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EVALUATION OF CLOSTRIDIUM PERFRINGENS AS AN INDICATOR ORGANISM TO ASSESS THE EFFICIENCY OF BIOSOLIDS DISINFECTION PROCESSES

Christine Bean
University of New Hampshire

EVALUATION OF *CLOSTRIDIUM PERFRINGENS* AS AN INDICATOR ORGANISM TO ASSESS THE EFFICIENCY OF BIOSOLIDS DISINFECTION PROCESSES

Principal Investigators: Dr. Christine Bean, University of New Hampshire

Problem and Research Objectives: Domestic sewage is treated to minimize the public health risk from pathogens in biosolids applied to land nationwide. The Environmental Protection Agency (EPA) has published regulations, the Part 503 Rule (EPA Part 503 Biosolids Rule), which establishes the processes and conditions required to minimize these risks. The pathogen regulations are intended to reduce the presence of pathogens to concentrations that should not cause adverse health effects. Pathogen standards include treatment requirements, site restrictions and monitoring requirements. Pathogens of concern include bacteria, viruses, protozoa and helminths. A complete list of principal pathogens of concern in domestic sewage and sewage sludge considered in establishing the Part 503 Rule is included as Appendix A.

Two categories of biosolids have been established and include Class A, which have no detectable pathogens and Class B, which have detectable concentrations of select pathogens. A combination of treatment and site restrictions for Class B biosolids are intended to result in reduction of pathogens and indicator microorganisms to undetectable concentrations prior to public contact (Southworth 2001). Class A biosolids are treated to reduce pathogen densities below the following detection limits for these organisms: less than 3 most probable number (MPN) per 4 grams of total solids for*Salmonella* sp.; less than 1 plaque-forming unit (PFU) per 4 grams of total solids for enteric viruses; and less than 1 viable *Ascaris* ova per 4 grams of total solids for helminths.

The routine examination for pathogens is time consuming and reliable methods do not exist for many organisms likely to be present in biosolids including emerging pathogens such as *Microsporidia* and *E. coli* 0157:H7. The human pathogen *Ascaris lumbricoides* is screened for when assessing the presence of viable helminth ova in biosolids.*Ascaris* was chosen as the parasite indicator organism in the 1970's when the EPA regulations were being written since these helminth ova have a long survivability in the environment and are easy to identify due to size. The problem with using only this organism to assess parasitic risk is that it is not ubiquitously present in biosolids due to a variable geographic distribution. Other parasites like the protozoa *Cryptosporidium* and *Giardia* have been detected in products of wastewater treatment and biosolids (Bean and Brabants 2001b) and appear to be more prevalent than Ascaris ova in some biosolids. Screening is not currently required for these organisms in the Part 503 EPA regulations.

Indicator organisms are certain species of organisms believed to indicate the presence of a larger set of pathogens that may be used to monitor whether the larger set of pathogens may be present. Fecal coliforms are used as indicator organisms in the Part 503 Rule to classify Class A biosolids and therefore determine health hazards. Fecal coliforms are also used to indicate wastewater treatment efficiency and are measured to determine if bacteria have repopulated when Class A biosolids are stored before land application. We have found that fecal coliforms underestimate pathogen health hazards in experiments performed to assess the effects of lime stabilization on biosolids (Bean and Brabants-see Related Research section). The oocysts of *Cryptosporidium parvum* are more hardy that fecal coliforms and survive longer when treated with lime to a pH of 12 (Bean and Brabants Related Research).

Clostridium perfringens has been suggested as a better indicator organism to assess the efficiency of biosolids disinfection processes than screening for parasites that may or may not be present. *C. perfringens* is a spore-forming bacterium and has been suggested as a tracer for less hardy indicators and for the absence of protozoan parasites or viruses during wastewater treatment (Payment and Franco 1993). This organism is found in densities of 10 6 colony forming units (CFUs) per gram of solids in raw or untreated biosolids and has been suggested as an excellent surrogate for the eggs of*Ascaris* (Reimers et al. 1991) in systems including composting and anaerobic digestion. *C. perfringens* spores were selected for monitoring *Ascaris* ova survival in chemically processed municipal sewage sludge, because both organisms appear to exhibit similar resistance to physical and chemical agents.

The Part 503 regulations lack a timely method to monitor indirectly for the inactivation of *Ascaris* ova and *Ascaris* inactivation is used to determine whether a disinfection process produces Class A biosolids. The direct method of assessing *Ascaris* ova inactivation currently requires recovering the eggs from biosolids, placing them in culture for 3 to 4 weeks and then examining the ova microscopically for viability. The method is time-consuming, costly and ova are not present in all biosolids consistently. An inexpensive, simple technique to monitor for inactivation of helminth eggs by surrogate microbes would be beneficial. *C. perfringens* may be a good indicator organism for *Ascaris* inactivation by anaerobic digestion.