Report for 2002WY6B: Real-time monitoring of E.Coli contamination in Wyoming

- Articles in Refereed Scientific Journals:
  - Johnson, P.E. 2002. Biodetection with flow cytometry: better, faster, cheaper, Biodetection Technologies, Knowledge Press, Brookline, Massachusetts, Volume 1: 71-83.

Report Follows:

Problem and Research Objectives:

The state of Wyoming supports 108,767 miles of rivers and streams and 325,048 acres of lakes (USEPA website). The Clean Water Act (CWA) requires states to designate uses for surface waters such as these and monitor the quality of the water in support of its use. Under section 305(b) of the CWA, Wyoming is required to include the results of these water assessments in biennial reports submitted to the United States Environmental Protection Agency (USEPA). Fecal coliform contamination is one water quality standard tested and included in these reports. Fecal coliform monitoring is an indicator of the sanitary quality of the water and can determine the extent of fecal contamination in the water from warm-blooded animals. Fecal contamination is important from a public standpoint when the surface water's designated use includes contact recreation such as beach use, boating, or swimming (USEPA report, 1986).

Wyoming's 2002 305(b) report to USEPA included a 303(d) list of the State's surface waters with water quality impairments not allowing those reaches to support their designated use(s). Reasons for impairments include metal contamination from copper, selenium, cadmium and silver, excess siltation, high phosphate and chloride levels, high pH, habitat degradation, oil deposits, and fecal contamination. Of the 45 water reaches included in the list, 22 are for fecal coliform exceedences, with all having contact recreation designated uses. This illustrates that fecal contamination of surface waters is an important issue for the State of Wyoming.

This project is developing and proving the concept of an economical system capable of real-time detection of individual *E. coli* in surface waters in Wyoming, including streams, rivers, and lakes, in order to quantify fecal coliform contamination. Our goal is a detection limit of < 5 *E. coli* cells per 100 ml of water in 15 minutes of analysis time, with a minimum detection efficiency of 50% and a false detection rate of < 1%. We are proving the concept of a low-cost, portable testing system that screens water in the field. This system will also support a more economical testing regime than any currently in use, allowing for more frequent and comprehensive water monitoring, thus minimizing human pathogen exposure, e.g. in contact recreation. This proof of concept allows for the design and fabrication of a remote monitoring system that will automatically screen water in real time. Alternative methods necessitate the shipping of bulk water samples or concentrates to laboratories and labor-intensive screening technologies, which may include bulk water concentration, incubation, and culturing. These factors combine to impede overall routine monitoring for fecal coliforms in the field and preclude widespread, routine screening of surface waters.

Based on USGS Year I funding (in partnership with the Wyoming Water Development Commission), we developed and tested a low-cost, portable, highly sensitive, self-contained single cell detection system for *E. coli* in surface waters, which will greatly exceed the current testing procedures in both speed and reliability. Project objectives are**:** 1) low-cost detection of *E. coli* in Wyoming surface waters, 2) allows rapid (<<1hour) enumeration of *E. coli* concentration, 3) sensitive, will allow for enumeration of concentrations < 5 cell/100 ml with minimal number of false positive detections, 4)

portable for field monitoring, 5) simple to use, does not require substantial training, and 6) development of proof of concept for remote monitoring.

Methodology:

The innovative technique employed in this research has the potential to detect foodborne and waterborne pathogens in real time at the level of a single bacterium in a volume of air or water through the use of specific fluorescent antibodies. The resulting fluorescence is detected with a CCD imager using a novel integration scheme called Fountain Flow (FF). Our system is called the Wyoming Biodetection System or WBS.

Our proprietary and patented (pending) WBS is based on immunofluorescence identification:

- An antibody specific to the cell species of interest is labeled with a fluorescent molecule or fluorochrome.
- The labeled antibody is mixed in solution with the cell species of interest. The antibodies attach to specific sites on the cells (antigens).
- The cells are passed in a stream of liquid toward a low-cost laser diode, which illuminates the fluorochromes causing them to fluoresce at a different wavelength.
- A low-cost CCD (charge coupled device) 2-D detector detects a burst of fluorescence emission each time a marked cell is illuminated by the laser diode while passing in front of the detector.
- The number of marked cells is then counted via computer. Antibodies can be chosen that are highly specific to the cells of interest.

In our current USGS/NSF-funded research, an operational CCD/FF apparatus was assembled and used to demonstrate the practical and economic feasibility of real time detection of *E. coli* to image and count (via computer) individual microorganisms. **The NSF-funded research focuses on detection of *E. coli* O157:H7 in ground beef, while the USGS-funded research involves detection of *E. coli* in Wyoming surface waters.** This device enabled the flow cytometry feasibility demonstrations to date and established the suitability of the FF cytometer to ***effectively detect single pathogenic microorganisms*.** Emphasis is placed on signal-to-noise enhancement. This allows for multicolor detection providing enhanced reliability using several markers. In this detection system, fluid is transported through a hole that is large enough (2-mm dia.) to prevent clogging. Our imaging technique allows automated measurement of individual cells while they transit this flow cell.

In Fountain Flow a sample of fluorescently-labeled bacteria suspended in a transparent or translucent aqueous solution flows up a tube toward imaging optics, where sample particles can be imaged onto a CCD camera and counted or measured photometrically. The imaging optics include, with a single color instrument, a filter isolating the wavelength(s) of fluorescent emission. The ideal situation occurs when a particle flowing up the flow tube is imaged in the same pixel(s).

Principal Findings and Significance:

Our US Geological Survey Year I proposal outlined the following five goals: 1) optimize fluorescent labeling of *E. coli*, 2) perform laboratory measurements on quantified *E. coli* samples to determine the detection efficiency and sensitivity of the monitoring system, 3) test methods of removing background detritus, 4) test methods of counting quantified samples of *E. coli* in a background matrix, and 5) enumerate *E. coli* in stream and lake water samples using both our proposed method and the standard method currently recommended by the USEPA. Progress toward these goals is listed below.

FLUORESCENT LABELING

The fluorescent labeling of *E. coli* was the first goal accomplished. A protocol was designed using an R-phycoerythrin (R-PE) labeled antibody to *E. coli* K12, the benign test microorganisms used in our preliminary research. The antigen/antibody reaction was optimized to ensure fluorescent emission strong enough to be detected with the system with as little waste of unattached antibody as possible. This optimization can be scaled up or down according to sample size.

E. COLI DETECTION METHOD AND EFFICIENCY OF FF

In order to determine the efficiency of the Fountain Flow (FF) technique, comparisons were made between FF and Whipple-grid counting. For FF, samples of fixed *E. coli* K12 were labeled with propidium iodide dye and flowed through the FF system at 2.2 ml/hr. A 13-mW 475-nm laser diode was used to illuminate the stained bacteria at an illumination angle of 45 degrees. The exposure time of the CCD imaging the flow was 400 ms. Multiple frames were recorded and software used to quantify the detections made. The original overnights of the *E. coli* K12 were dilution-plated and counted with a Whipple grid to obtain the total number of cells in the sample. This number was compared with the number of cells counted by our system, the Wyoming Biodetection System or WBS. Efficiencies of ~50% are obtained with cells and ~90% when comparisons are made with hemocytometer counts of microbeads, even when the microbeads are fainter than cells. This discrepancy is largely due to cell clumping, which doesn't significantly affect our Whipple-grid results, but has a large effect on FF counts. Typically, one ml of flowing liquid produces 900 images. This large amount of data necessitates counting in real time so that data archiving is not necessary. Detection efficiency is computed by comparing the counts from the WBS with the Whipple-grid counting method or by the mTEC filtration method (described in the next section).

FILTERING BACKGROUND DETRITUS

The USEPA introduced the mTEC enumeration method for *E. coli* in 1986. This method is currently employed by the USGS Water Resource Office in Cheyenne, WY and was demonstrated to us by USGS staff hydrologist Melanie Clark in a visit to our site. This membrane filter method provides a direct count of *E. coli* in water, based on the development of colonies that grow on the surface of the membrane filter. We first established that filtration through a 10-µm filter was necessary to remove any autofluorescing material from the water sample. We then used the mTEC method to test the efficiency of bacteria recovery after this initial filtration. An overnight culture of *E.*

coli K12 was diluted by a factor of 10 using Crow Creek Reservoir (Wyoming) water as the diluent. (Because it is non-pathogenic, K12 was used in the preliminary research.) Crow Creek water simulates water samples that will be collected in the field, which contain sand, other dirt particles, and a small percentage of organic material. Fifteen ml of the sample was vacuum filtered through a 10-µm filter to remove debris, and then subsequently filtered through a 0.45-µm filter to separate bacteria from the solute. The 0.45-µm filter was then placed onto the surface of an mTEC agar plate. A second 15 ml sample, the control, was filtered only through the 0.45-µm filter. This 0.45-µm filter was also placed onto an mTec agar plate. Both plates were incubated at 35.2^{o}C for two hours and then at 44.5^{o}C for 22 hours. The colonies were enumerated the next day. Tween 80 was added to some samples to attempt to increase the recovery fraction. Similarly, sonication was also applied to some samples. **Results from spiked Crow**

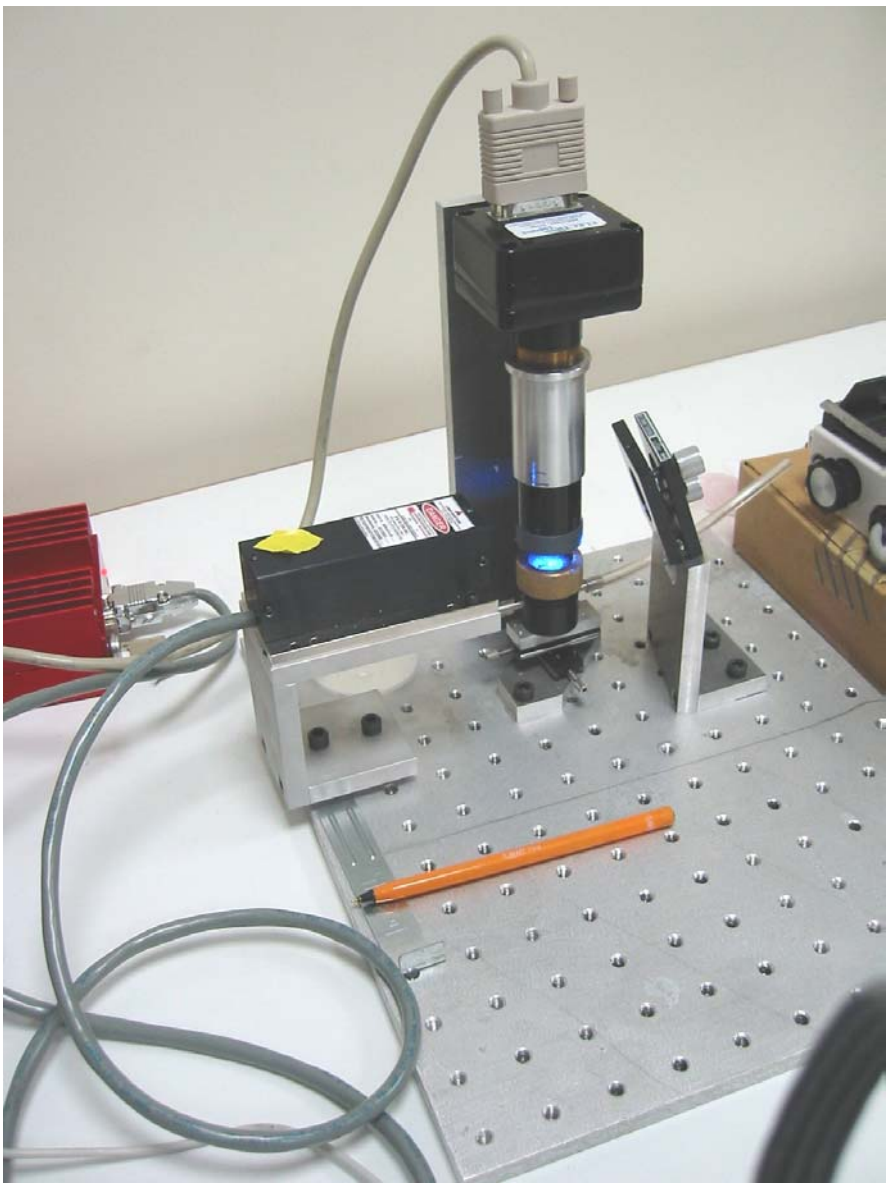

Figure 1. *The Wyoming Biodetection System with a 475 nm solid-state 13-mW laser.*

Creek Reservoir water are summarized with the following mean bacteria separation efficiencies: 1) 62% (±5%) with no surfactant, 2) 33% (±2.5%) in a 2% Tween 80 solution, and 3) 33% in 2% Tween 80 following 10 minutes of sonication.

QUANTIFYING SAMPLES OF *E. COLI* IN VARIOUS BACKGROUND MATRICES

We have demonstrated successful recovery of *E. coli* spiked Crow Creek water. However, testing of different water sources is necessary to encompass the diversity of Wyoming's waters. It is anticipated that bacteria recovery will be different in waters containing other types of soil and detritus. For this it may be necessary to employ techniques other than simple filtration to recover adequate amounts of bacteria without background interference. Alternative techniques include: addition of surfactants, recovery of bacteria using immunomagnetic beads, and centrifugation or gravity separation. Immunomagnetic bead separation, based on using fluorescently tagged magnetic beads coated with an antibody specific for the target bacteria, shows promise. The beads are added to the sample, mixed for a determined period allowing for bacteria attachment to the antibody, and recovered through their magnetic properties. The beads are then placed into a transparent aqueous solution and flowed through the biodetection system. Water samples from various locations throughout the state will be taken and analyzed for bacteria recovery, testing the previously mentioned techniques to improve efficiency. Sampling will be coordinated with USGS sampling. These additional techniques are being explored in Year II.

ENUMERATION OF *E. COLI* IN STREAM AND LAKE WATERS

The latter part of Year II research will encompass end-to-end testing (filtration and FF) of various water sources in Wyoming using the WBS. Water samples from locations throughout the state will be taken and analyzed for endogenous bacteria. Results from the system will be compared with counts obtained from the mTec method currently employed by the USGS Water Resource Office in Cheyenne, WY. Sampling will be coordinated with USGS and results compared with theirs.

SUMMARY OF GOALS FOR YEAR II RESEARCH

In preliminary testing, our team has demonstrated success in proving the concept of the WBS. Bacteria labeling, recovery of bacteria from authentic Wyoming surface water, and enumeration efficiency of the WBS have all been successfully accomplished. Year II plans include: 1) developing techniques to efficiently recover *E. coli* bacteria from surface waters throughout the state, 2) end-to-end sampling of those waters to detect endogenous *E. coli,* and 3) comparison of results to with the current USEPA techniques to test the efficiency of the WBS.

Students Supported Through This Project:

During the last year, the P-I employed two undergraduate and two graduate students, in this research. The interaction among personnel of varying backgrounds (including microbiology, pharmacy, and physics) has provided a highly educational experience for everyone in research biodetection technology.