**Protocol for testing water disinfection system**

**Purpose:**

To create a water disinfection system that utilizes electroporation to lyse bacterial cells in water.

**Background:**

**Water Quality in Charles River:**

According to the Charles River Watershed Association (CRWA), which takes monthly water samples at 37 sites along the Charles for analysis, the average concentration of escherichia coli (E. coli) found in the Charles in 2005 is approximately 27,200 cfu per 100mL, or in other words, 272 cfu/mL. To put this value into more perspective, the U.S. Environmental Protection Agency (EPA) standard for E. coli is 126 cfu/100mL for swimming and 630 cfu/100mL for boating; the standards for fecal coliform set by the Massachusetts Department of Environmental Protection (DEP) are 200 cfu/100mL for swimming and 1,000 cfu/100mL for boating. Here in this study we will be primarily looking at E. coli concentrations in water, as water quality standards are slowly being shifted from fecal coliform bacteria to E. coli bacteria.

**Electroporation:**

A water disinfection system utilizing electroporation has been devised in the past. From these researches, electric field strength required for cell lysis was determined to be 1-5kV/cm. The minimum residence time for cell lysis was found to be 17 µsec under high voltage application. Therefore for a system at a relatively low voltage of 25 V, a much longer residence time would be expected.

**System Design:**

Our system consists of two 5cm x 10cm metal electrodes (Ti or stainless steel), connected to a power supply. When using Ti electrodes, one of the electrodes will be anodized to increase resistance to corrosion. 4mm diameter holes will be drilled into
the electrodes to serve as inlets and outlets for water flow. PVC tubes are connected to the holes to allow water transfer. Syringes are attached to the inlet tubes, and the outlet tubes lead into a collection beaker kept on ice. The syringes are clamped to a stand. A shimstock frame of 127µm thickness is placed between the two electrodes to control the distance between the two electrodes. The electrodes and the shimstock are sealed by glue to prevent leakage. Below illustrates the basic design schematic:

Sample preparation:

**LB broth:**

A stock of LB broth will be prepared by dissolving 10g Bactotryptone, 5g Yeast extract, and 10g NaCl into 800mL distilled water. The pH of the solution will be adjusted to 7.5. Additional distilled water will be added to make the total volume of the mixture to be 1L. The mixture will then be autoclaved for 40 minutes at 250°F and 1 atm in slow exhaust mode. The broth will be cooled at room temperature.
E. coli stock culture:

A 100mL E. coli stock culture at a bacterial concentration of approximately 1360 cfu/mL will be prepared. 2mL of LB broth will be inoculated with a single E. coli colony picked from a freshly streaked plate and allowed to grow overnight at 37°C. The next day, 10µL of overnight culture will be transferred into a tube containing 10mL of fresh LB broth and allowed to grow under shaking at 37°C for an additional day. The culture will then be diluted down to allow for measurement by a spectrophotometer to determine bacterial concentration. Depending on the concentration of the culture, dilutions can then be made to achieve our target 100mL stock at 1360 cfu/mL.

Water sample for testing:

Two types of systems will be tested:
  1. system with 2 water inlets/outlets
  2. system with 1 inlet/outlet.

For system (1), a 45mL water sample with a final concentration of 272 cfu/mL will be prepared by mixing 9 mL stock culture (1360 cfu/mL) into 36 mL distilled water. 20 mL of the water sample will be loaded into each of the two syringes, and any air bubbles trapped within the syringes removed. The remaining 5 mL of water sample will be saved and kept on ice for bacterial concentration quantification.

For system (2), a 35mL water sample with a final concentration of 272 cfu/mL will be prepared by mixing 7 mL stock culture (1360 cfu/mL) into 28 mL distilled water. 30 mL of the sample will be loaded into the syringe, and the remaining 5mL kept on ice for bacterial concentration quantification.

Testing procedure:

1. Setup system design and attach onto a stand.
2. Prepare water sample for testing.
3. Take absorbance measurement of water sample (pre-treatment) to determine bacterial concentration.
4. Load water sample (20mL/syringe in 2-syringe system; 30mL in 1-syringe system) into syringe(s) and remove air bubbles.
5. Clamp syringes onto stand to stabilize.
6. Set voltage at 25 V.
7. Apply load onto syringe(s) and allow entire volume of sample to run through system.
8. Transfer 1mL of sample (post-treatment) from collection beaker into a clean cuvette.
9. Take absorbance measurement of water sample (post-treatment) to determine bacterial concentration.

**Flow rate control:**

Flow rate will be adjusted to ensure that the bacteria residence time in the system is substantially higher than 17µsec, at around 200-300 ms. The flow rate of water will be controlled by applying a constant load to the two syringes, which will be achieved by putting a weight on top of the two syringes that will pump water into the system. The applied force would therefore be equal to the mass times gravitational acceleration (F=mg). A calibration curve of flow rate vs. applied load will be constructed before testing to allow conversion from applied load to flow rate. During experimentation, the applied weights will be adjusted to determine the optimal load and flow rate for cell lysis.

**Electrochemical condition:**

The system will be operated at 25V at 1A in a DC current.

**Bacterial concentration quantification:**

Quantification will be achieved by spectrophotometer absorbance measurement at a wavelength of 600nm. A sample of 200µL LB broth (no bacteria) in 800µL distilled water will be used to blank the instrument before measurement. For each measurement, 1mL of sample will be loaded into a clean cuvette and the absorbance recorded. Quantification measurements will be taken for each water sample before and after an experiment run (through the system). Samples must be kept on ice and measurements taken as soon as possible to eliminate any additional bacterial growth.