Sunlight Inactivation of Bacteria in Open-Water Unit Process Treatment Wetlands: Modeling Inactivation Rates

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Constructed wetlands can be used as a natural, low cost and low energy technology for wastewater treatment. These treatment systems have the ability to remove waterborne viruses[1] and bacteria[2] from wastewater effluent through various disinfection processes, of which photo inactivation is one of the most important. This is especially true in open water systems.

To promote sunlight inactivation, open water wetland cells are designed to have a shallow, clear water column, and are lined by geotextiles or cement to inhibit vegetation growth. Previous studies[2] have found pathogen removal in regular vegetated cells to be minimal, mainly due to the limiting amount of sunlight inactivation which occurs when vegetation shades the water surface.

The ultimate goal of this research project is to create photoaction spectra of wastewater bacteria that can be used in combination with sunlight irradiance data to predict their photo-inactivation rates. Photoaction spectra are functions that consist of wavelength-specific sensitivity coefficients [$P(\lambda)$] that describe the biological response of an organism to radiation[3]. Currently, there are no practical photoaction spectra which can be applied to a variety of organisms, presenting an issue when attempting to model pathogen inactivation in open water systems.

Data was collected from multiple experiments conducted using a solar simulator. Experiments were conducted in open top, black painted, batch reactors that were filled with phosphate buffered saline solution, which was spiked with lab grown *E. faecali*s and *E. coli* and exposed to simulated sunlight. Samples were collected periodically over the course of 48-h long experiments, and plated using the spread plate method within 1 h of sampling. *E. faecalis* was plated on mEnteroccous agar and incubated at 34°C for 48 h. *E. coli* were plated on mTEC agar, with incubation for 20-28 h at 37°C. Duplicate plating for t = 0 samples, frequent sampling, as well as the plating of numerous dilutions for each sample ensured the accuracy of plate counts. Varying glass cutoff filters were placed on top of each reactor during experiments to determine how different wavelengths affect inactivation. Data collection and analysis are ongoing, and will be used to develop photoaction spectra for *E. faecalis* and *E. coli*.

Once developed, the E. *faecalis* and *E. coli* photoaction spectra will be validated using previously collected monitoring data from a pilot scale open water treatment wetland in Discovery Bay, CA. However, the monitoring data was collected using Colilert *E. coli* media with Idexx Quanti trays, instead of mTEC agar, which was used for developing the photoaction spectra. Therefore additional experiments were conducted to determine how different growth media affect the apparent inactivation rate of the cultured bacteria. A separate set of solar simulator experiments were conducted, without the use of glass cutoff filters, simulating natural sunlight; inactivation was observed in triplicate batch reactors. This experiment looked at the inactivation rate of *E. coli* on three different growth media: mTEC agar, Luria Burtani (LB) agar with kanamycin, and Colilert with Idexx Quanti trays. This information will help us determine if

we can use the monitoring data collected with Colilert to validate photoaction spectrum. *E. coli* inactivation rates were determined for mTEC agar and Luria Burtani (LB) agar using a multiple-target inactivation model[4] that accounts for the initial shoulder in the inactivation curve. The inactivation rate for Idexx was calculated using the first order exponential decay equation for the linear portion of the curve.

The results found in this experiment show that there is a difference in the apparent inactivation rate of *E. coli* on the three different growth media (Figures 1 and 2). Plating *E. coli* on LB with kanamycin resulted in the lowest measured inactivation rate, while Idexx had the highest. Similar inactivation rates between mTEC agar and Idexx media were observed. It can be concluded that non-selective media (i.e. LB with kanamycin) is less damaging to bacterial growth, resulting in a lower observed inactivation rate. Finally, given the similarity in observed inactivation rates between mTEC and Idexx media, it is possible to use monitoring data found in previous studies[2] to validate our photoaction spectrum model.

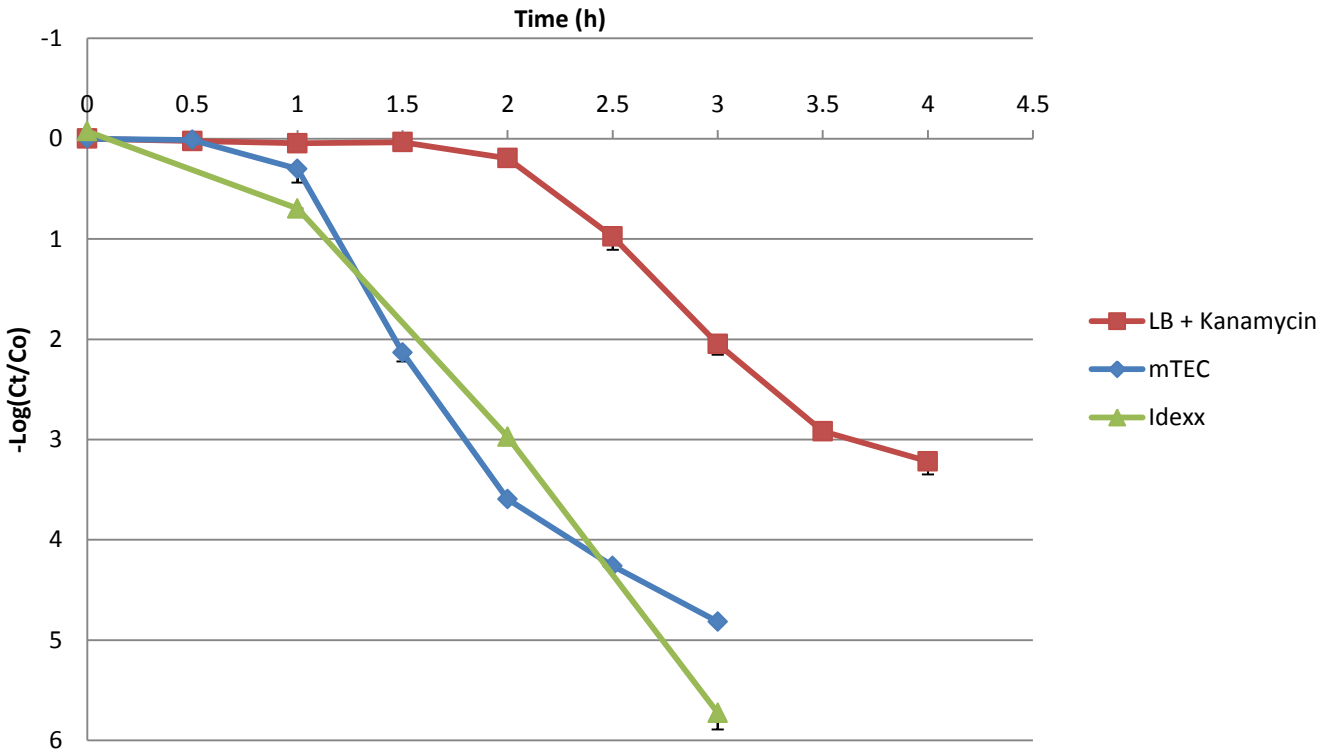
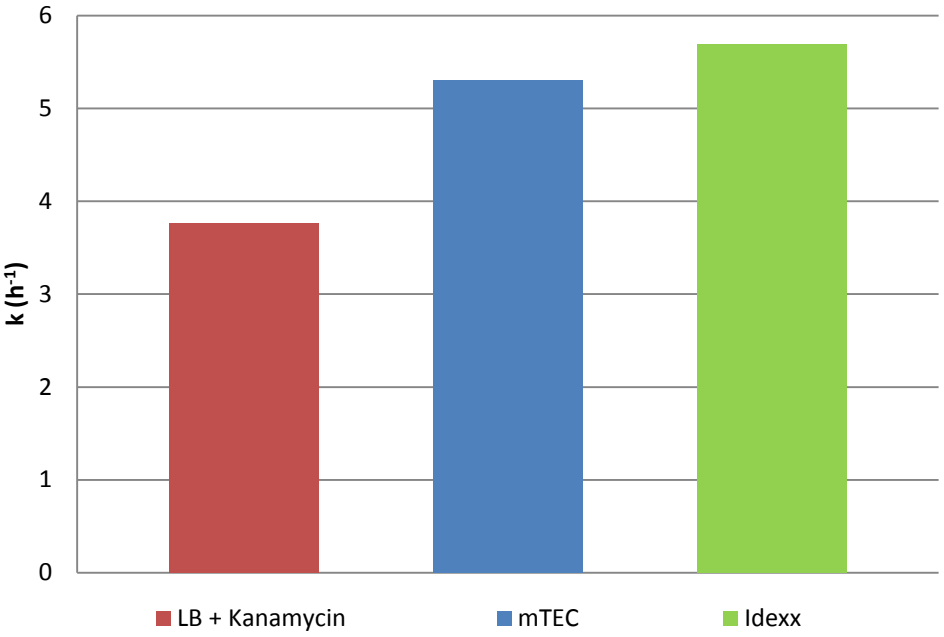


Figure 1. Inactivation curves of *E. coli* cultures on LB agar with kanamycin, mTEC agar, and Idexx Quanti trays. Data are average of triplicate reactors. Error bars are standard error and are smaller than the data symbols for most time points.



Growth Media	k observed
LB + kanamycin	3.8
mTEC	5.3
Idexx	5.7

Figure 2. Observed inactivation rates (k) of *E. coli* plated using LB agar with kanamycin, mTEC agar, and Idexx Quanti trays.

References

(1) Silverman, A. I.; Nguyen, M. T.; Schilling, I. E.; Wenk, J.; Nelson, K. L. Sunlight inactivation of viruses in open-water unit process treatment wetlands: modeling endogenous and exogenous inactivation rates. *Environmental Science and Technology*. **2015**, 49 (5), 2757−2766.

(2) Nguyen, M. T.; Jasper, J. T.; Boehm, A. B.; Nelson, K.L. Sunlight Inactivation of fecal indicator bacteria in open-water unit process treatment wetlands: modeling endogenous and exogenous inactivation rates. *Water Research*. **2015**, 83:282−292.

(3) Fisher, M. B.; Love, D. C.; Schuech, R.; Nelson, K. L. Simulated sunlight action spectra for inactivation of MS2 and PRD1 bacteriophages in clear water. *Environmental Science and Technology*. **2011**, 45 (21), 9249−9255

(4) Wegelin, M.; Canonica, S.; Mechsner, K.; Fleischmann, T.; Pesaro, F.; Metzler, A. Solar water disinfection: scope of the process and analysis of radiation experiments. *Journal of Water Supply: Research and Technology - Aqua*. **1994**, 43 (3), 154−169.