Electronic supplementary information (ESI) for

Cu_3P Nanowire Enabling High-Efficiency, Reliable, and Energy-Efficient Low-Voltage Electroporation-Inactivation of Pathogens in Water †

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1. Experimental section

1.1. Electrode fabrication and device construction.

The Cu mesh (Alfa Aesar, 100 mesh, 0.11 mm as wire diameter) was washed with 1 M HCl (Sigma) and subsequently with deionized water for 3 times to remove surface impurities. The washed Cu mesh was then anodized in an alkali solution (1.5 M NaOH, Sigma) for 20 min under 1.5 mA cm^{-2} to fabricated the $Cu(OH)_2$ nanowire-assisted copper mesh ($Cu(OH)_2$NW-Cu) electrode using a potentiostat (BioLogic, VMP3). To prepare Cu_3P nanowires (Cu_3PNWs), excess sodium hypophosphite (Sigma) was placed at the center of the tube furnace. After flushed with Ar for 15 min, the center of the furnace was elevated to 300 °C and the $Cu(OH)_2$NW-Cu electrode was placed at the downstream with temperature about 100 °C. After 2 h, the furnace was cooled down naturally to room temperature under Ar atmosphere and the Cu_3PNW-assisted copper mesh (Cu_3PNW-Cu) electrode was obtained. Two of the Cu_3PNW-Cu or $Cu(OH)_2$NW-Cu electrodes were then fitted into a plexiglass coaxial electrode holder to prepare an electroporation disinfection cell (EDC). The distance between the two electrodes was fixed at 100 μm.

1.2. Material characterization.

The morphology of the electrodes was characterized by a scanning electron microscope (SEM, Zeiss, LEO 1530) and a scanning transmission electron microscope (STEM, Tecnai, G2 F30), respectively. The corresponding element distribution was analyzed by energy dispersive X-ray (EDX) spectroscopy on the SEM (Zeiss, LEO 1530) and the STEM (Tecnai, G2 F30). The atomic ratio of Cu and P was analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES, PerkinElmer, Optima 8000). A piece of 1 cm^2 prepared Cu_3PNW-Cu electrode underwent

ultrasonic treatment in 5 mL water for 5 min. Removed the electrode and added 5 mL of HNO_3 (2 M; Sigma) into the solution to dissolve the detached Cu_3PNWs, and the Cu and P concentration was measured using the ICP-OES (PerkinElmer, Optima 8000). The crystalline structure of the samples was analyzed by X-ray diffraction (XRD, PANalytical, Alpha 1 MPD). The chemical compositions were analyzed by an X-ray photoelectron spectroscopy (XPS) with an Axis Ultra instrument (Kratos Analytical, K-alpha) under ultrahigh vacuum ($<10^{-8}$ Torr) and by using a monochromatic Al Kα X-ray source.

1.3. Bacterial inactivation performance analysis.

Bacterial inactivation by EDC operation was demonstrated using four model bacteria *Escherichia coli* (*E. coli*, ATCC 15597), *Enterobacter hormaechei* (*E. hormaechei*, ATCC 700323), *Enterococcus durans* (*E. durans*, ATCC 6056), and *Bacillus subtilis* (*B. subtilis*, ATCC 6051). Bacteria samples were cultured in a tryptic soy broth (TSB, Sigma) to log phase (12 h) and harvested by centrifugation at 1500 g and suspended in deionized water. After washed with deionized water for 3 times, bacteria cells were suspended in deionized water to achieve the desired concentration of ~10^7 colony forming units per milliliter (CFU mL^{-1}). The *E. coli* were also dispersed into the lake water (after 0.2 μm membrane filtration) to ~10^7 CFU mL^{-1}. Each water sample flowed through the EDC device at a designated flow rate. Varying voltages (1 to 5 V) applied by Keithley 2400 sourcemeter were applied across the two electrodes. Given that the cross-section area of the electrode (φ 0.375 inch) was 0.713 cm^2, flow rates were kept in the range of 1.2-19.2 mL min^{-1}, corresponding to flux of 1.0 to 16.0 m^3 h^{-1} m^{-2}. After treated for 4 min, the bacteria samples were collected. The bacteria concentrations in the influent (C_{in}) and effluent (C_{eff}) samples

were measured using a standard spread plating technique.[1] Each sample was serially diluted and each dilution was plated in triplicate. All the results for each sample were averaged and the standard deviation was calculated. Bacterial inactivation efficiency was calculated [$E = -\log (C_{eff}/C_{in})$] to evaluate the inactivation performance.

1.4. Long-term bacterial inactivation test.

The long-term inactivation performance of the EDC was tested by treating the prepared bacterial samples (*E. coli*, log phase, ~10^7 CFU mL^{-1}) for a continuous 12-h period and monitoring the bacterial inactivation efficiency over time. The applied voltage was fixed at 1 V and the flux was fixed at 2.0 m^3 h^{-1} m^{-2}. The long-term inactivation performance of the EDC was also evaluated by treating bacterial samples (*E. coli*, log phase, ~10^7 CFU mL^{-1}) for different cycles. During each cycle, the Cu_3PNW-Cu electrode treated the bacterial sample for 30 min. After a 30-min operation, the bacterial inactivation efficiency was analyzed. The electrode was taken out carefully dried in a desiccator (VWR) for 2 h. After the drying process, the electrode was then put into the EDC for the next cycle's operation. The applied voltage was fixed at 1 V and the flux was fixed at 2 m^3 h^{-1} m^{-2}.

1.5. Bacterial inactivation mechanism analysis.

The bacterial inactivation mechanism was analyzed by using a waveform generator (Rigol, DG5352) generating electric pulses (fixed peak voltage of 1 V) with different frequencies ranging from 1 to 3.5×10^8 Hz to the electrodes during the operation. The bacteria inactivation efficiency was analyzed at different frequencies. The bacteria morphology was analyzed by SEM (Zeiss, LEO 1530). All bacterial samples for SEM were harvested by centrifugation at 1500 g for 15 min at 15 °C and supernatants were removed. Then bacteria were fixed overnight in the fixative

containing 0.1 M phosphate-buffered solution (pH 7.3; Sigma), 2% glutaraldehyde (Sigma), and 4% paraformaldehyde (Sigma) at 4 °C. Samples were then dehydrated with increasing concentrations of an ethanol solution (50, 70, 90, and 100%; Sigma) and dried in 100% *tert*-Butyl alcohol (Sigma). Samples were dispersed on a metal grid in preparation for SEM characterization. The bacteria membrane integrity was analyzed by PI dye staining experiment. Bacterial samples (*E. coli*, log phase, ~10^7 CFU mL^{-1}) before and after EDC operation were collected and 10 μL of 1 mg mL^{-1} propidium iodide (PI; Sigma) dye solution was added into 10 mL of each bacteria sample for a final concentration of 1 μg mL^{-1}. Samples were examined using fluorescent microscopy.

1.6. Electrode release mechanisms analysis.

The electrode release process was investigated for a continuous 12-h period EDC operation (1 V and 2.0 m^3 h^{-1} m^{-2}). The released Cu concentration in the EDC effluent was measured as follows: (1) a 1-mL aliquot of effluent was collected and dosed in 1-mL HNO_3 (2 M; Sigma) ensuring the final HNO_3 concentration to 1 M and analyzed by the Copper Test Kit (HACH, porphyrin method) to determine the total Cu concentration (C_T); (2) another 1-mL aliquot was centrifuged (HITACHI RX2 series) at 14500 rpm, corresponding to 17600 g, for 15 min under 15 °C, and the Cu concentration in the supernatant was measured by the Copper Test Kit to determine the dissolved Cu^{2+} concentration (C_{dis}); (3) the suspended Cu particles caused by detaching (C_{det}) was then calculated [$C_{det} = C_T - C_{dis}$]. The electrodes after operation were taken out from the EDC carefully and dried in a desiccator (VWR) overnight. After drying, the Cu_3PNW-Cu electrodes were characterized using SEM (Zeiss, LEO 1530), XRD (PANalytical, Alpha 1 MPD) and XPS (Kratos Analytical, K-alpha) to determine the morphology, the structure, and composition changes,

respectively.

2. Figures

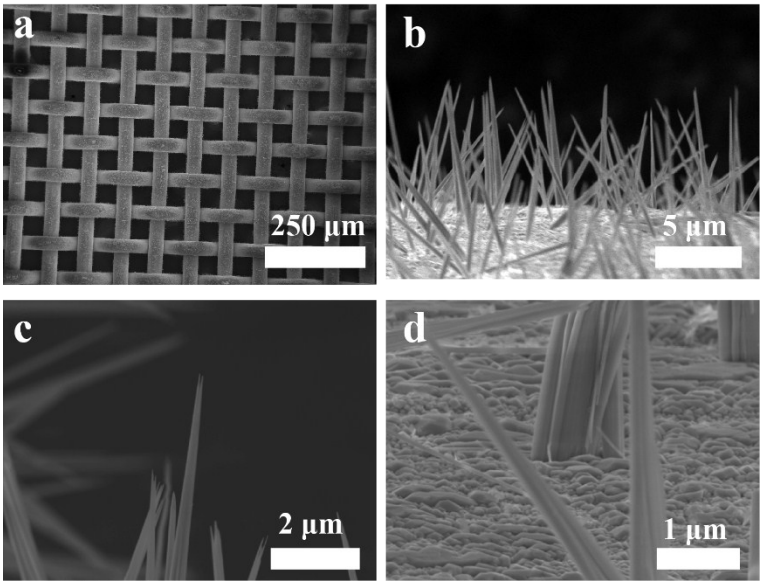

Fig. S1. Scanning electron microscope (SEM) images of $Cu(OH)_2$ nanowire-assisted copper mesh ($Cu(OH)_2$NW-Cu) electrode.

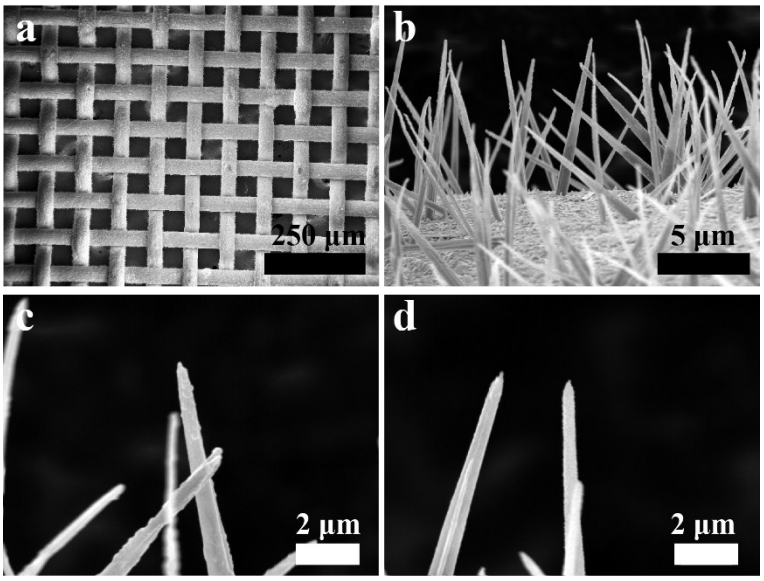

Fig. S2. SEM images of Cu_3P nanowire-assisted copper mesh (Cu_3PNW-Cu) electrode.

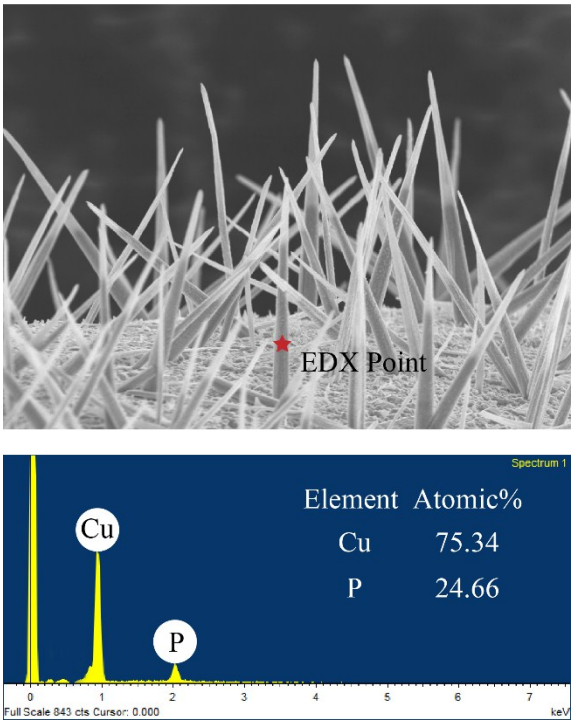

Fig. S3. SEM image and the corresponding energy dispersive X-ray (EDX) elemental analysis of Cu and P for a Cu_3PNW.

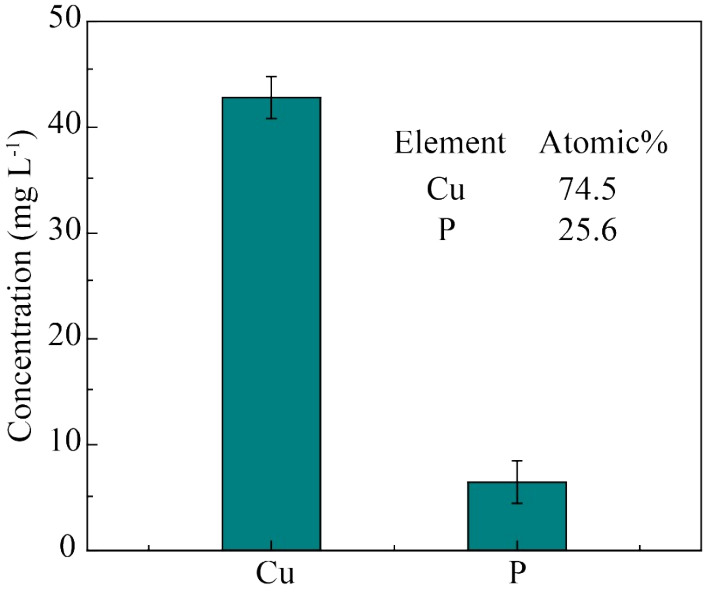

Fig. S4. The elemental analysis using inductively coupled plasma optical emission spectrometry (ICP-OES) for Cu_3PNWs.

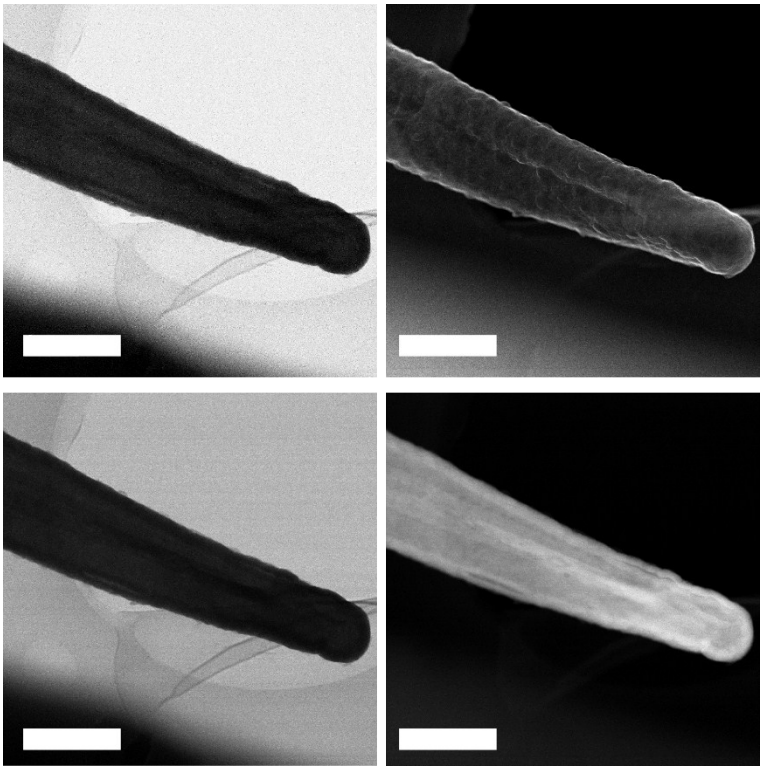

Fig. S5. The scanning transmission electron microscope (STEM) images for a Cu_3PNW. The scale bar is 0.5 μm.

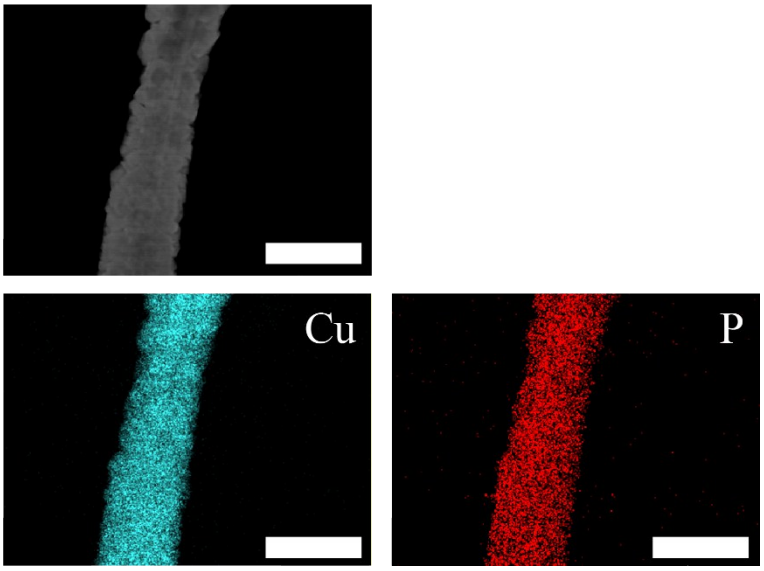

Fig. S6. The STEM images and the corresponding EDX elemental mapping images of P and Cu for a Cu_3PNW. The scale bar is 0.5 μm.

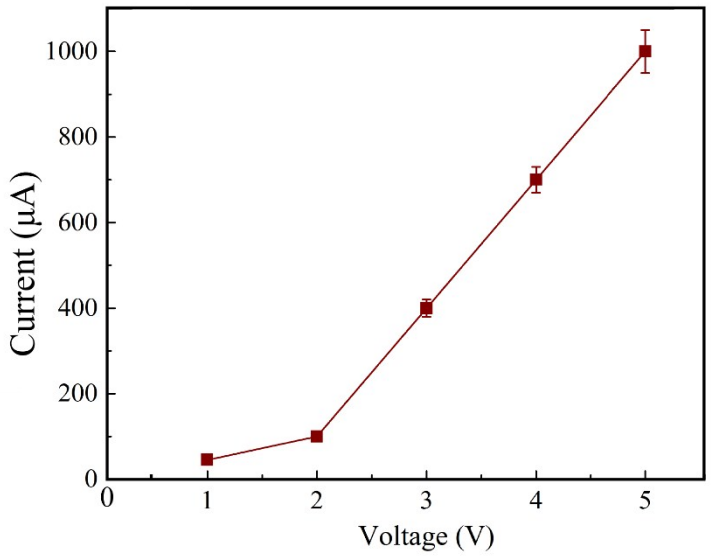

Fig. S7. Electric currents during EDC operation. Applied voltages were ranging from 1 to 5 V and the flux was fixed at 2 $m^3 h^{-1} m^{-2}$.

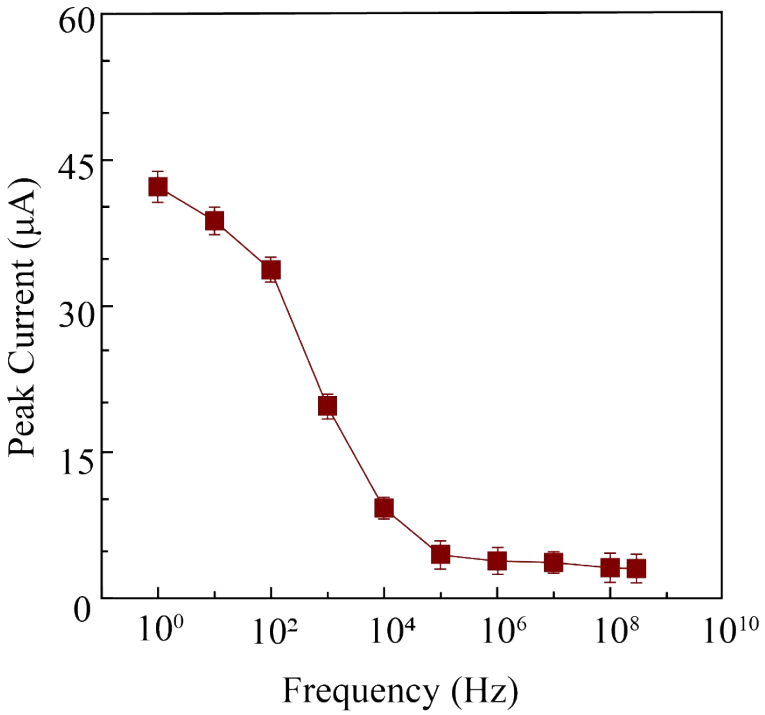

Fig. S8. Electric currents (peak currents) with alternating current (AC) with frequencies varying from 1 to 3.5×10^{8} Hz. The peak voltage was fixed at 1 V and the flux was fixed at 2 m^3 h^{-1} m^{-2}. As the frequency increased, current decreased. This indicated fewer electrochemical reactions occurred.

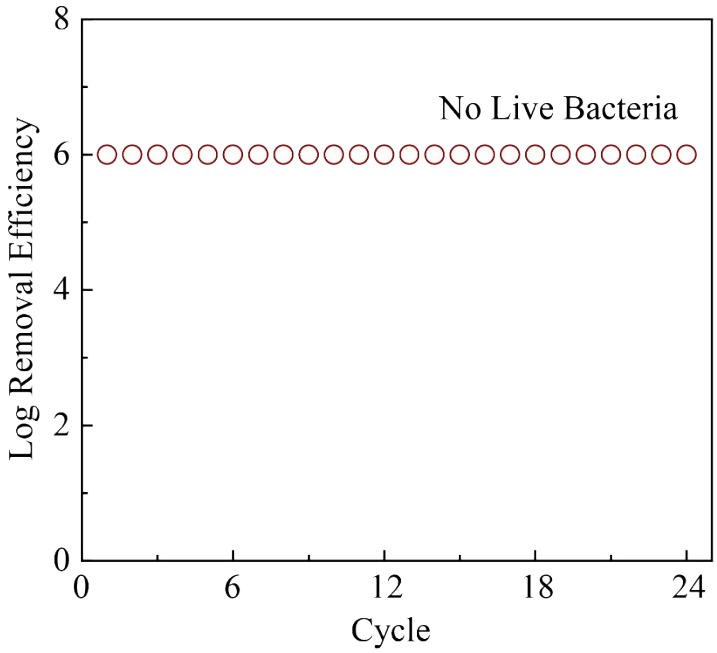

Fig. S9. The bacterial inactivation efficiency of the Cu_3PNW-Cu electrode for different operation cycles. During each cycle, the Cu_3PNW-Cu electrode treated bacterial samples (*E. coli*, log phase, ~10^7 CFU mL^{-1}) for 30 min. The applied voltage was fixed at 1 V and the flux was fixed at 2 m^3 h^{-1} m^{-2}.

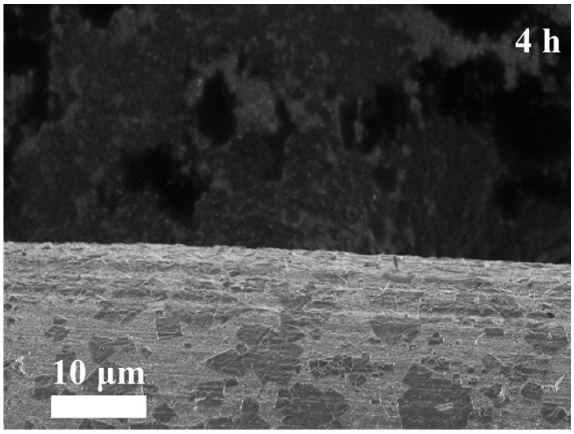

Fig. S10. SEM image of the $Cu(OH)_2$NW-Cu electrode after 4-h EDC operation.

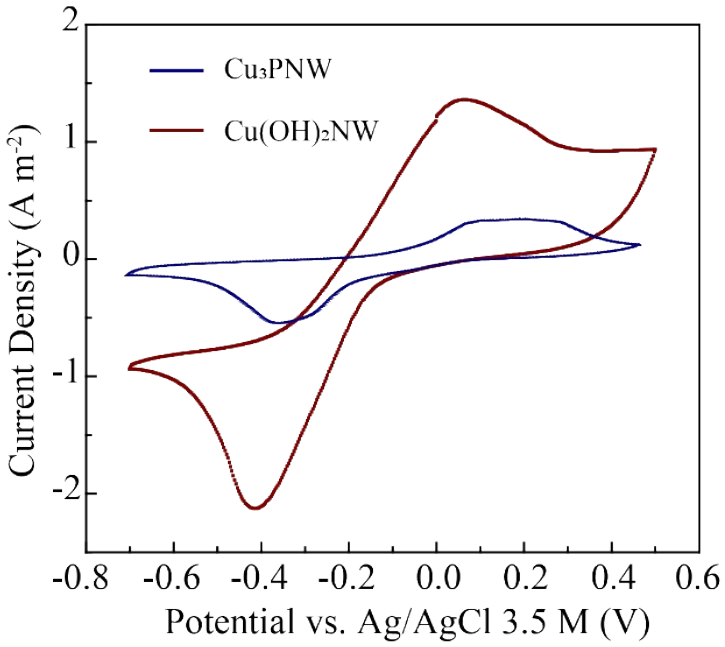

Fig. S11. Electrochemical characterization. Cyclic voltammograms (CV) test for the $Cu(OH)_2$NW-Cu and Cu_3PNW-Cu electrodes were performed using a BioLogic VMP3 potentiostat. A double junction Ag|AgCl|KCl (3.5M) reference electrode (RE) was used in the measurement. CV test was carried out in the potential range -0.7 V to 0.5 V vs. RE under a sweep rate of 10 mV s^{-1}.

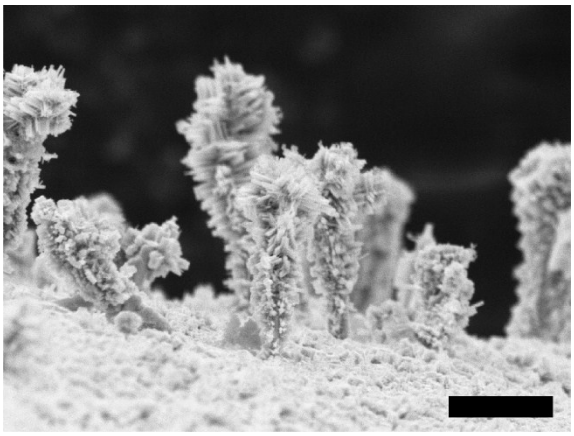

Fig. S12. SEM image of the Cu_3PNW-Cu electrode serving as negative electrode after 12 h EDC operation. The scale bar is 1 μm.

3. Tables

Table S1. General information of the lake water.

Name	Location	pH	Total dissolved solids (mg L^{-1})	Conductivity (uS cm^{-1})
Allatoona Lake	34°08'27.6"N 84°40'37.6"W	6.7	83	170

Table S2. Energy consumption of the EDC operation.

Flow rate[a] (mL h^{-1})	Applied voltage (V)	Current (μA)	Energy Consumption[b] (J L^{-1})
142	1	45	1.2

[a] Flow rate = Flux × area

Flux = 2000 $L \cdot h^{-1} \cdot m^{-2}$; r = 0.475 cm; Area = $\pi \times r^2$ = 0.708 cm^2

[b] Energy Consumption = (Applied Voltage × Current) / Flow rate.

Reference:

1. Allen, M.J., S.C. Edberg, and D.J. Reasoner, *Heterotrophic plate count bacteria—what is their significance in drinking water?* International journal of food microbiology, 2004. **92**(3): p. 265-274.