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Emerging investigator series: Locally Enhanced Electric Field Treatment (LEEFT) with Nanowire-Modified Electrodes for Water Disinfection in Pipes

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Abstract

Chlorine disinfection inevitably generates carcinogenic by-products. Alternative non-chlorine-based techniques in the centralized treatment plants cannot produce residual antimicrobial power in water disinfection systems. Here, we propose locally enhanced electric field treatment (LEEFT) for chemical-free water disinfection in pipes. A tubular LEEFT device with coaxial electrodes is rationally developed for easy adaption to current water distribution system as a segment of the pipelines. The center electrode is modified with perpendicularly grown nanowires, so that the electric field strength near the tips of the nanowires is significantly enhanced for pathogen inactivation. We have demonstrated >6-log inactivation of bacteria with 1 V, a small voltage that can be generated *in situ* by flowing water.

Environmental significance

Water-borne pathogenic diseases pose a threat to public health. Pathogen inactivation in water distribution systems, *i.e.*, secondary disinfection, is equally important with that in water treatment plants. The most common chlorination provides residual disinfectant for pipelines, but inevitably generates carcinogenic disinfection by-products. The successful bacterial inactivation using the LEEFT device with a coaxial-electrode configuration provides a new solution for the secondary disinfection to substitute residual chlorine. The implementation of the LEEFT in water distribution systems will prompt the centralized water treatment facilities to adopt non-chlorine-based disinfection techniques with no residual antimicrobial effect. Relying on electroporation for pathogen inactivation, the LEEFT is potentially chemical free, which minimize the use of chemicals and impact on the environment. As aging infrastructure has become a global problem, it is a great opportunity to test and implement "smart" pipes with additional functions, e.g., killing pathogens.

1. INTRODUCTION

Disinfection is essential to protect human from pathogenic infection [1, 2]. A drinking water system for developed urban areas typically contains primary and secondary disinfection phases. The primary disinfection inactivates or removes pathogens in a centralized unit (*i.e.*, a treatment plant), while the secondary disinfection refers to maintaining microbicidal effects in the distribution systems (*i.e.*, pipelines) [3]. The most popular disinfection method is chlorination attributed not only to its low cost and high primary disinfection efficiency, but also to its capability of providing secondary disinfection effect via the remaining free chlorine and/or chloramines [4]. Nevertheless, chlorination and chloramination inevitably generate carcinogenic disinfection by-products that threaten human health [4, 5]. This problem can be solved by using non-chlorine-based techniques such as ultraviolet (UV) or membrane filtration [6, 7]. With the accumulation of technology development and industrial experiences, these alternative techniques have become more robust and cost-effective, showing great potential to substitute chlorination in centralized water facilities. However, none of these methods produces residual antimicrobial power. Microbial regrowth in pipelines becomes the major obstacle against the adoption of UV, ozonation, and membrane filtration in centralized water treatment facilities [8]. Therefore, disinfection technologies are urgently needed to provide continuous antimicrobial effect throughout the water distribution system [9, 10].

Potential technologies should be easily incorporated into existing water distribution systems. In addition to being low cost, low energy consumption, and high efficiency, the preferred technology should require as low maintenance as possible, considering that most of the pipelines are underground [11]. Locally enhanced electric field treatment (LEEFT) has emerged as a promising water disinfection technique [12]. When biological cells are exposed to a high-strength

electric field, the permeability of the cell membrane dramatically increases, and electroporation may occur [13, 14]. When the strength of the external electric field reaches a sufficiently high level (1-10 kV/cm), the electroporated pores on the cell membrane become irreversible, resulting in cell inactivation [14, 15]. Traditionally, a high voltage (>1 kV) must be applied to realize the high-strength electric field, leading to intensive energy consumption and operating risks [16]. Recently, such problem has been resolved by the LEEFT with nanowire-modified electrodes. The nanowires can enhance the local electric field near the tips by several orders of magnitude, and thus enable pathogen inactivation with very low voltage (1-2 V) applied [12, 17]. Taking advantage of this phenomenon, a series of nanowire-assisted electrodes have been developed, and LEEFT devices have achieved high inactivation of various bacteria and viruses[17-19]. The energy consumption of the LEEFT can be as low as ~1 J/L [19], which is significantly lower than that of conventional electric field treatment (typically >150 kJ/L) and other aforementioned water disinfection processes (UV, 20-60 J/L; ozone, 50-100 J/L; membrane, 500-5000 J/L) [20].

Here, we propose to apply the LEEFT for water disinfection in pipelines (Fig. 1A). Compared with the residual chlorine, the LEEFT is a chemical-free process and doesn't generate any disinfection by-products. In addition, there is no concern of over-treatment, because the LEEFT is a physical process that has little impact on the physical and chemical property of the treated water. In this study, we introduce a new LEEFT configuration with a cylindric treatment chamber and coaxial electrodes: a tubular outer electrode and a nanowire-modified center electrode (Fig. 1B). Such configuration allows LEEFT devices to be directly adapted to current water distribution system as segments of the pipelines. When water is flowing through a LEEFT pipe, potentially existing pathogens will be sent towards the center electrode by various forces, *e.g.*, hydrodynamic force, electrophoresis force, and dielectrophoresis force [21]. Subsequently, the

pathogens are inactivated by irreversible electroporation due to the enhanced electric field near the tips of the nanowires, even though a low voltage is applied.

2. MATERIAL AND METHODS

2.1 Construction of the prototype and scaled-up coaxial-electrode LEEFT devices

The coaxial-electrode LEEFT device was composed of a hollow cylinder as the outer electrode, a coaxial wire in the center of the cylinder cross-section serving as the center electrode, and a reactor holder for assembling. The outer electrode was a copper cylinder (interior diameter, 0.95 cm; length, 12.7 cm), while the center electrode was a fine wire (diameter, 76 μm; length, 12.7 cm) with nanowires modified (see the detailed method in Section 2.2). For the scaled-up reactor, a commercially available aluminum tube (interior diameter, 0.77 cm; length, 183 cm) was used as the outer electrode. A 183-cm-long center electrode wire (diameter, 76 μm) was fabricated using the same procedure of the one in the prototype reactor. Similar acrylic reactor holder is used to assemble the parts together.

2.2 Fabrication and characterization of nanowire-modified electrodes

The fine copper wire serving as the center electrode was modified with copper oxide nanowires (CuONWs) using the methods developed in our previous study (Fig. S1) [22]. Specifically, after being washed with HCl solution (1 M) and rinsed with DI water to remove the oxidation layer, the copper wire was heated at 400 °C in air for 2 hours and let cool down to the room temperature, which allowed CuONWs to grow perpendicular to the electrode surface. Subsequently, the prepared CuONW-Cu wire was immersed into a dopamine solution buffered with Tris (0.01 mol/L, pH 8.5) under 40 °C to be coated with a polydopamine protection layer.

After the coating process, the electrodes were gently washed with DI water, dried in the air, and ready to use.

The morphology of the center electrode was characterized with a scanning electron microscope (Zeiss Ultra60 SEM) and a transmission electron microscope (Hitachi HT-7700 TEM). The length and diameter of the nanowires were measured on the SEM and TEM images and used for the electric field analysis.

2.3 Bacterial inactivation experiments

Four strains of model bacteria, *Escherichia coli* (*E. coli*, 10798), *Enterobacter hormaechei* (700323), *Bacillus subtilis* (6051), and *Staphylococcus epidermidis* (14990), were purchased from the American Type Culture Collection (ATCC) and used for the bacterial inactivation experiments. The model bacteria were cultured aerobically in the according broth media at 35 °C to log phase (6-12 hours). The bacteria solution was then centrifuged at 5000 rpm for 5 min and washed using DI water for three times to remove the potential interference of the background media. The harvested bacteria solution was diluted with DI water to a concentration of ~1×10^7 colony-forming units (CFU)/mL [23]. During the inactivation experiments, the bacterial solution flowed through the LEEFT device with a fixed flow rate (0.7 to 10 mL/min). Different waveforms of the voltage were applied between the positive and negative electrodes. The direct-current (DC) voltages (0-2 V) were created by a Keithley 2400 Sourcemeter and the square wave pulses were generated by a Keysight 33500B Waveform Generator. The waveform parameters controlled in the experiments included frequency (10^5 Hz), lead edge (8.4 ns), trail edge (8.4 ns), high voltage (1 V), and low voltage (0 V). The current during DC operation was measured by the Sourcemeterand recorded at the sampling point. The river water matrix was first filtered with a membrane (0.2 μm diameter) and then dosed with *E. coli* of ~10^7 CFU/mL. The bacterial

concentration (c_{in} for influent and c_{eff} for effluent) was measured using the spread plating technique and the inactivation efficiencies were calculated by Equation (1):

$$\text{Log inactivation efficiency} = -\log_{10}\left(\frac{c_{eff}}{c_{in}}\right) \quad (1)$$

The effluent copper concentration was measured. After being collected and acidified with HNO_3 (2 % w/w) solution, the water samples were analyzed by the Copper Test Kit (HACH, porphyrin method 8143) with a HACH DR6000 spectrophotometer.

2.4 Electric field simulation

The electric field distribution was simulated by finite element method using COMSOL Multiphysics. A 3D model of the chamber was set up, and a single nanowire was built to demonstrate the electric field around the tip area. Electrostatic module was used for the simulation, where the electric field was defined by Equation (2).

$$E = -\nabla \mathrm{V} \quad (2)$$

where V was the electric potential. The values used for the simulation represented the real configuration and operation condition of the prototype LEEFT device (Table S1).

2.5 Live/dead bacterial staining experiments

Water samples were collected before and after the disinfection process (voltage, 1 V; flow rate, 1 mL/min). After being added the same amount of PI dye (10 μL, 3 μM), the samples (1 mL) were stored in the dark for 1 hour and then rinsed with DI water to wash off the extra dye. Subsequently, the stained samples were examined by an Axio Observer 7 Inverted Live-Cell Research Microscope under both florescence and differential interference contrast (DIC) modes.

3. RESULTS AND DISCUSSION

The as-constructed prototype of the coaxial-electrode LEEFT device is shown in Fig. 2A. The copper wire is modified with CuONWs and coated with a polydopamine protection layer (Figs. 2B, S1, and S2) [22]. As shown in Fig. 2B, the surface of the copper wire electrode is uniformly covered with nanowires with diameters around 100 nm and lengths around 5 μm. The diameters of the bare CuONWs are about 30 nm at the tips, while the thickness of the polydopamine protection layer is about 12 nm (Fig. S3). The polydopamine coating, even with a thickness up to 30 nm, and the aggregation of some nanowires at the tips have little effect on the distribution of the electric field strength (Figs. S4 and S5). Similar CuONW-modified copper mesh and copper foam electrodes have demonstrated good microbial inactivation performance in our previously reported LEEFT devices, and the polydopamine coating has effectively enhanced the electrode stability and reduced the copper release to the treated water [12, 24].

Attributed to the rational design, the coaxial-electrode LEFFT device with nanowire-modified electrodes enables two levels of electric field enhancement. As shown in the simulation results in Fig. 2C, the electric field is first enhanced because of the layout of the coaxial electrodes. The electric field strength near the surface of the center electrode is higher than that near the inner surface of the cylindric outer electrode. The enhancement factor (*f*) is determined by the geometry of the device and can be calculated by Equation (3) [25].

$$f = \frac{R}{r} \cdot \frac{1}{\ln\frac{r}{R}} \qquad (3)$$

where *R* and *r* are the radii of the outer and center electrode, respectively. Higher enhancement effect can be achieved by reducing the diameter of the center electrode. For our LEEFT devices equipped with a 76 μm diameter center electrode, the enhancement factor is about 26. The

second level of electric field enhancement is due to the lighting rod effect of the nanowires, which has been harnessed in previous LEEFT devices [12]. With such two levels of enhancement, the electric field strength near the center electrode of our devices is high enough for irreversible electroporation, even when the applied voltage is only 1 V (Fig. 2C).

The coaxial-electrode LEEFT devices have demonstrated outstanding performance for water disinfection (See experimental set-up in Fig. S6). As shown in Fig. 3A, when the prototype LEEFT device is applied to treat water samples containing 10^7 CFU/mL *E. coli*, over 6 logs bacterial inactivation (no living bacteria detected in the effluent) is achieved with an applied voltage larger than 1 V and a flow rate lower than 1 mL/min. The bacterial cell membrane is disrupted during the LEEFT, indicated by the propidium-iodide staining results (Fig. S7): propidium iodide only stains cells who have lost their membrane integrity [12]. Under the operation of 1 mL/min flow rate and 1 V DC voltage, the copper concentration in the treated water is only ~24 μg/L, a concentration that is too low to cause significant antimicrobial effect [26, 27] (Fig. S8). Meanwhile, the level of copper concentration is much lower than the maximum contaminant level goal (MCLG) of 1.3 mg/L set by U. S. Environmental Protection Agency for drinking water. The high inactivation efficiency (~5.6 logs) still maintains (Fig. S9) when a pulsed voltage with a high frequency of 10^5 Hz (on for 5 μs and off for 5 μs) rather than a DC voltage is applied to power the LEEFT. At such a high frequency, the electrochemical reactions are largely eliminated, which rules out the microbial inactivation contributed by direction oxidation and electrochemically-generated reactive oxygen species [28]. The inactivation efficiency is negligible when no voltage is applied (Fig. 3A), which suggests few bacterial cells stick on the surface of the electrodes. Control experiments using a polydopamine coated copper wire as the center electrode show no significant bacterial inactivation (Fig. S10), indicating that the nanowire

structure is crucial to the disinfection process and the antimicrobial effect of polydopamine can be neglected [29]. Thus, nanowire enabled irreversible electroporation is believed to be the main mechanism for microbial inactivation.

Since the high-strength electric field is limited to the vicinity of the nanowire tips (Fig. 2C), it is critical to send bacterial cells to these areas, *i.e.*, the surface of the center electrode. Although the electroporation process can be trigged by a strong electric field in a few microseconds, slow enough flow rate, *i.e.*, long enough treatment time, is required for the transportation of the bacterial cells (Fig. 3A). Considering that most bacterial cells (including the four model bacteria tested in our study) are negatively charged in water with neutral pH, the center copper wire of the coaxial-electrode LEEFT device is typically set as the positive electrode so that the electrophoresis force is driving the cells towards the center of the device [26, 30]. Reversing the direction of the electric field, *i.e.*, setting the copper wire as the negative electrode, significantly reduces the inactivation efficiency (Fig. S11). Under this condition, the treated water has a similar low concentration of copper, which suggests again that copper is not a major inactivation mechanism during the LEEFT (Fig. S12). The dielectrophoresis force also plays an important role in delivering the cells. Because the conductivity of the bacterial cytoplasm is usually higher than that of the water matrix, the dielectrophoresis force directs the cells towards where the electric field strength is higher, i.e., the center of the device (Fig. 2C) [31].

When the LEEFT is performed at 1 V and the flow rate is 1 mL/min, the electric current during the operation is ~24 μA (Fig. S13). Thus, the energy consumption is estimated to be as low as 1.4 J/L (Table S2), similar to that of previous LEEFT devices [18, 19]. Under the same operating condition (1 V and 1 mL/min), the prototype device has been tested to treat water continuously for 16 hours. As shown in Fig. 3B, the inactivation efficiency maintains higher than

5 logs with slight fluctuation throughout the testing period, indicating the high stability and reliability of the treatment. Such long-term performance is in accordance with that achieved in our previous study [22].

The coaxial-electrode LEEFT prototype device also performs well to kill other bacteria, including both gram-negative (G^-) and gram positive (G^+) bacteria. As shown in Fig. 3C, similar to *E. coli* (G^-), all the other three bacteria tested, *Enterobacter hormaechei* (G^-), *Bacillus subtilis* (G^+), and *Staphylococcus epidermidis* (G^+), can be effectively inactivated (>6 logs), when the applied voltage is larger than 1 V and the flow rate is 1 mL/min. Fig. 3C also shows the inactivation efficiency when applying the prototype device to treat a natural river water sample (see water quality characteristics in Table S3) that has been dosed with *E. coli*. The results suggest that bacterial inactivation is slightly affected by the property of the water matrix. Nevertheless, the impact is not significant, and high inactivation efficiency is still achievable [12].

When the scaled-up LEEFT device (Fig. 3D insertion) with a much longer treatment chamber is applied for water disinfection, higher water treatment throughputs can be achieved. As shown in Fig. 3D, the flowrate can be increase to 6.0 mL/min while maintaining the high inactivation efficiency (>6 logs) with the same low voltage (1 V) applied. Such results indicate the great scalability of the coaxial-electrode LEEFT devices.

LEEFT is a chemical-free process and operates solely on electricity. Although the energy consumption is low (~1.4 J/L), having reliable access to electricity is critical for LEEFT. To drive LEEFT in pipelines, the most convenient and economic way is getting electricity from the power grid, which is usually close to the water grid. Another option is to harvest the kinetic energy directly from the flowing water in the pipes and convert it to electricity. This has been demonstrated in this study by using a commercially available electromagnetic generator whose

rotor is connected with the water impellers (Fig. S14). When water is flowing through the turbine electric generator with a flow rate of 4 L/min, the power output is about 95 mW with a voltage of 5 V and a current of 19 mA. The power generated is enough to drive ~4000 sets of the prototype LEEFT devices treating water at a flow rate of 1 mL/min each (Fig. 4). Thus, the total treatment speed is about 4 L/min, which is in line with the flow rate applied for the electricity generation. Such flow rate match suggests the feasibility of powering LEEFT in pipelines without external energy sources.

4. CHALLENGES AND CONCLUSIONS

We have proposed to apply the LEEFT for water disinfection in water distribution systems and have successfully demonstrated a high-performance, low-energy, and scalable coaxial-electrode LEEFT device for such purpose at bench scale. Nevertheless, great challenges still exist for the practical implementation the LEEFT at large scale. For example, the mechanical strength of the electrodes should be elevated to endure the high flow rate and high pressure in water pipes. Meanwhile, particles in water may introduce a shielding effect making bacteria more difficult to get close to the tips of nanowires and be inactivated.

With further investigation and improvement, the coaxial-electrode LEEFT may substantially change the water disinfection strategy and existing systems. In practical uses, segments of pipelines can be replaced by the LEEFT device every certain distance to provide consecutive antimicrobial power. Potential locations for easy replacement include maintenance wells and pumping stations. As the aging water infrastructure has become a serious concern and caused severe problems, e.g., the Flint water crisis, a lot of pipes need to be replaced in the next few

decades. Thus, it is a great opportunity to test and implement smarter pipes that can have additional functions in addition to conveying water, such as killing the pathogens.

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Author contributions: X.X. and J.Z conceived the idea and designed the experiments. J.Z. fabricated the device and electrodes, conducted the disinfection experiments, and designed the self-powered system. T.W. conducted the electric field analysis and dye test. W.C. took the TEM images of the material. B.L and J.Z. constructed the scaled-up device and schematic figures. X.X. and J.Z. cowrote the paper in consultation with all authors.

Competing interests: The authors declare no competing financial interests.

Supporting information:

Figs. S1-S14

Tables S1-S3

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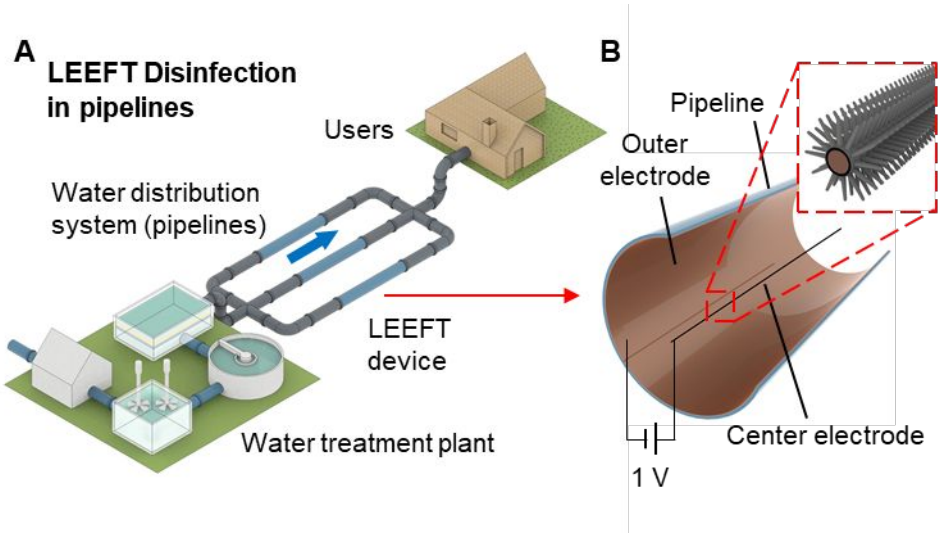


Fig. 1. Schematic showing (**A**) the LEEFT disinfection in pipelines and (**B**) the configuration of the coaxial-electrode LEEFT device.

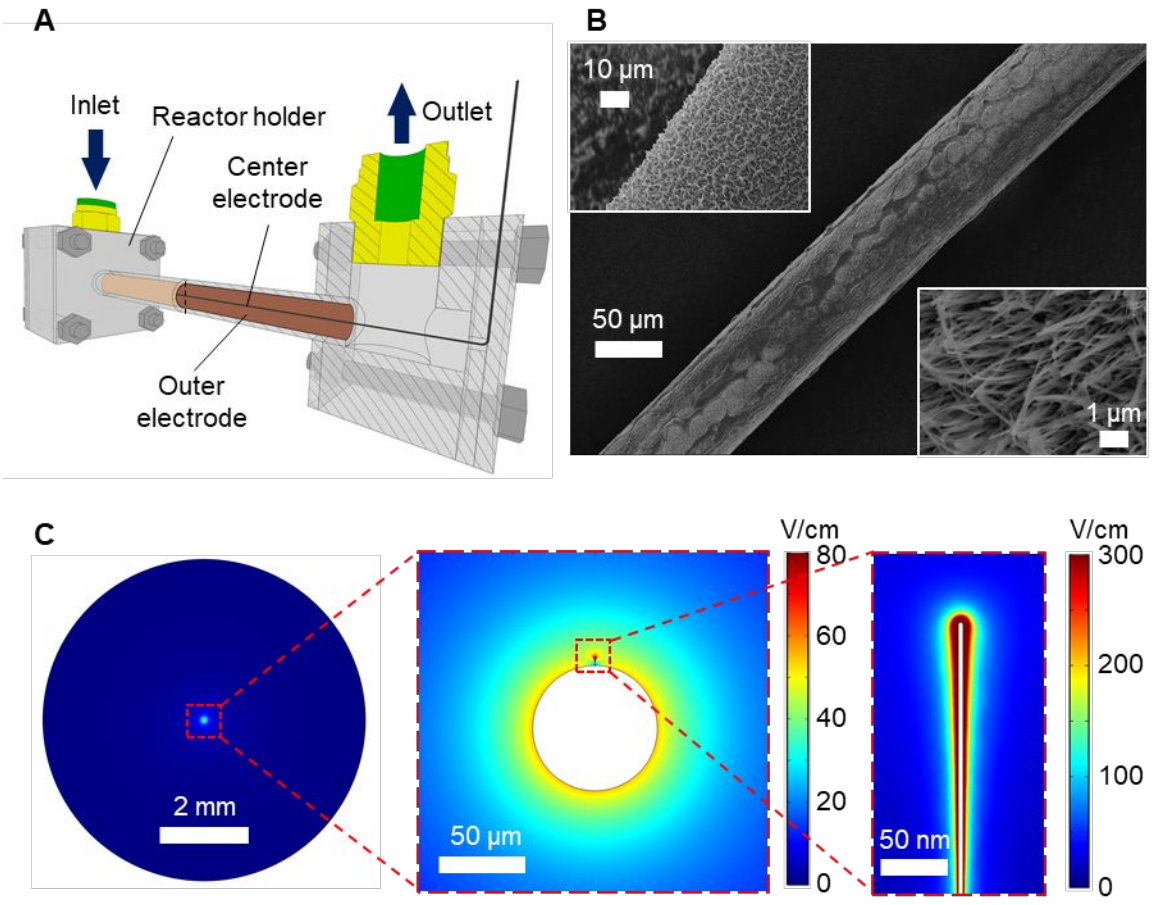


Fig. 2. The rational design of coaxial-electrode LEEFT device. (**A**) 3D schematic shows the device set-up. (**B**) Scanning electron microscopy images show the polydopamine coated CuONW-Cu center electrode. (**C**) Electric field simulation on the cross-section of the device showing the non-uniform distribution of the electric field with a two-level strength enhancement.

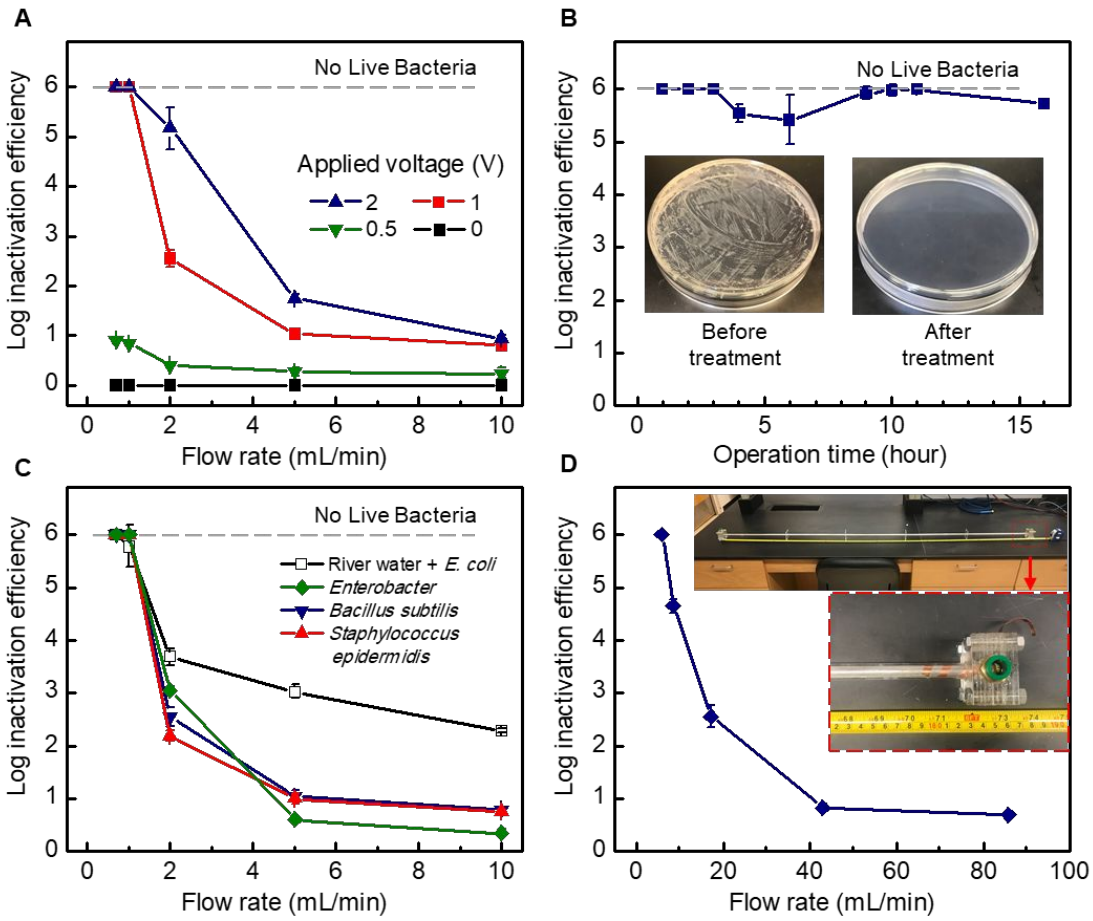


Fig. 3. Coaxial-electrode LEEFT disinfection performance. (**A**) Comparison of the disinfection performances of *E. coli* under different applied voltage implying a threshold exists to enable inactivation. (**B**) Long-term treatment against *E. coli* showing a stable and superior disinfection performance for 16 h. (**C**) Inactivation efficiencies of *E. coli* in the river water, *Enterobacter*, *Bacillus subtilis*, and *Staphylococcus epidemidis* with varied flow rates and fixed voltage (1 V). (**D**) *E. coli* inactivation performance in a 6-feet-long scaled-up LEEFT device. Insertion shows the photographs of the scaled-up device.

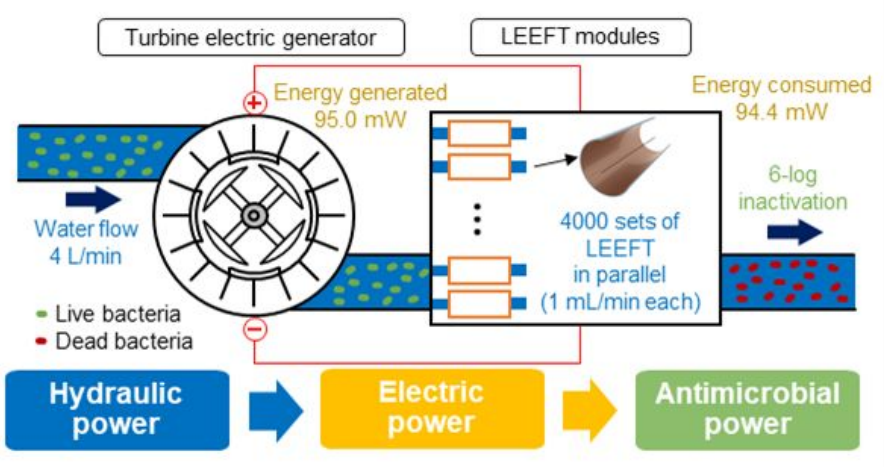
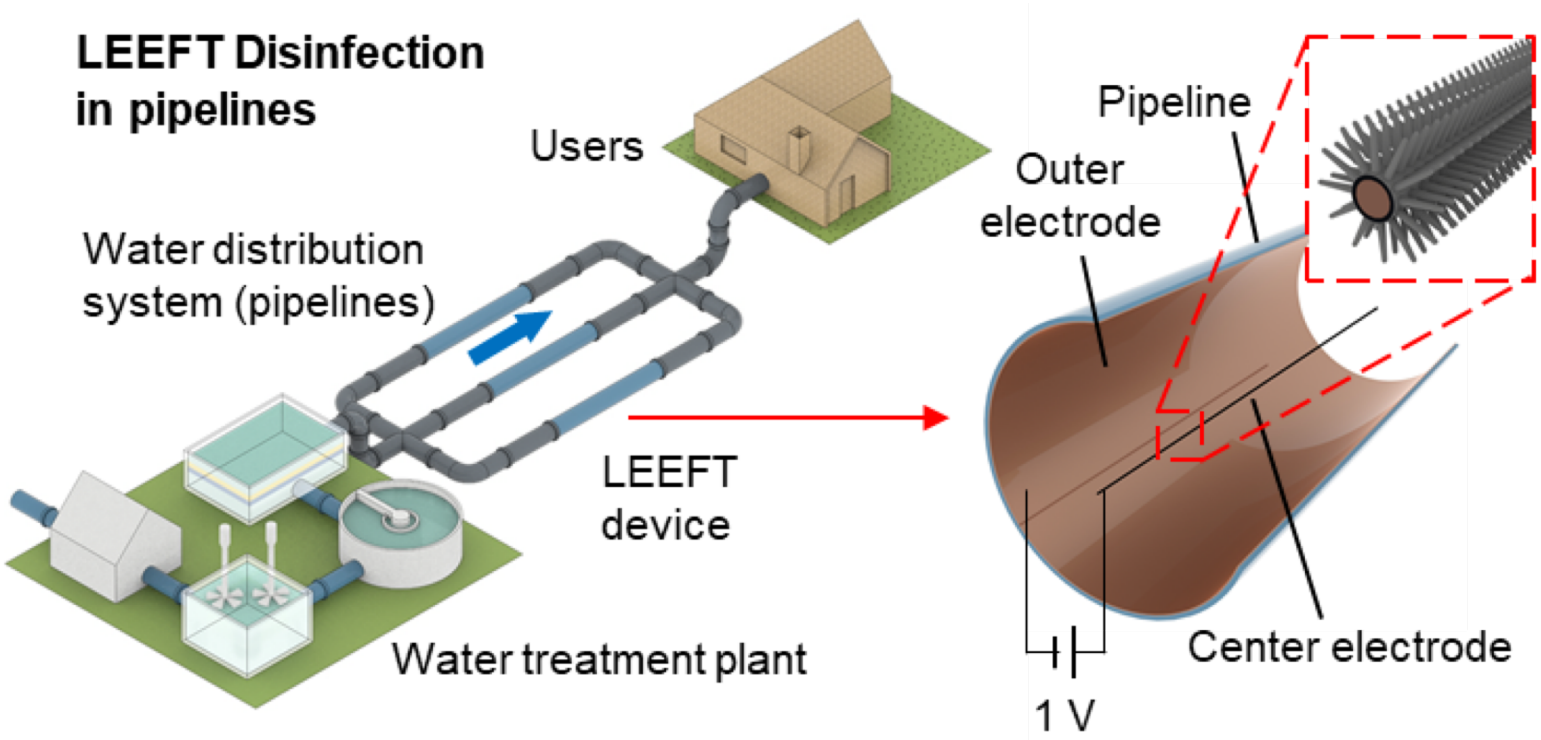


Fig. 4. Powering the LEEFT devices with a turbine electric generator. When the water (4 L/min) flow through the turbine electric generator, the energy generated (95.0 mW) is sufficient to power the LEEFT modules (4000 sets with a flow rate of 1 mL/min each) to disinfect the water.



Assisted by perpendicularly-grown nanowires, a coaxial-electrode locally enhanced electric field treatment (LEEFT) device achieves high disinfection in pipes.