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Session 2B (Biology)

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MICROSCALE ELECTROKINETICS FOR PHYTOPLANKTON ANALYSIS

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There are many cases where the analysis of individual cells within a population gives insight into environmental conditions (ex: phytoplankton) or human health (ex: red blood cells). Many techniques have been developed through the advancement of microfluidic devices. Microscale electrokinetic techniques, which have been under continuous development under the past few decades for lab-on-a-chip systems, can be used for sample enrichment, selective particle sorting, and/or sample analysis. These techniques generate relatively large electric fields (~10^6 V/m) using submillimeter electrodes connected to a benchtop waveform generator.

This presentation will begin with an introduction to dielectrophoresis, a popular microscale electrokinetic technique that uses non-uniform electric fields for microparticle manipulation. Next, the current status and recent work of a collaborative research project, recently supported by NSF Instrument Development for Biological Research, will be highlighted. This microscale electrokinetic platform operates using a unique electrode geometry which generates a constant dielectrophoretic force thereby applying a constant force (and thereby constant velocity) to all exposed particles. By tracking the trajectory and speed of particle motion microscopically, the dielectric spectra of individual particles can be acquired; such data will ultimately be able to differentiate subpopulations of cells. To date, proof-of-concept tests have been performed on polystyrene particles. We will expand tests to subpopulations of freshwater phytoplankton. High throughput of algal cells allows extraction of cellular dielectric properties and will provide insight into cellular morphology and physiological condition. Our objective is to expand the use of microscale electrokinetic techniques to the cells of several freshwater algae taxa as a potential diagnostic of the health status of aquatic ecosystems.

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SELENIUM REDUCTION BY A CO-CULTURE OF *PANTOEA VAGANS* STRAIN EWB32213-2 AND *SHIGELLA FERGUSONII* STRAIN TB42616

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Background

Selenium occurs naturally in environment, distributed widely but unevenly in the earth crust. Though selenium concentration in ambient water is commonly less than 1μg/L, a high concentration ranging from 10 to 100 μg/L may be detected and sometimes even exceeding 1000 μg/L (Lemly 2004). Selenium toxicity can be caused at high-level exposure. Selenium should be removed from the environment due to its toxicity at high-level exposure, especially from waters considering its significant damage to fish or other aquatic life (Eisler 1985). Microbial selenium reduction has been studied for many years. In 1992, a *Pseudomonas stutzeri* isolate was reported capable of both Se(VI) (selenate) and Se(IV) (selenite) reduction to Se(0) (elemental selenium) (Lortie, Gould, Rajan, McGready, & Cheng, 1992). In 1996, two strains, i.e. *Pseudomonas fluorescens* and *Bacillus subtili*, were found able to reduce Se(IV) to Se(0) (Garbisu, Ishii, Leighton, & Buchanan, 1996).

In our previous study (Ji and Wang 2016), Se(IV) generated from Se(VI) reduction using an *E. Coli* strain was observed to accumulate in the reactor because of the significantly faster rate of Se(VI) reduction than Se(IV) reduction. In this study, a Se(IV)-reducing strain, *Pantoea vagans* EWB32213-2, was co-cultured with *Shigella fergusonii* TB42616 in a batch reactor to enhance Se(IV) reduction rate. Wide pH and temperature ranges were also investigated in order to determine the optimal condition for selenium reduction in the co-culture.

Materials and methods

Bacterial Strains: The *Pantoea vagans* strain EWB32213-2 and the *Shigella fergusonii* strain TB42616 were isolated from a coal slurry pond at E.W. Brown Generating Station in Harrodsburg, Kentucky, and the aeration basin in Town Branch Wastewater Treatment Plant in Lexington, Kentucky, respectively. They were subsequently identified by 16S rRNA sequencing. Both strains were preserved on agar plates at 4°C and then grown at 30°C with constant agitation in Erlenmeyer flasks with nutrient broth for 16 hours. Cells were harvested by centrifugation and washed three times.

Analytical method: Se(IV) and Se(VI) were measured according to the colorimetric method (Rice et al. 2012). The cell density was measured by a cell counter, and the pH was measured with a pH meter.

Results and Discussion

No significant selenium reduction was observed in the control experiments, indicating that no chemical reaction occurred between the inactive cells of both strains and the medium.

The evaluation of pH effect on selenium reduction of both strains was performed at 30°C after flushed with nitrogen supplemented with 5 g/L glucose in the Erlenmeyer flasks. As shown in Table 1, the optimal pH for *Pantoea vagans* strain EWB32213-2 of Se(IV) reduction was at pH 7 with a highest reduction rate of 90.05% from Se(IV) to Se(0) after a 4-day incubation. As for *Shigella fergusonii* strain TB42616, the optimal pH was determined as pH 8 with an approximate selenium reduction rate 79% from Se(VI) to Se(0) after 4 days. The temperature effect on selenium reduction is shown in Table 2. An optimal temperature of 30°C was also observed according to the highest selenium reduction of 91.91% for *Pantoea vagans* strain EWB32213-2. While, the selenium reduction peaked for *Shigella fergusonii* strain TB42616 at 40°C with a rate of 92.3%.

Future study will focus on batch experiments of selenium reduction using a co-culture of *Pantoea vagans* strain TB42616 and the *Shigella fergusonii* strain EWB32213-2. The factors including bacterial composition and Se(VI) and Se(IV) concentrations will be investigated.

Table 1. The selenium reduction rate of *Pantoea vagans* EWB32213-2 strain and *Shigella fergusonii* strain TB42616 at different pHs after a 4-day incubation.

Strain	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9
Pantoea vagans	13.27%	20.93%	54.08%	90.05%	87.17%	86.24%
Shigella fergusonii	0.11%	2.28%	1.67%	30.95%	78.72%	57.76%

Table 2. The selenium reduction rate of *Pantoea vagans* EWB32213-2 strain and *Shigella fergusonii* strain TB42616 at different temperatures after a 4-day incubation.

Strain	20℃	30℃	40℃	50℃
Pantoea vagans	55.85%	91.91%	77.41%	9.29%
Shigella fergusonii	33.51%	66.95%	92.30%	21.45%

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EFFECTS OF TEMPERATURE AND MACRONUTRIENT RATIOS (N:P) ON *Microcystis aeruginosa* TOXIN PRODUCTION AND GROWTH

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Microcystis aeruginosa is a potentially toxic cyanobacteria, capable of harming and killing both aquatic and terrestrial fauna. Toxic algal blooms have become prevalent in Kentucky due to nutrient loading and global climate change. Current literature regarding Microcystin production and its correlation with total nitrogen to total phosphorus ratios (TN:TP) is conflicting. We investigated what macronutrient ratios were more conducive to algal growth and potential toxin production. We analyzed toxin production and *M. aeruginosa* growth in environmental chambers. *M. aeruginosa* was grown in TN:TP ratios of 2.3, 5, 10, and 20 in temperatures of 20, 22, and 24°C. Axenic cultures were maintained at ~30 µmols photons m^{-2} s^{-1} in a 16/8 hour light/dark cycle. An Abraxis Adda Elisa was used to assess [Microcystin] *in vivo* after 14 days of growth. Algae growth was assessed using both cell counts and [chlorophyll *a*]. Greatest algal growth was observed at 24°C with TN:TP = 20; while lowest growth occurred at 20°C with TN:TP = 2.3. No significant correlation existed between toxin production and TN:TP ratios, regardless of temperature. All conditions resulted in [Microcystin] > 1.64 µg L^{-1} (measured as Microcystin-LR). Proper watershed management practices should be implemented to lower nutrient concentrations, especially in waterways used as, or draining into, public drinking water supplies.

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