

Cell-based sensors for screening toxins

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Major Accomplishments: Contamination of the bioreactor, the heart of the wastewater treatment plant, from chemical dumping can have a devastating impact on wastewater processing. In fact, system shut-down caused by bioreactor failure often results in the diversion of large amounts of raw untreated sewage to rivers and streams with an obviously detrimental effect. Several different classes of chemicals, including electrophilic toxins, feeding into water treatment plants have been known to cause bioreactor degradation leading to system processing failures. Recent results from Love's group have led to the hypothesis that sludge deflocculation or biofilm detachment, a mechanism by which the bioreactor can fail, occurs through the activation of the glutathione-gated K^+ efflux (GGKE) system stimulated by electrophilic toxins. Therefore, prior to biofilm detachment, bacterial cells expel large amounts of potassium from inside of the cell to the outside. In this collaborative effort, we have designed a cell-based microfluidic biosensor to detect the presence of electrophilic toxins in water streams entering wastewater treatment plants in an effort to prevent failure of the bioreactor. The approach is to immobilize bacterial cells in a microfluidic system and monitor cell response, in particular potassium efflux, as the water flows through a small bed of immobilized cells. In essence, we have created a micro-bioreactor. The behavior of our micro-bioreactor is then used to predict the behavior of the process bioreactor when exposed to the same water supply. A negative response in the microsystem can be used to decide when to divert part of the incoming stream to prevent process failure. The microbioreactor has several key components including an immobilized bed of live bacterial cells (*Escherichia coli* K-12); an optode film that is responsive to potassium ions; an optical detection unit; and microfluidic channels to move cell media and the water samples through the cells and past the optode film. This year, we have focused our attention on two aspects of this project: (1) cell immobilization in polymer microchannels and (2) development of a miniaturized optical detection component.

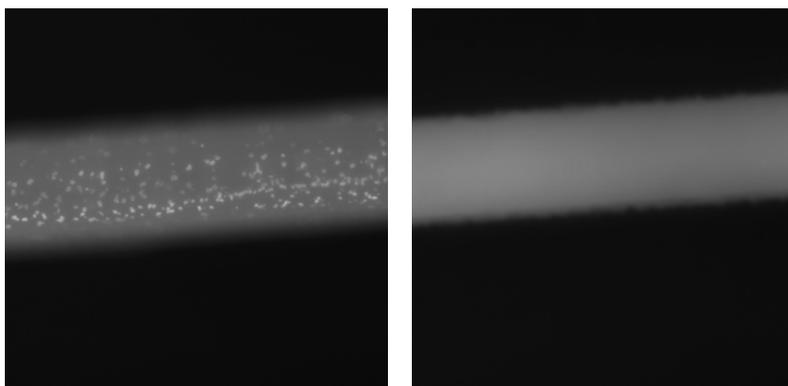


Figure 1. Fluorescence image of PETG microchannels after exposure to *E. coli* cells. Fluorescent dots indicate the presence of live cells. A) Uncoated PETG channel, and B) channel coated with polystyrene sulfonate.

Love's group hypothesized that cell adhesion to an abiotic polymeric surface would be influenced by several factors including the chemical characteristics of the bacterial membranes; the physiological state of the cells at the time of adhesion; and the characteristics of the polymer material used to fabricate microfluidic

channels. Earlier work¹ was performed to determine the characteristics of the bacterial membrane, specifically the relative hydrophobicity of *E. coli* K-12 cells in different growth media, that could affect cell adhesion. Experiments showed that cells grown in an N-limited medium (20:1 C:N) exhibited greater cell surface hydrophobicity than a balanced C:N media supporting previous findings². Based on these results, polymers were chosen that were expected to enhance cell adhesion and these were tested in cell binding experiments. *E. coli* cells were also immobilized onto polymer surfaces coated with polyelectrolyte multilayers. It was determined that the cells adhered better to uncoated polyethylene terephthalate (PETG) surfaces than to PETG surfaces coated with polyelectrolyte (shown in Figure 1).

The detection system was also miniaturized this year to make the device more suitable as a prototype field device. A microfluidic device was mounted on a small holder and a miniaturized light source was coupled to the channel using fiber optics. Light was collected through fiber optics into a hand-held spectrometer (Ocean Optics) coupled to a laptop computer for data processing. This device is now being evaluated to measure the optode film response in the presence of varying concentrations of potassium.

¹ van Loosdrecht, Mark, C.M, Lyklema, Johannes, Norde, Willem, Schraa, Gosse and Zehnder, Alexander J.B *Applied and Environmental Microbiology*, **53** (8) 1898-1901, **1987**.

² Cowell, B.A., Willcox, M.D.P., Herbert, B., and Schneider, R.P. *Journal of Applied Microbiology*. **86** 944-954, **1999**.