Pathogen Risk Indicators for Wastewater and Biosolids

This project set out to develop indicators to determine treatment efficacy rather than to detect the presence of fecal contamination. The team investigated the presence of pathogens and potential indicators in wastewaters and sludges from a range of climatic zones in Australia, South Africa, and the United Kingdom, noting that use of appropriate indicators to measure treatment performance is a better approach for risk management of pathogens compared with endpoint measurement of an indicator. Treatment performance involves measurement of the same indicator before and after treatment to determine treatment efficacy. The two waste matrices targeted were wastewater and biosolids.

While it is recognized that the detection and enumeration of all pathogens is not feasible, there is a need to identify and use indicators of their presence in waste matrices.

Criteria for Choosing Representative Pathogens and Indicators

The researchers conducted a detailed literature review to identify representative pathogens and candidate indicators. The four groups considered to pose the greatest health risk include: bacteria, viruses, protozoa, and helminths. The review considered potential indicators, both microbial and chemical, applicable to each group of pathogens, including less typical indicators, such as the inflammatory potential elicited from red blood cells. For pathogens and indicators, the criteria for inclusion in the study incorporated the importance to the water industry, geographic and seasonal distribution, similarity to other pathogens, presence in sufficient numbers and persistence in the relevant waste matrix, and analytical requirements. Chemical indicators were considered but ultimately, because their behavior was unlikely to mimic that of organisms in the environment, analyses were deemed too expensive and their presence was dependent on climatic and socioeconomic factors.

The chosen suite of pathogens and indicators was verified by analyzing wastewater and biosolid samples from plants treating primarily domestic wastewater in Africa, Australia, and Europe. Samples were taken from tropical through temperate climatic regions. The study excluded pathogens and indicators lacking sufficient numbers.

The removal efficiency of the resulting representative pathogen-indicator pairs were further examined in laboratory-scale activated sludge and tertiary treatment processes. Tertiary treatment processes included alum flocculation, dissolved air flotation, rapid sand filtration, flat bed ultrafiltration, and disinfection by ultraviolet light. Representative pathogen-indicator pairs in biosolids were similarly assessed using laboratory-scale processes modeled on full-scale processes, including lime amendment, air-drying, aerobic digestion, and anaerobic digestion.

BENEFITS:

- Identifies particle profiling as the most promising indicator for pathogen presence in wastewater.
- Identifies the pathogens and indicators consistently present and that are best suited in primary and secondary treated wastewaters and sludges using a range of climatic zones.
- Provides information from laboratory-scale experiments on pathogen and indicator removal for a range of tertiary treatment processes.
- Identifies limitations of currently used indicators.

RELATED PRODUCTS:

Understanding Viability of Pathogens during Disinfection (00HHE1)

Reduction of Pathogens, Indicator Bacteria, and Alternative Indicators by Wastewater Treatment and Reclamation Processes (00PUM2T)

Molecular Alternatives to Indicator and Pathogen Detection: Real-Time PCR (01HHE2a)

Assessing the Fate of Emerging Pathogens in Biosolids (01HHE3)

Biosolids Samples Processing for Analyses of Pathogens (02HHE2)

Evaluation of Bacterial Pathogen and Indicator Densities after Dewatering of Anaerobically Digested Biosolids: Phases II/III (04CTS3T)

RELATED ONGOING RESEARCH:

Determining the Relationship of Microbial Pollution and Associated Health Risks at Freshwater and Saltwater Beaches of Florida (PATH3C09)

Measuring Water Ingestion during Water Recreation (PATH5R09)

Concentration Dynamics of Fecal Indicators in Hawaiian Coastal and Inland Sand, Soil, and Water During Rainfall Events (PATH6R09)

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Wastewater

The results of the initial analysis of primary and secondary wastewater effluents found numbers representative of bacterial pathogens and indicators consistent with those reported in the literature. Log removals of vegetative bacteria by full scale-scale activated sludge processes were similar to literature values. *Giardia* and *Clostridium* spores were present in primary effluent from all sites, while *Cryptosporidium* was present at all sites except the one in the United Kingdom. Enterovirus genomes, hepatitis A, and reovirus were initially considered, but ultimately excluded from the study due to insufficient numbers detected in the primary and secondary effluents. The removals for the different pathogenic viruses and indicators were variable. No helminths were detected at any of the sites. Of the alternative indicators evaluated, inflammatory potential of red blood cells did not prove useful for measuring the effectiveness of secondary treatment processes. The most promising alternative indicator for pathogen presence in wastewater was particle profiling. Only one site had sufficient data to allow a detailed comparison of pathogen numbers with different particle size classes. There was generally no correlation between the removal of any particular size class with the removal of similarly sized pathogens, but the data suggested a direct correlation between the volume of particles and the numbers of pathogen in primary or secondary treated wastewater. The observed correlations appeared to be site-specific.

Biosolids

For biosolids, the numbers of alternative indicators (enterococci, F-specific coliphage) and pathogens (adenovirus) in this study were consistent in all samples from four different climatic zones in Australia and were in the ideal range for use as pathogen indicators. Lime stabilization was most effective in reducing pathogen numbers to below detection limits, with the highest reductions measured for bacteria and F-specific bacteriophage. Adenoviruses were the most stable and could be a valuable model microorganism for monitoring enteric virus inactivation during sludge treatment processes. Adenovirus removal was similar to that of *Salmonella* (with the exception of lime treatment, where Adenovirus proved more resistant to treatment). *Clostridium perfringens* spores were highly resistant to inactivation by all treatment processes, with the exception of lime amendment, where all spores present were completely inactivated. Further research is required to evaluate their potential as indicators for protozoan inactivation.

Final Synopsis

A key finding from this study was that few of the microbiological indicators evaluated are good predictors of pathogen treatment efficiency (removal) for full-scale treatment of wastewater and biosolids. The best bacterial indicator for coliforms was *E. coli* removal. The behavior of viruses, both indicators and pathogens, was variable depending on the treatment process, with large differences in removal efficiencies. Polyomavirus was the most promising virus indicator, behaving similar to adenovirus and being a very conservative indicator (lower removal than the pathogen) of removal for rotavirus. Sulphite reducing clostridia spores were conservative indicator of removal for *Cryptosporidium and Giardia.* In the case of biosolids, the best bacterial predictor for the removal of *Salmonella* was the indicator adenovirus.

The most important result for alternative indicators was to confirm the 2004 Chavez et al. report, proposing that measurements of particle volume for specific particle sizes are correlated with the absolute numbers of a particular pathogen. Good correlations found between the volume of particular size classes of particles and the number of bacterial or protozoan pathogens in this research suggests a relationship between the amount of fecal material and the numbers of pathogens in a wastewater sample. However, this correlation is site specific. The precise relationship between particle volume and a particular pathogen should be examined site specifically including the impact of seasonal changes.

Should particle profiling prove to be robust, then it has the potential to offer a cost effective method for predicting pathogen presence, once the site-specific relationships between particles and pathogens have been determined.

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