, release heat when hydrated, and the temperature rise kills microorganisms. Alkaline amendments can be applied as a dry dust and, if used in high proportions, can decrease the moisture content of dewatered biosolids to a low level (e.g., less than 40% moisture) that impedes microbial growth. The effectiveness of heat, pH, and ammonia in causing pathogen kill increases when these parameters are elevated over a period of time measured in hours and several days. But time can begin to work against pathogen control goals when the toxic characteristics imposed by pH and heat return to ambient conditions.

*Character of Alkaline Products.* Reactivity is a key parameter of alkaline amendments. A highly reactive alkaline product releases substantial heat when water is added to it, as the oxygen associated with the calcium ion reacts with water to create carbonate and the hydroxyl ion. The reactivity of an alkaline product is expressed in comparison to pure calcium oxide (CaO). Quick lime is nearly pure calcium oxide and can be procured at 95% CaO reactivity. At the other end of the spectrum, lime kiln dust may be available at no cost to an agency, but its reactivity may be less than 20% the reactivity of quick lime. Some residual ashes have substantial alkalinity; for example, ash from coal combustion may be high in alkalinity arising from use of lime to control air emissions. But other ashes may not have adequate alkalinity; coal combustion products from the western United States do not typically have high alkalinity.

The reactivity of alkaline residuals is also affected by handling practices. A material such as lime kiln dust is affected by moisture absorbed from the air; outdoor stores of dust are low in reactivity compared with freshly produced dust (which may be as high as 60% CaO reactivity). In deriving a formula for an alkaline amendment, the reactivity of the amendment at the time of delivery must be known because the target dosage of additive for controlling fecal coliform regrowth should be expressed as a percentage CaO on a CaO kilogram to dry kilogram (pound to dry pound) of biosolids basis.

Particle size varies in the alkaline amendments available for fecal coliform regrowth control. Alkaline products vary from the size of large gravel to the finest

of dusts. Agricultural lime is typically purchased as granules. The granular form is not dusty and is less expensive than pulverized lime. However, complete mixing is difficult to achieve with granules, and the granules take time to dissolve and fully react. A fine powdered lime is immediately reactive but may require energy intensive mixing equipment to fully blend the materials and affect the highest level of efficiency. Use of gravel-sized lime is possible when used in conjunction with pulverizing equipment used to resize the lime product before blending with biosolids.

Lime can also be used as a liquid slurry, also called *slaked lime*. When quick lime is added to water, the calcium forms a hydroxide salt that effectively raises pH in the biosolids when blended together. Lime slurry products are typically safer to handle than dry lime sources.

Various alkaline amendments suitable for fecal coliform regrowth mitigation may be available in a community at low or no cost. Power-generating facilities, cement plants, and lime kilns generate high-alkaline dusts that are residues of their operations. The reuse of these residual alkaline products may be regulated by U.S. state governments; therefore, contaminant characterizations should be reviewed before use with biosolids. An advantage of using ash residuals may be their low cost. Whereas a pulverized quick lime may cost up to \$100 per ton delivered, some alkaline dusts may be delivered at no charge. Again, the key factor is the CaO reactivity of the amendment being used. An additive with low reactivity will add significantly to the mass of biosolids

shipped for land application, thus increasing operational costs.

*Points of Application and Mixing Options.* Alkaline amendments for fecal coliform regrowth mitigation may be added upstream of or after dewatering. If added upstream of dewatering, the liming agent should be made into a slurry and applied as a liquid to the feed line before the dewatering device. This method ensures good mixing but is likely to result in loss of lime to the centrate and may alter the performance of the polymer used for dewatering. Additionally, ammonia release from the elevated pH levels in the biosolids feed need to be considered in the capture and treatment of resulting fugitive emissions.

Alkaline amendments may be blended into biosolids after dewatering. Careful consideration needs to be given regarding the alkaline amendment dosage required and the mixing method used. The following describes potential mixing options.

The most basic form of mixing may be the use of a front-end loader to blend liming additives with dewatered biosolids. However, a large open pad area is needed to perform such an operation and this type of operation will be hampered during precipitation events unless performed under cover. Metering of desired quantities of biosolids and lime/alkaline amendments is typically more difficult to accurately achieve with this method of mixing. Because of the relative inefficiency of thoroughly blending with a front-end loader, higher dosages of lime should be expected to obtain an equal level of fecal coliform reduction compared

with other methods of mixing.

Batch mixing in mix boxes is another means of mixing. Mix boxes are large portable or stationary devices with capacities from 9 to 23 m<sup>3</sup> (12 to 30 cu yd). Most are equipped with weigh scales to allow for accurate measurement of batches of biosolids and alkaline amendments. A front-end loader is typically used to fill the mix boxes with a known recipe of biosolids and alkaline amendments. Horizontal counter-rotating helical mixing augers then blend the materials for several minutes to obtain a homogeneous mixture. Once blended, a side-discharge door is opened and the contents are emptied out onto a stacking conveyor or holding bin to allow loading to transport vehicles for land application or to a storage area awaiting land application. These devices are amenable for use with any type of alkaline amendment in liquid or dry form. They have the advantage of being readily available from vendors and can be installed in a short period of time with minimal infrastructure investment.

Continuous feed mixing can be accomplished using enclosed screw conveyors after the dewatering device or high-energy, continuous-feed mixers. Enclosed screw conveyors work well if sufficient residence time is provided and biosolids are discharged on a continuous basis. Continuous-feed mixers include pug mills or similar devices that have counter-rotating shafts with mixing blades attached to promote folding of lime additives into the biosolids. The degree of mixing is controlled by the number of paddles, the angle of paddles, the speed of the paddles, the residence time in the mixer, and a number of other variables that

are all important in delivery of the proper amount of energy to the mixing of the materials. These systems are typically effective and achieve the greatest efficiency of blending when correctly sized. They are typically used in larger wastewater treatment plant operations and require significant engineering and capital expenditure to build.

*Dosage Calculations.* A common expression of dosage of alkaline amendment to biosolids is the equivalent percentage of reactive CaO to each dry kilogram (dry pound) of biosolids treated. The dosages of alkaline amendments for accomplishing control of fecal coliform regrowth in dewatered digested biosolids are lower than typically needed to meet post-lime-stabilization requirements for Class B biosolids or advanced alkaline stabilization requirements for Class A biosolids. Class B biosolids prepared from wastewater solids may require lime (CaO) dosages in the 10 to 20% range. The same raw solids would require more than 20% CaO to achieve the time–temperature requirement for Class A pasteurization. Experience by a number of utilities has shown that effective control of fecal coliform regrowth can be achieved with CaO dosages in the 3 to 9% range. Storage of limed biosolids used in conjunction with low level lime dosing for a period of several weeks can also be an effective means of controlling fecal coliform regrowth.

*Performance Parameters.* A key performance measure is mixing effectiveness. Thorough mixing is necessary for ensuring that the goal of pathogen control is achieved. The percentage solids in the biosolids affects the amount of “mixing energy” needed to accomplish a complete blend. The higher the solids content in the biosolids, the more energy needed. The speed of the mixer, the configuration of paddles, and the residence time in the mixer are variables that may be adjusted to accomplish necessary mixing energy goals.

An operator planning for a suitable mixing ratio of alkaline amendment and biosolids can use several field measurements to help ensure uniformity and consistency. These include making visual inspection for uniformity and taking measurements of temperature and pH in the biosolids/alkaline blend.

A good lime mix with biosolids will not have large clods that, when broken open, reveal black, unmixed centers. If lime has not thoroughly penetrated the biosolids mass, then pockets of neutral pH will exist that can reseed microorganisms and permit regrowth over time. A good lime mix will show a significant rise in temperature over a short period of time if dry lime is used.

An adequate lime dosage will show a pH rise in the mix that is in the basic range, but not so high as to cause total suspension of biological activity. For pathogen suppression, a pH in the biosolids/amendment mix of between 9.0 and 10.0 may be adequate to prevent fecal coliform regrowth. In some cases, a pH of 8.5 to 9.0 has shown effective fecal coliform reduction. Testing of fecal coliform levels and pH can be used to establish a target pH range to achieve control. A

substantially higher lime dosage will result in a higher pH in the range of 10.5 to 11. The higher the pH, the greater the release of ammonia gas, which can result in significant localized odors and worker safety concerns. This should be considered in the capture or dispersion of these ammonia gases to minimize worker exposure to high ammonia concentrations.

In those cases in which treated biosolids will be held for several days before land application, biosolids samples can be taken by the operator or land applier to demonstrate suppression of fecal coliform indicators. U.S. EPA-recommended sampling procedures should be followed.

**Storage Options for Reducing Fecal Coliforms.** The Part 503 regulations allow for three different ways to meet Class B pathogen requirements. One of the requirements, Alternative 1: Monitoring of Fecal Coliform [503.32(b)(2)], requires that seven samples of treated biosolids be collected and that the geometric mean fecal coliform density of these samples be less than 2 million CFU/g cake solids (dry weight). Some generators choose to use this method of meeting Class B pathogen requirements in lieu of process methodology.

It has been documented that isolating and storing biosolids over a length of time (that varies with ambient temperature conditions) allows fecal coliforms to achieve required density levels. Segregation is critical to ensure that the entire stockpile has come into compliance before land application. Storage parameters will vary depending on operational constraints of the generator, characteristics of

the biosolids, and ambient conditions.

A large southeast utility has used storage for two years to test for fecal coliform reduction. After analyzing research data, the utility discovered that ambient temperature strongly affected the length of storage required to reduce pathogens. The storage piles were in an enclosed, unheated building. The storage method was used for research purposes as the utility does not have enough storage space for this method to be a practical long-term solution for total biosolids production. However, during the warmer months, the storage method has been effective in reducing fecal coliforms to an approved level and allowed the less expensive option of land application for approximately 30% of the biosolids. For purposes of stockpile management and recordkeeping, the following has been shown to be effective at this utility.

- A diagram of the storage facility was developed;
- Locations of the stockpiles within the facility are numbered;
- The pile dimension (width, length, and average height) of each location has been defined;
- The locations have physical markers denoting their number for ease of operational direction.

The samples collected include the location of the stockpile, such as S-1, and the date of the last addition to the stockpile, such as 040706. This is

necessary for ongoing tracking of the stockpiles that are land applied from each particular location. During the course of the year, for example, 20 S-1 stockpiles could be land applied but only one would be S-1-040706.

Storage pile testing frequencies are based on documented trends. All tests are used to document compliance and are the geometric mean of seven samples.

Sampling frequencies are scheduled on a sliding scale basis. If the first test does not pass, another test is scheduled typically in seven-day increments. In cooler months, the stockpiles are tested beginning at 21 days from the final addition of product to the pile. If the initial result is more than 2 million CFU, the next test is scheduled for the 28th day and so on, until the result is a compliant geometric mean. In warmer months, the opposite occurs. Testing begins at 21 days but can be reduced to 7 days from final addition of product.

This case study demonstrates the effectiveness of a utility in achieving consistent fecal coliform regrowth through documented and measured storage procedures.

## **FUTURE WATER ENVIRONMENT RESEARCH FOUNDATION RESEARCH ON THIS ISSUE**

The Water Environment Research Foundation provided the following information on the status of the Phase II project, Understanding Mechanisms and Methods to

Mitigate Survival, Reactivation and Regrowth of Viable but Nonculturable Coliforms.

**Present Status.** Since the inception of the WERF Phase II Project in March 2005, the project has selected sites to sample, organized a sampling plan, and performed sampling at seven sites. In addition, the project team has performed a number of mechanistic studies to examine methods to grow viable but nonculturable bacteria as well as begun two laboratory-scale, digestion time–temperature experiments (WERF, 2006b). Another time–temperature experiment is currently ongoing. The project is approximately 80% complete and the research will be completed by December 2006, with a draft report being completed March 2007. The following sections list the accomplishments and results to date.

*Survey.* A survey of anaerobic digestion facilities has been expanded to include additional facilities with mesophilic digestion to investigate the extent of reactivation and regrowth phenomena. This survey includes a repeat analysis of few of the processes from Phase 1 to validate the reactivation and regrowth phenomena. The survey includes seven different plants, two with thermophilic anaerobic digestion and five with mesophilic anaerobic digestion. The thermophilic digestion plants include one with three reactors in series and one with parallel, single-stage reactors. Both used centrifuge dewatering. The

mesophilic plants included single- and two-stage reactors. Also, three of the mesophilic plants use BFP dewatering and two used high-solids centrifuges.

*Mesophilic Digestion Reactivation and Regrowth.* For the solids from two mesophilic digestion plants with centrifuges, modest reactivation was measured; in other words, the difference in *E. coli* density measured by real-time PCR and standard culturing methods were about one order of magnitude or less. However, the solids from these facilities showed significant regrowth during biosolids storage to surpass fecal coliform regulatory target levels. Fecal coliforms can grow rapidly and reach a peak concentration of approximately  $10^7$  to  $10^8$  organisms/g dry solids within one to three days of storage.

*Die-off during Storage.* Mesophilic or thermophilic digestion facilities that have extended storage capabilities can reduce culturable fecal coliforms with storage, often within 5 to 15 days of storage to meet fecal coliform regulatory targets. The amount of storage time varies among facilities. The regrowth appears to diminish rapidly after five days. Thus, the solids from facilities with anaerobic digestion and high-solids centrifuges appear to rapidly reactivate within 20 minutes of centrifuge solids retention time (SRT), slowly regrow over one to three days of storage, and more slowly die off thereafter. After reaching a peak concentration during biosolids storage, fecal coliform densities decrease over time and are near or below detection limits after 30 to 60 days.

*Belt Filter Press Dewatering.* Solids from facilities with mesophilic anaerobic digestion and dewatering with BFPs did not experience significant reactivation or regrowth.

*Reactor Hydraulics.* Thermophilic digesters operating in three reactors-in-series mode were able to produce solids that meet process to further reduce pathogens (PSRP) requirements with no reactivation or regrowth. This process configuration continues to hold promise to produce Class A biosolids with improved destruction after three stages in series. More work is needed to understand this phenomenon. It should be noted that, for thermophilic digestion, complete fecal coliform disinfection may be required to prevent regrowth thereafter.

*Process to Significantly Reduce Pathogens Time–Temperature.* A laboratory mesophilic digester SRT study to examine the effect of digester SRT on culturable and viable but nonculturable indicator organisms showed that *E. coli* destruction followed approximate first-order decay, and (similar to field results) approximately one order of magnitude difference between culturable and nonculturable *E. coli* was measured. A digester SRT of 20 days is sufficient to achieve PSRP requirements using both culturing and molecular methods.

*Process to Further Reduce Pathogens Time–Temperature.* Preliminary

laboratory experiments are underway to examine the effect of batch thermophilic time–temperature to meet PFRP fecal coliform requirements using culturing and molecular methods.

*Sampling.* Quality-control experiments demonstrated that storage on ice for 24 hours (to simulate overnight shipping on ice in a cooler) does not affect fecal coliforms and *E. coli* counts measured by standard culturing methods.

*Mechanistic Studies.* A number of mechanistic studies were performed to determine if amendments could be added to the standard culturing media that would enhance the recovery of viable but nonculturable organism. In addition, several washing experiments were performed to remove possible inhibitors to culturing. In these experiments, the maximum increase in recovery was as high as one order of magnitude for a thermophilic digested sample. Therefore, this approach was shown not to replicate the full-scale reactivation seen from field samples.

**Future Directions.** According to WERF, future and on-going work will include (1) sampling of additional field sites, which will ideally include acid/gas digestion, additional plants with thermophilic digestion, and additional plants with mesophilic digestion; (2) performance of laboratory-scale batch digestion tests to

further examine the role of reactor hydraulics on bacteria entering the viable but nonculturable state and their ability to be reactivated; (3) examination of several biosolids amendments such as low dose lime to control regrowth in the biosolids; and (4) examination of regrowth mechanisms.

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