An Experimental Model of Anthrax in Drinking Water for the Development of Effective Decontamination Procedures

NIST scientists are developing technologies to approximate the way biothreat agents, such as anthrax might populate drinking water systems. Bacterial spores are inherently resistant to chemical disinfectants and other means of decontamination. The interaction of Bacillus anthracis spores (the causative agent for the disease Anthrax) *with native water system organisms, known as biofilms, provides additional protection from commonly used disinfectants. Using a unique method to sensitize the spores to disinfectants, NIST scientists have developed a method to remove spores from water system biofilms without the use of large quantities of dangerous chemicals. The new method is an important step in helping to safeguard our critical drinking water infrastructure.*

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Spores of *Bacillus anthracis* have been used as biological weapons and the continued threat of their use requires constant surveillance and research to protect human health. An act of terrorism to our public water systems can result in damage to human health and our nation's infrastructure causing severe economic cost.

The objective of this work was to elucidate the fate of *Bacillus anthracis* spores in the complex environment of a treated water system biofilms and evaluate commonly used disinfectants for decontamination in the event of a bioterror attack on a public water system. Biofilm communities predominate at water/surface interfaces common to nearly all ecosystems including drinking water systems and are known to harbor potential pathogenic bacteria.

Advanced experimental modeling and bioimaging methods are enabling NIST to gain a clear understanding of how bacterial bioterrorism agents function.

NIST ressearchers have been developing laboratory models for the growth of biofilms on plumbing materials and pipes for the past two years. PVC and copper pipe materials were used in either a continuously stirred tank reactor (CDC reactor) for controlled shear or a pipe loop system. Native water system biofilms were accumulated on pipe material surfaces with synthetic water containing humic acids as a carbon source. Once the biofilms were established, *Bacillus anthracis* Sterne or *Bacillus thuringiensis* (kurstaki and ATCC 33679, used as simulants) spores were added to the water system. Pipe surfaces were studied for biofilm accumulation and spore adhesion. Commonly used disinfectants, sodium hypochlorite and monochloramine disinfection of spores in solution and adhered to and biofilm organisms on the pipe materials were quantified using plate count methods.

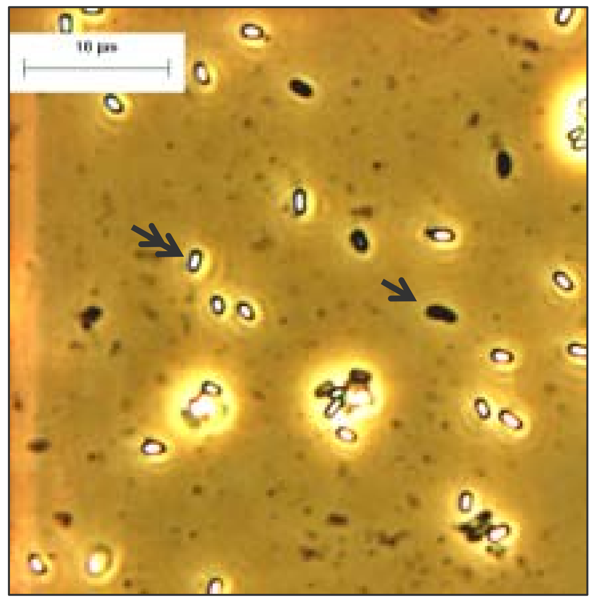

***Figure 1**. Phase microscopy image of Bacillus thuringiensis spores. Germinated (or permeable) spores appear as black rods (single arrow), ungerminated spores are phase bright (double arrow). Image captured with an Olympus BX50 Microscope, 100X magnification.*

Bacillus spores are normally highly resistant to disinfection. We have found that spores readily adhere to the biofilm surfaces on plumbing materials at high levels, and when adhered they are more difficult to disinfect with chlorine and monochloramine than in free solution. Spore retention was proportional to the biofilm accumulation ($R^2 = 0.955$). The high concentrations and long contact times required for disinfection with chlorine and monochloramine make their use impractical and even create an additional environmental hazard through the release of high concentrations of toxic disinfectants and disinfection byproducts. An alternative approach we are developing is to trigger germination of the spores, a process known to change the permeability of the spores, with millimolar concentrations of an amino acid, L-alanine and a nucleoside, inosine. Germination of biofilm associated spores resulted in the release of attached spores from the pipe surfaces subsequently significantly increasing the sensitivity of both free and attached spores to chlorine and monochloramine.

Global Impacts: Enhancing the disinfectant efficacy through germination provides a means to use greatly reduced concentrations of disinfectant and lower contact times to achieve complete decontamination of the spores from the pipe surfaces. The knowledge gained from this research will allow us to better determine the extent of a deliberate contamination event and to safely remediate a water system in the event of a bioterror attack.

Future Research: We are continuing this research to better understand the complex interactions between introduced biological threats and water system biofilms and develop measurements methods. We are extending our measurements to *Escherchia coli* O157:H7, *Francisella tularensis* (the bacteria that causes tularemia), and the toxin, ricin. Future work will focus on elucidating the long-term fate and possible persistence of biological threats in water systems using Confocal Laser Scanning Microscopy (CLSM) in addition to the methods described here.