Deactivation of Parasitic Nematodes for Improved Water, Sanitation, and Hygiene

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This project aims at assessing energy utilization during electroporation employed as a back-end blackwater treatment process for helminth remediation. Results from our initial work have demonstrated the effectiveness of this technique for permeabilizing ova of *Caenorhabditis elegans* (*C. elegans*) nematodes, a helminth surrogate, through apparent pore formation in the lipid-rich permeability layer within the eggshell. The permeability barrier is crucial to the well-being of the embryo and, therefore, this achievement represents a key milestone towards helminth deactivation. This work will determine optimal treatment conditions according to minimal energy requirements.

Supported by the

Bill & Melinda Gates Foundation
'Reinvent the Toilet Challenge'

Background

Posited as a public health risk by the World Health Assembly in 2001, helminths are a virulent family of parasites prominent in the developing world with various species thought to have had infected over half of the world's population [1]. Helminth eggs are incredibly resilient, possessing the ability to survive changing environmental factors and exposure to various chemical treatments [2,3]. While conventional sanitization methods are able to inactivate the eggs [4,5], they are largely inefficient in doing so. Other studies have expanded the capabilities of conventional methods (i.e., chlorination or oxidation) by enhancing and expediting their effects with photochemistry [4,5]. Ongoing work in our laboratory follows a similar trend by combining electrochemical treatments with electroporation under the prospect of finding a cost effective and sustainable means of sanitization. While preliminary results in Duke and Elon laboratories have been promising, no information on energy utilization has been determined.

Scope

Electroporation will be performed in plastic cuvettes inserted into a BTX ECM830 Square Wave Electroporation System. The cuvettes are fitted with two opposing stainless-steel electrodes positioned 0.4 cm apart and serve as the reservoir for the nematode test solutions, as illustrated in Figure 1. Phosphate-buffered saline (PBS) and blackwater will be used as test solutions. Blackwater (BW) obtained from our Duke University collaborators will be filtered to remove larger organic aggregates, yet otherwise will contain feces, urine, and water. Use of PBS of varying salt concentrations and blackwater enable determination of effects of solution composition and ionic concentration on energy consumption.

Preliminary Results

The author's electroporation results indicate the feasibility of electropermeabilizing nematode eggshells (citation below). Fluorescence microscopy showed increasing dye labeling of embryonic DNA and red emission intensity with EP pulse amplitude, as shown in Figure 2. The fluorescence images in Figures 4 d,e,f show increasing red emission intensity for *C. elegans* electroporated for 3 min of total EP duration using 1500 V/cm, 1750 V/cm, and 2000 V/cm pulsed electric fields, respectively. Fluorophore uptake was observed for all pulse amplitudes showing greater reaction-diffusion kinetics with electric field magnitude and total EP duration, as shown in Figure 3. The standard deviation error bars shown in this figure reveal greater variability for the shorter EP treatment times possibly attributed to different sizes and developmental stages of the eggshells and embryos. Another important observation was that active and healthy *C. elegans* worms prior to EP were destroyed upon exposure to the intense electric fields. Dye uptake was observed in permanently immobilized worms and showed similar fluorophore uptake kinetics to ova fluorescence, while "stunned" worms displayed no fluorescence and regained mobility shortly after the treatment.

M. H. Dryzer, C. Niven, S. D. Wolter, C. B. Arena, E. Ngaboyamahina, C. B. Parker, and B. R. Stoner, Journal of Water, Sanitation and Hygiene for Development (2019) 9 (1): 49–55.

Experimental approach

A parametric study involving EP parameters listed below and associated response data (power utlization, energy utilization) will be evaluated using SAS JMP® software. This will enable evaluation of the multi-dimensional factor space, revealing the interplay between important EP treatment parameters. An oscilloscope will be used to monitor the voltage and current pulse train using a voltage divider circuit and wide band current monitor, respectively. The voltage and current waveforms will be captured from the oscilloscope. Further, capacitive loading as a function of EP treatment parameters will be observed by transient response in the current waveform. Data outcome will provide determination of the 'sweet spot' among all EP treatment parameters for energy efficient EP treatment. This will contribute to the ultimate design of an electroporation module for use in the prototype toilet at Duke University.

BTX electro porator parameters:

- Pulsed electric fields of 250 V/cm to 2500 V/cm
- Pulse duration of 10 µsec to 10 ms
- Pulse interval of 100 ms to 10 s
- Total EP treatment duration of 10s to 10min
- Monopolar and bipolar electric pulses

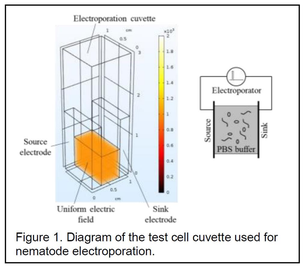

Figure 1. Diagram of the test cell cuvette used for nematode electroporation.

References

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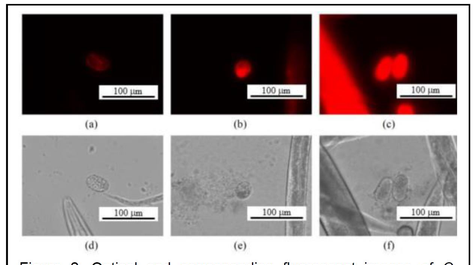

Figure 2. Optical and corresponding fluorescent images of *C. elegans* eggs post-EP. The images display increasing red fluorescence for nematode eggs electroporated for 3 minutes at three different field amplitudes: 1,500 V/cm (a,d), 1,750 V/cm (b,e), and 2,000 V/cm (c,f).

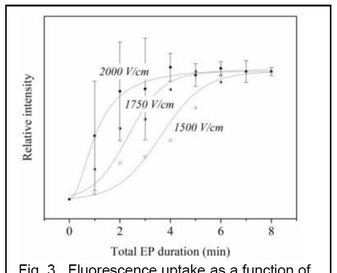

Fig. 3. Fluorescence uptake as a function of total EP duration for pulsed electric fields of 1500 V/cm, 1750 V/cm, and 2000 V/cm. Standard deviation error bars show greater variability for shorter treatment times.

Experimental Results