Report as of FY2006 for 2006DE73B: "Detection of Salmonella in Biosolids using PCR"

Publications

- Water Resources Research Institute Reports:
  - Smith, S., and D. Herson, 2007, Detection of Salmonella in Biosolids Using PCR, Delaware Water Resources Center, University of Delaware, Newark, Delaware, 12 pages.
- unclassified:
  - Boyd, A., ed., 2006, Delaware Water Resources Center WATER NEWS Vol. 6 Issue 2 Nine DWRC Internship Winners for 2006 2007, http://ag.udel.edu/dwrc/newsletters/Summer2006.pdf, p. 6-7.

Report Follows

Undergraduate Internship Project #4 of 9 for FY06

Samantha Smith built on her ***DWRC*** internship research of last year, in which she compared the effectiveness of a combination of techniques versus a new method proposed by the EPA to detect and count *Salmonella* in treated biosolids. Her FY06 internship project, titled *"Detection of Salmonella in Biosolids Using PCR"*, was advised by Diane Herson, ***University of Delaware (UD)*** Department of Biological Sciences, and was co-sponsored by the ***DWRC*** and ***UD Institute of Soil and Environmental Quality (ISEQ).*** Samantha worked to develop a short-term, effective procedure using PCR (polymerase chain reactions) that decreased the length of time and cost of detection of these pathogens.

Abstract

Biosolids are produced as byproducts of waste water treatment. *Salmonella* spp. are major pathogens of concern in this material. Recently, the EPA proposed a new procedure for the detection of *Salmonella* in biosolids. In the newly proposed Method 1682, after an initial enrichment in a Trypticase Soy Broth (TSB) 15 tube Most Probable Number (MPN) assay, selection of *Salmonella* spp. occurs on modified semi-solid Rappaport Vassiliadis (MSRV). This medium contains an antibiotic (novobiocin) and a dye (malachite green) to inhibit non-*Salmonella* species. Method 1682 requires several days due to the multiple cultural steps involved. The standard polymerase chain reaction (PCR) assay is a molecular assay that can be used for the detection of *Salmonella* spp. This method takes less time, but DNA from dead as well as live organisms is amplified, and false positive results may be obtained. Other concerns are that DNA amplification may be inhibited by the presence of coliforms or by inhibitors present in biosolids. Our studies first tested biosolids samples using the cultural EPA method 1682 and compared them to the PCR assays run on the same samples. The results indicated that we could not use the TSB MPN samples in PCR assays because of inhibitory substances present in biosolids. Inhibition was again observed when cells isolated from MSRV plates were used as a source of DNA in the PCR assay. We determined that this inhibition was due to the MSRV media components. We then tested several different ways of separating cells or DNA from the inhibitors present in MSRV. It was found that heating the sample taken from MSRV in a hot water bath for 1-2 minutes to melt the agar, followed by centrifugation and removal of the supernatant prior to the addition of Instagene, gave results in the PCR assay consistent with those obtained from the cultural method.