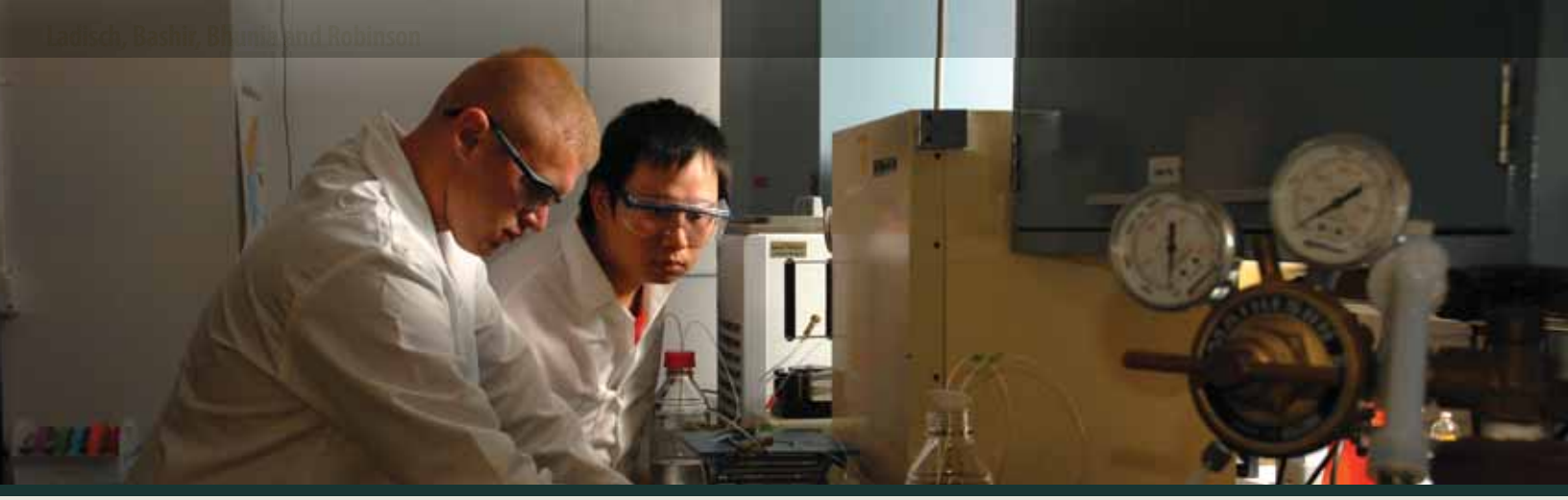

Engineering of biosystems for the detection of *Listeria monocytogenes* in foods

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Project Rationale

Current methods for detecting *L. monocytogenes* rely upon enrichment procedures to increase bacterial numbers for detection. The food or food extract is incubated in a special growth medium for 12 to 24 hours, and the resulting culture is tested for *L. monocytogenes* using procedures that require an additional 3 to 24 hours. An overall time of 2 to 3 days is typical of the time that elapses between when the food is sampled and when the test results are available. The elapsed time, referred to as time to result or TTR, is problematic since some perishable, ready-to-eat foods are consumed before test results would be available. Rapid and affordable technologies to detect low numbers of *L. monocytogenes* cells directly from food and to distinguish living from dead cells are needed.

This multi-disciplinary, multi-departmental research project is addressing the fundamental engineering and science required for developing a microchip that is capable of rapidly detecting *L. monocytogenes* at the point of use. Our primary goals are to establish microscale detection of *L. monocytogenes* on a real-time basis with a time to result of 4 hours and to reduce the time of bacterial culture steps with rapid cell concentration and recovery based on membrane technology.

Our multidisciplinary research team is addressing the development, engineering and validation of the microchip system that combines bioseparation and bionanotechnology. Bioseparations technology will allow rapid concentration and recovery of microbial cells. Bionanotechnology enables the construction of systems capable of interrogating fluids for pathogens. The combination of the two technologies facilitates devices for rapid processing and detection of food pathogens.

Project Objectives

- Develop a system for rapid cell concentration and recovery. Improve membrane chemistry and methodology for handling complex food samples presented by blended hotdog, hamburger, vegetables, milk, and meat and decrease the volume in which the cells are captured by selecting or constructing the appropriate membrane design and combining with other bioseparation techniques.
- Correlate media composition to changes in growth characteristics and metabolism of *L. monocytogenes* cells (recovery of stressed cells, capture from mixed cultures, increase sensitivity through decreasing media conductance) and develop media that enhance pathogenic cell response to detection methods. We also are interested in developing low conductivity media for enhanced capture and detection of stressed cells by antibodies and other bioreceptors.
- Combine antibody-based capture and growth detection for the biochip. Integrated devices have been designed and microfabricated to combine ATP, pH, and/or direct nucleotide and antibody-based detection on-chip using multi-channel, multi-functional designs. The intent is to obtain biochips that sense multiple parameters simultaneously and improve DEP (di-electrophoresis)-based selective capture of *L. monocytogenes* and other pathogens in mixtures of cells. Then we will test for sensitivity of detection and selectivity of capture.

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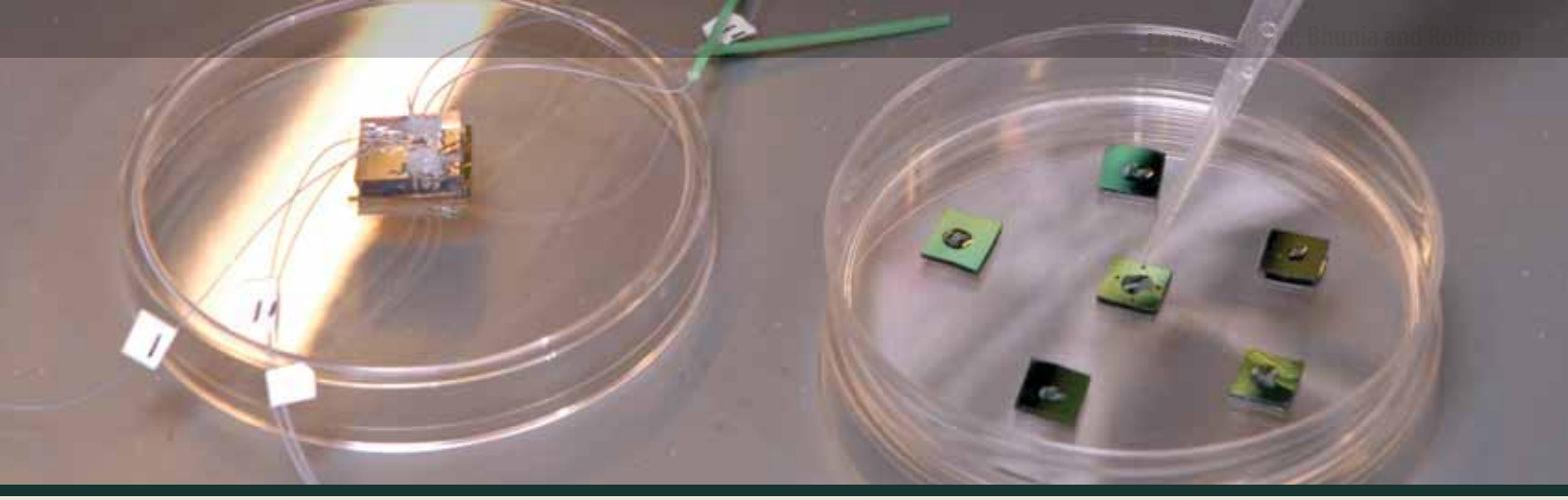

Project Highlights

The concentration and recovery of microbial cells for analysis requires rapid recovery of viable cells while minimizing retention or loss of cells during processing. This work further extended the methods for achieving rapid concentration of homogenized or stomached food samples. We showed the utility of using a small hollow fiber module with an internal volume of 23 or 101 µL. The module is operated in a dead-end mode for the rapid concentration and recovery of various types of microbial cells in a small sample volume that is compatible with micro-scale detection systems. Tests were carried out with *Bacillus thuringiensis* DUP-6040, *Escherichia coli* K12, *Listeria innocua* FY248, *Pseudomonas fluorescens* ATCC13525, and *Streptococcus faecalis* CG110 in PBS at initial concentrations of 100 cfu/mL as well as *E. coli* or *L. innocua* in washing solution from hotdogs (i.e., massaged hotdog solution). After treating massaged hotdogs with protease and lipase, up to 53% recovery of viable cells at a concentration of 10^5 to 10^6 cfu/mL in a 100 µL sample was achieved when *E. coli* and *L. innocua* cells were concentrated from a 250 mL massaged hotdog solution containing an initial microbe concentration of 600 to 900 cfu/mL. The design and fabrication of hollow fiber modules and membrane/microbe interactions during cell concentration and recovery was developed to achieve reproducible devices and rapid concentration of the cells.

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