Fact Sheet

Water Environment Research Foundation
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Study Finds Fecal Coliforms Appear to Reactivate in Centrifuge Dewatered Solids At Four of Seven Facilities Tested

In a recent study of anaerobically digested solids from seven wastewater treatment facilities, counts of fecal coliform bacteria increased after dewatering at four of the facilities tested. Immediately after centrifugation, fecal coliform counts increased from very low or nondetectable levels, often by as much as several orders of magnitude, at the four facilities where increases were observed. This study only looked at seven facilities and only facilities using anaerobic digestion and high-solids centrifugation for dewatering; numerous other stabilization and dewatering processes are also widely used.

The Water Environment Research Foundation (WERF) and its research partners initially undertook this study in response to reports of increases of fecal coliform counts following anaerobic digestion and dewatering at a few wastewater treatment facilities. A number of utilities (such as our utility research partner in this study) are also in the process of spending millions of dollars to upgrade or build new digestion and dewatering facilities to produce biosolids using the types of processes studied here. These processes include both mesophilic and thermophilic digestion for stabilization and high-solids centrifugation for dewatering. This research, and ongoing work to further address this issue, demonstrates the leadership of the nation's wastewater community as supported by WERF in continuing to ensure scientifically sound and environmentally safe biosolids management practices.

The study identifies some potential control methods and mitigation strategies that utilities could use to reduce coliform levels. Developing specific information on mechanisms for reactivation and regrowth, as well as control methods and operational changes, is the focus of further research which is already underway.

Why Is This Observed Increase in Coliforms an Important Issue?

Regulations developed to protect public health rely on indicator organisms such as fecal coliform bacteria to estimate the levels of pathogens in wastewater and solids. Reliably estimating the levels of pathogens in wastewater solids by testing for indicator organisms is one means of measuring compliance with these regulations. The concentration of fecal coliform bacteria in wastewater and wastewater solids also is used to indicate the effectiveness of treatment processes used to reduce levels of pathogens.

What Processes Are Used to Reduce Pathogens and Moisture Content in Biosolids?

Anaerobic digestion processes are widely used at wastewater treatment plants to stabilize sludges and reduce the number of pathogens, often in order to meet Federal and other regulatory requirements. Anaerobic digestion processes are generally classified as either mesophilic or thermophilic, depending on the temperature at which they operate. Mesophilic processes operate at 30-40°C (86-104°F), while thermophilic processes typically operate at 53-60°C (127-140°F). Temperature-phased anaerobic digestion (TPAD) combines both a mesophilic and a thermophilic process in separate stages.

Dewatering processes are often used after digestion to reduce the moisture content of the

sludge. A high-solids centrifuge is a type of dewatering device which produces lower moisture sludges by achieving greater centrifugal forces in the unit. Anaerobic digestion followed by high-solids centrifugation is one combination of treatment processes used to produce biosolids for land application.

What Was Learned from this Research?

Of the seven facilities tested during this study, researchers observed large increases in fecal coliforms at four facilities after anaerobically digested solids were processed using high-solids centrifuges. In some cases these increases were as much as several orders of magnitude. Two of these facilities used mesophilic digestion, one used thermophilic digestion, and one used TPAD. The other three facilities (one mesophilic, one thermophilic, and one TPAD) did not show an increase in fecal coliforms following high-solids centrifugation.

Researchers used two methods to estimate coliforms, including *E. coli* (a non-pathogenic coliform used to indicate the presence of pathogens). The standard culturing method (SCM), sometimes used to measure regulatory compliance, showed decreased coliform levels after digestion, followed by increases after dewatering at four facilities.

A second microbial measurement method, quantitative polymerase chain reaction (PCR) was also used. PCR is a method that measures the number of copies of DNA present, rather than counting the number of organisms that are actively growing. PCR is not currently used for regulatory purposes at wastewater facilities; however it is commonly used as a microbial detection tool in other fields, including the food and medical industries.

Measurements using PCR consistently showed less reduction in the concentration of coliform bacteria during digestion compared to decreases measured by SCMs. The differences observed by these two methods may be explained by two related hypotheses: creation of a "viable, but non-culturable" condition for indicator organisms in digestion followed by a "reactivation" of these organisms during dewatering.

Why Is This Happening?

Based on the data collected in this study, researchers determined that more coliforms may have actually been present after anaerobic digestion but were not picked up by the standard culturing method. Researchers suspect that certain configurations of anaerobic digestion processes lead to conditions which inhibit the ability of the coliforms to grow and be measured by SCMs. In other words, indicator organisms may "hibernate" and become "non-culturable." This phenomenon is referred to as "viable but non-culturable". The issue of viable but non-culturable (VBNC) bacteria was advanced in the 1980s, and gained significant interest in medicine, the food industry, and many other fields.

Researchers further theorize that after centrifuge dewatering, conditions change so that these VBNC organisms come out of "hibernation", begin to grow, and can be enumerated by SCMs. The process by which bacteria become culturable after the VBNC state is called resuscitation or reactivation. Under ideal conditions, coliforms take 20-30 minutes to double in numbers as a result of cell division (regrowth). Reactivation is suspected here since the solids residence time in the centrifuge, typically 20-30 minutes, is too short for regrowth to account for the several orders of magnitude increase in fecal coliforms observed at the four facilities.

The mechanisms that result in reactivation are currently unknown. Researchers think possible explanations for reactivation include changes in environmental conditions, removal of growth inhibitors, or shear forces during centrifugation. The completed study did not evaluate how widespread this occurrence is or the potential role of different digestion and dewatering processes. It did however show that not all facilities using anaerobic digestion with high-solids centrifugation demonstrate this phenomenon.

How Can Utilities Use the Results of this Study?

The full results are available in the study report, *Examination of Reactivation and Regrowth of Fecal Coliforms in Centrifuge Dewatered, Anaerobically Digested Sludges.* The report provides information on the following:

- Procedures and test methods that can be applied to assure measures for pathogen reduction are being achieved.
- Potential mitigation options (e.g., changes to digester hydraulics, dewatering chemical additions, or longer-term storage) that might be considered by a facility to achieve desired reductions in both pathogenic and indicator organisms.

What Are the Next Steps?

A few of public wastewater utilities are conducting additional testing to determine if there is an increase in measured fecal coliforms following dewatering. Where this is occurring, additional test procedures and operational changes are being evaluated and implemented on a case-by-case basis. WERF is planning and conducting additional research to better understand what is happening and to provide more specific information to wastewater utilities.

Follow-on studies by WERF and its research partners are already underway and include five additional wastewater facilities. These studies are addressing the following questions that will help to better define the conditions under which these increases in coliforms are likely to occur, the extent of this phenomenon, and options for wastewater treatment facilities to consider if it is observed:

- What combinations of wastewater process technologies lead to this observed increase in fecal coliforms?
- What specific process designs and operating conditions contribute to this increase in fecal coliforms?
- What are the mechanisms for reactivation?
- What conditions contribute to regrowth?

WERF will continue to report on the results from these follow-on studies; they are expected to be completed in late 2007. WERF is committed to ensuring that the science around this issue is fully addressed. Please contact WERF using the information provided below if your facility would like to participate in this research.

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