Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Electrochemical Inactivation of Enteric Viruses MS2, T4, and Phi6 using doped Laser-Induced Graphene Electrodes and Filters

Akhila M. Nair[1], Ashish Kumar[2], Najmul H. Barbhuiya[2], Swatantra P. Singh[1,2,3]

[1] Centre for Research in Nanotechnology & Science (CRNTS), Indian Institute of Technology Bombay, Mumbai 400076, India

[2] Environmental Science and Engineering Department (ESED), Indian Institute of Technology Bombay, Mumbai 400076, India

[3]Interdisciplinary Program in Climate Studies, Indian Institute of Technology Bombay, Mumbai 400076, India

Corresponding author's email: swatantra@iitb.ac.in

Scheme S1. Phage Purification, Propagation, and Enumeration

Bacteriophages MS2 and T4 were propagated and enumerated using the following steps. Initially, the host *E. coli* was grown to an exponential phase (OD_{600} = 0.6-0.8) in TSB at 37 °C. Then, the phages were propagated with the cultured *E. coli* suspension, wherein the phage-host mixture was added to the TSA media soft Agar (0.5 % agar). This mixture was overlaid onto the TSA media bottom agar (1.5 % agar), and incubated overnight at 37 °C. For further phage purification, the suspension medium (SM) buffer (NaCl, $MgSO_4.7H_2O$, 1 M Tris-pH 7.5, 2% gelation) was added to the plates that underwent complete lysis post-incubation and kept under shaking conditions at 4 °C for 2-3 hrs. The SM buffer with the virus particles was collected and centrifuged at 16,000 rpm for 30 minutes. Again, the supernatant containing the phage was collected and passed through 0.22 μM bacteriological filters to obtain a completely purified phage and stored at 4 °C. The purified viral stocks (10^{11} PFU mL^{-1}) were quantified using the double-layer agar method and adjusted to a concentration of ~10^9 PFU mL^{-1} in 0.9 % saline solution for further experiments. [1]

Bacteriophage Phi6 was propagated and enumerated using a similar protocol as mentioned above. However, the host was grown to an exponential growth phase (OD_{600} = 0.6-0.8) in TSB at 25 °C, and further, the phage-host mixture was added with the TSA media soft Agar (0.5 % agar) and then overlaid onto the TSA media bottom agar (1.5 % agar), and incubated overnight at 25 °C. The purified phage stocks (10^8 PFU mL^{-1}) were quantified using the double-layer agar method and stored at 4 °C for further experiments.

Scheme S2. Fabrication of LIG and titanium suboxide LIG electrodes and filters

Fabrication of LIG and LIG-TiO_x electrodes and filters was carried out, as explained in our earlier studies.[2,3] Briefly, for LIG electrodes, thin films of PES and TiO_2-doped PES sheets of three different concentrations (2.5%, 5%, and 10 % wt. of PES) were synthesized. LIG was made on the surface using a VLS 2.30 (Universal Laser) laser platform equipped with a 10.6 μm CO_2 pulse laser (30 W, 2.0 in. Lens Kit). LIG and LIG-TiO_x electrodes with dimensions 3 cm x 1 cm and 0.5 cm gap between anode and cathode were fabricated onto the PES and TiO_2 doped-PES sheets, respectively to a laser setting with an image density (ID) of 6, 140 PPI (pulses per inch), 15% power, and 25% scan rate under ambient conditions. The conductive copper tape was attached to the electrodes using carbon conductive ink (EnK, Tineshwar Labs Pvt Ltd) followed by the application of epoxy (Araldite-fast set) to protect and strengthen the electrical connection.

For LIG filters, PES membranes were synthesized using the phase inversion technique and lased with the laser settings of 1000 PPI, ID 6, 9% power, and 25% scan rate under ambient conditions. In the case of LIG-TiO_x filters, the filters were synthesized by the addition of 10% TiO_2 (10% wt. of PES) to the PES solution. Circular coupons of LIG filters with 46 mm diameter were fabricated and connected to the carbon thread using carbon-based glue. Epoxy was applied on the top to protect and strengthen the connection.

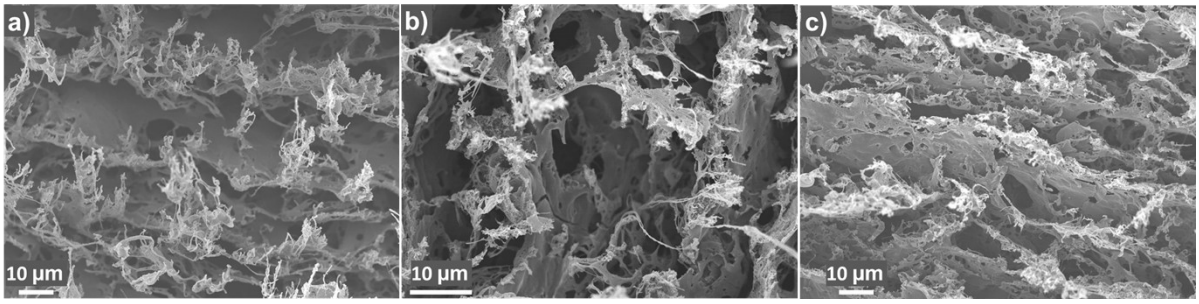

Figure S1. SEM images of (a) LIG (b) LIG-TiO_x2.5 (c) LIG-TiO_x5

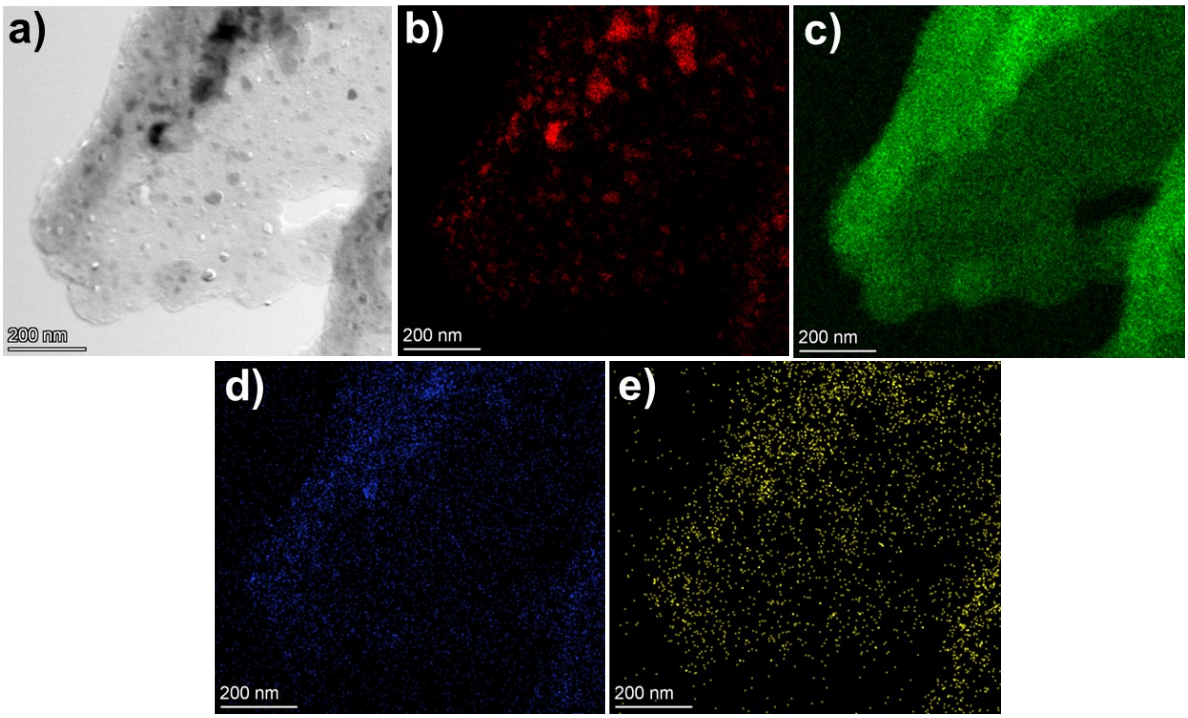

Figure S2. EDS mapping of LIG-TiO_x10 electrode. (a) TEM image of the mapping area. (b) Titanium mapping. (c) Carbon mapping. (d) Oxygen mapping. (e) Sulfur mapping.

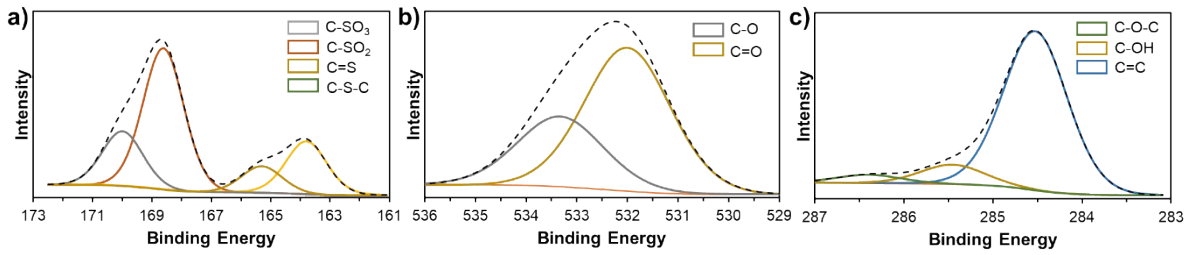

Figure S3. XPS narrow scan spectra of (a) S 2p (b) O 1s (c) C 1s for LIG electrodes.

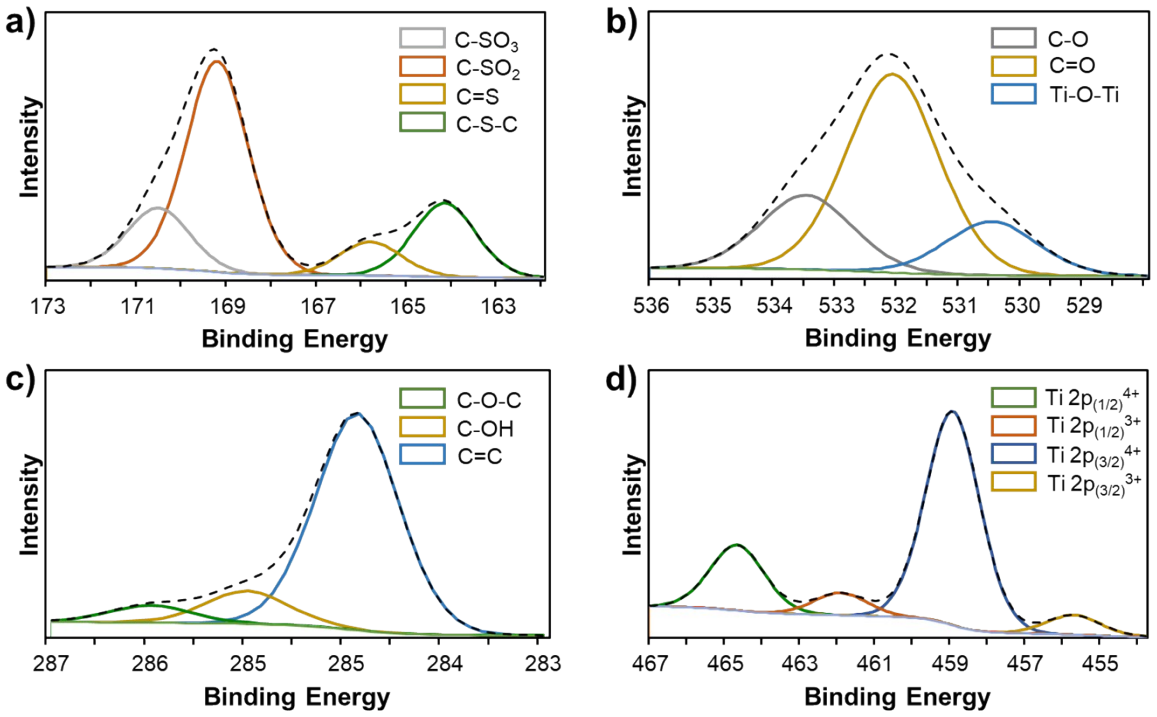

Figure S4. XPS narrow scan spectra of (a) S 2p (b) O 1s (c) C 1s (d) Ti 2p for LIG-TiO_x10 electrodes.

Scheme S3. Electrochemically Active Surface Area (EASA)

The electrochemically accessible surface area can be estimated from the double-layer capacitance (C_{dl}) obtained from the cyclic voltammetry measurement.[4] The CV curves were plotted as a function of various scan rates (20, 40, 60, 80, 100 mV/s) (Figure S3), and the C_{dl} was calculated. The linear regression slope of the difference in the current density (Ja-Jc/2) in the centre of the potential window versus scan rates calculates the C_{dl}.

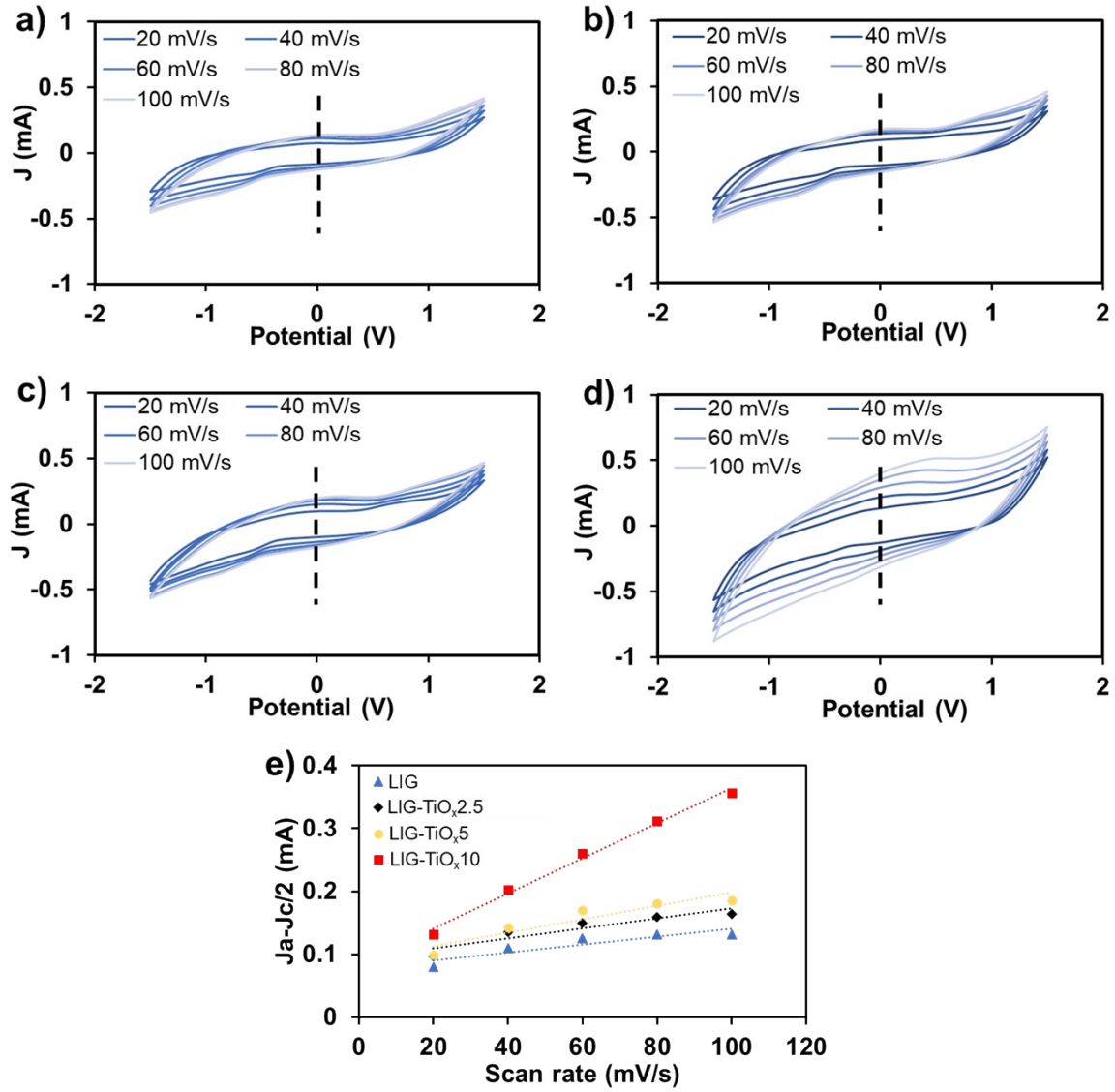

Figure S5. CV curves at a potential window from -1.5 V to 1.5 V at scan rates of 20, 40, 60, 80, 100 mV/s for (a) LIG (b) LIG-TiO_x2.5 (c) LIG-TiO_x5 (d) LIG-TiO_x10. (e) Linear regression of the difference in the current density in the centre of the potential window for LIG and all doping concentrations.

Table S1. Double layer capacitance of LIG and LIG-TiOx electrodes

Samples	Double-layer capacitance (C_{dl}) (mF cm^{-2})
LIG-TiO_x10	2.79
LIG-TiO_x5	1.06
LIG-TiO_x2.5	0.79
LIG	0.63

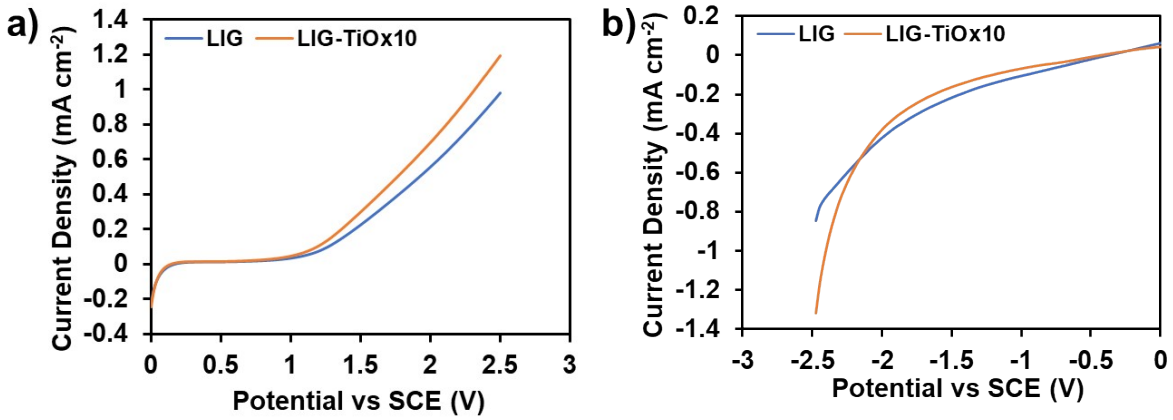

Figure S6: LSV of the LIG and LIG-TiO_x electrodes (a) OER (b) HER at a scan rate of 5 mV/s

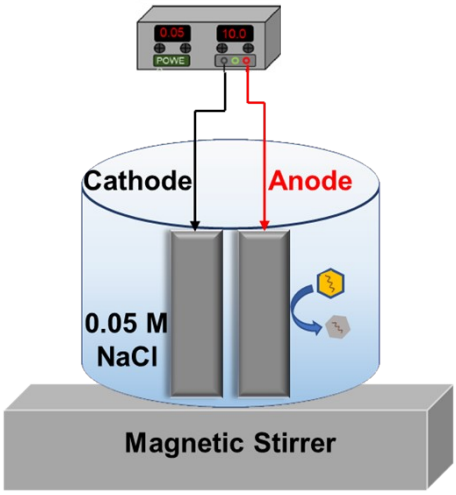

Figure S7. Schematic illustration of batch setup using LIG electrodes.

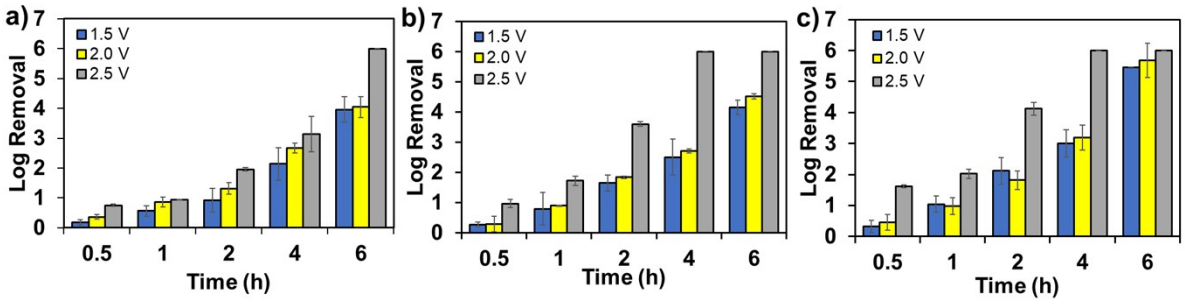

Figure S8. Inactivation of MS2 phage at 1.5-2.5 V using (a) LIG (b) LIG-TiO_x2.5 (c) LIG-TiO_x5 electrodes.

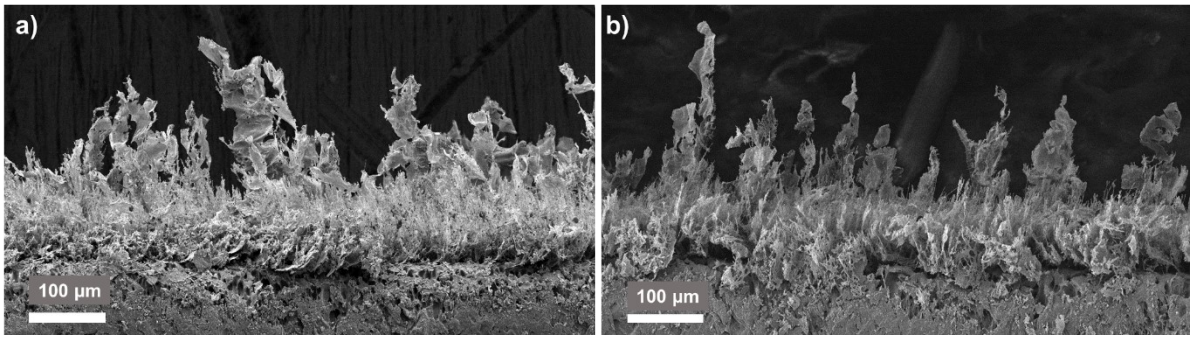

Figure S9. Cross section SEM image of (a) LIG-TiO_x10 (b) LIG electrodes.

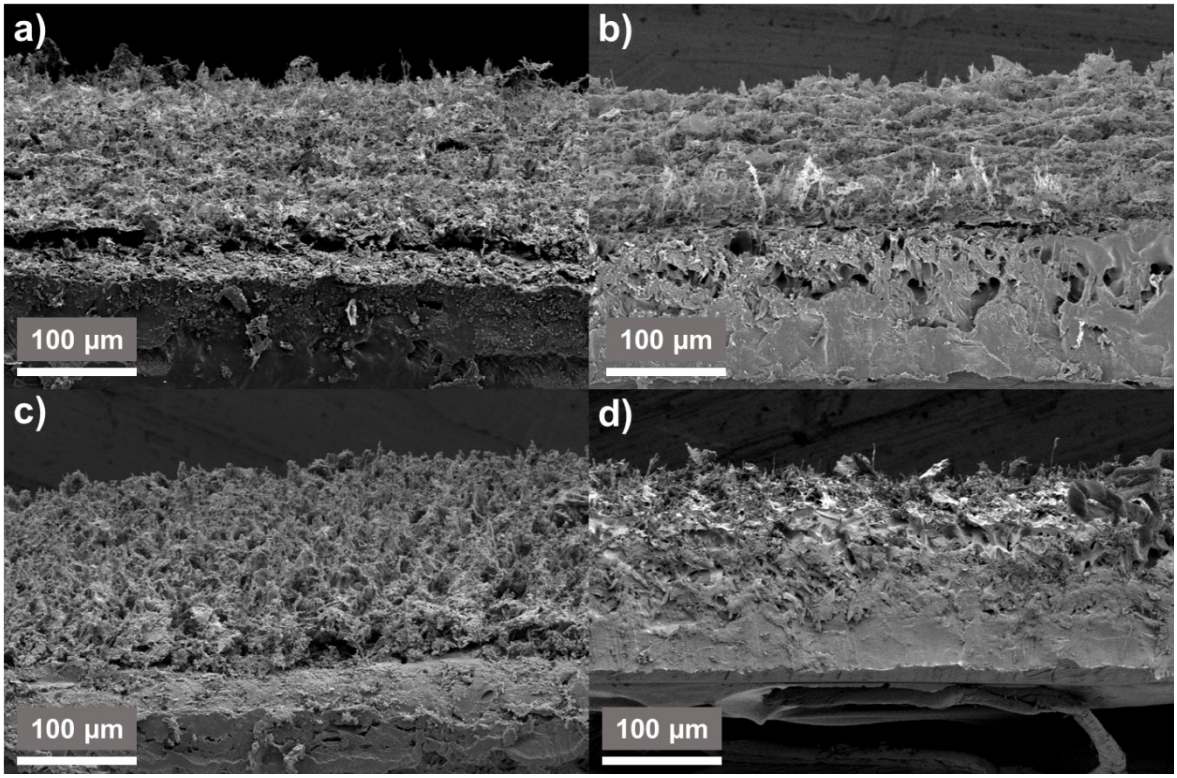

Figure S10. Cross section SEM image of LIG-TiO_x10 electrodes after 6 hours of operation at 1.5 V (a) anode (b) cathode and 2.5 V (c) anode (d) cathode.

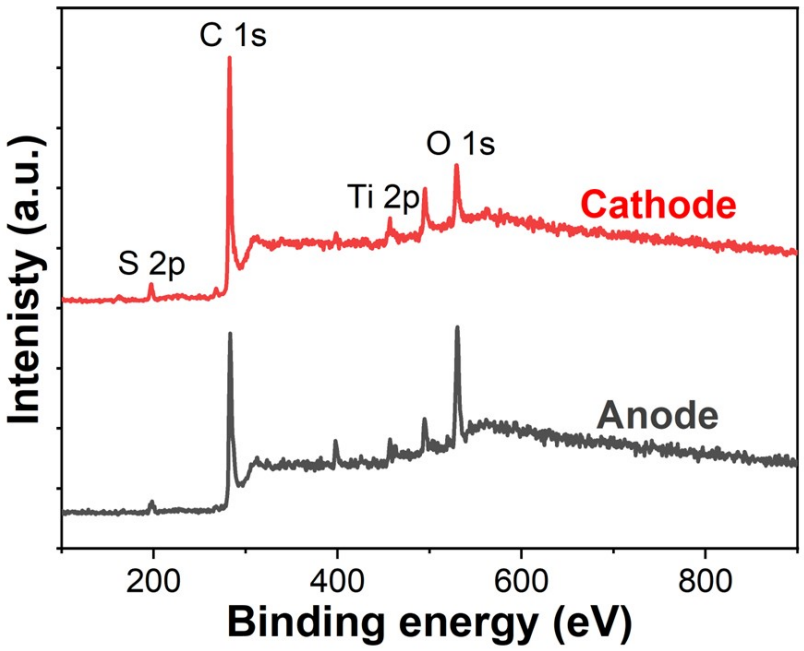

Figure S11. XPS wide spectra of LIG-TiO_x10 electrodes after 6 hours of operation at 1.5 V.

Table S2: Surface chemical composition of LIG-TiO_x10 electrodes.

Surfaces	Carbon (%)	Oxygen (%)	Sulfur (%)	Titanium (%)
Normal LIG-TiO_x	85.53	11.35	1.77	1.36
Anodic LIG-TiO_x10	75.65	22.35	0.3	1.65
Cathodic LIG-TiO_x10	83.6	14.2	0.9	1.3

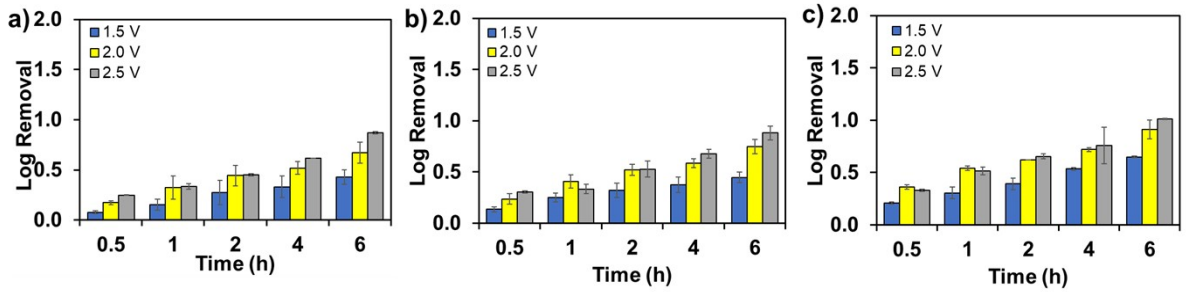

Figure S12. Inactivation of T4 phage at 1.5-2.5 V using (a) LIG (b) LIG-TiO_x2.5 (c) LIG-TiO_x5 electrodes.

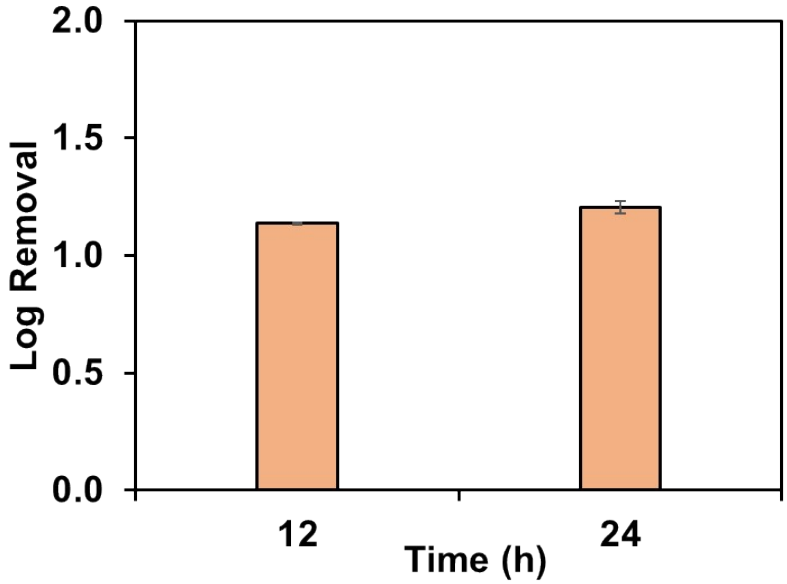

Figure S13. Inactivation of T4 at 2.5 V using LIG-TiO_x10 for prolonged treatment time.

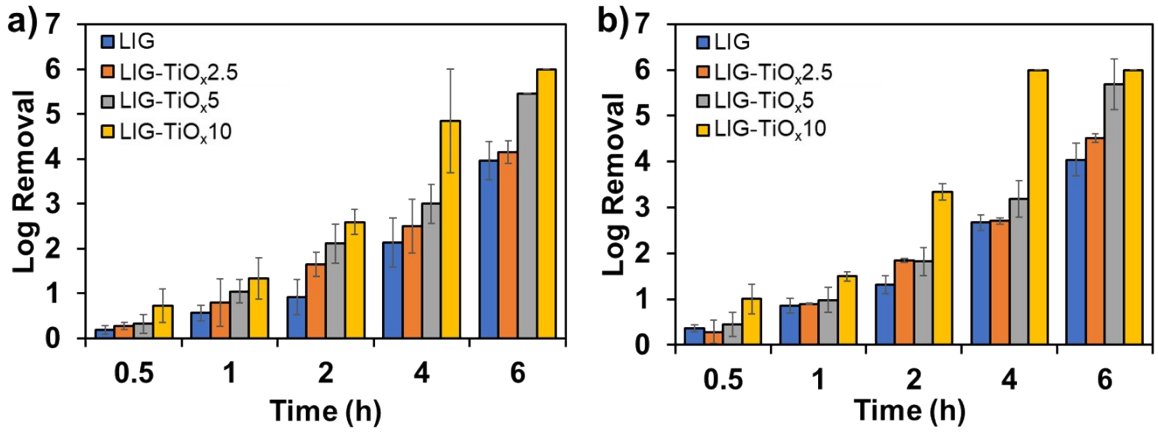

Figure S14. Inactivation of MS2 phage using LIG and doped LIG-TiO_x electrodes at (a) 1.5 V (b) 2.0 V.

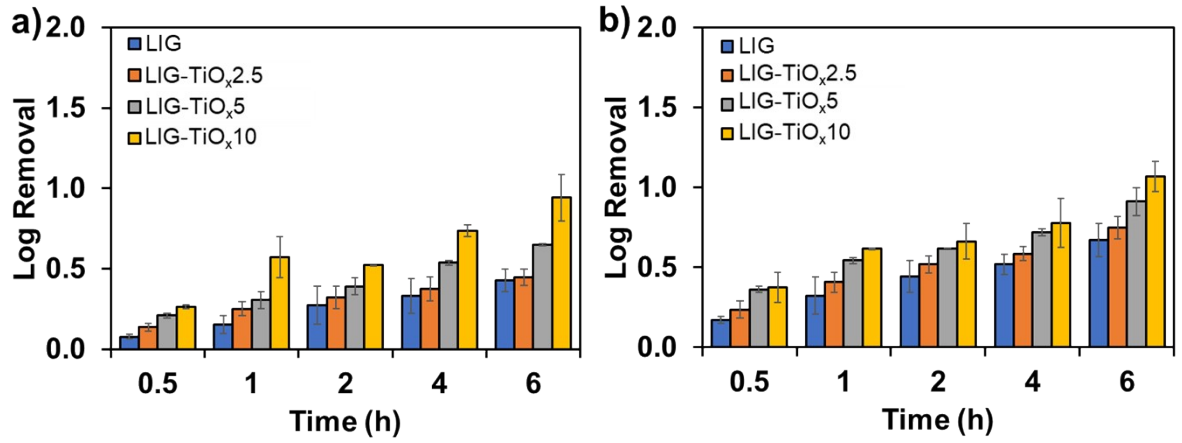

Figure S15. Inactivation of T4 phage using LIG and doped LIG-TiO_x electrodes at (a) 1.5 V (b) 2.0 V.

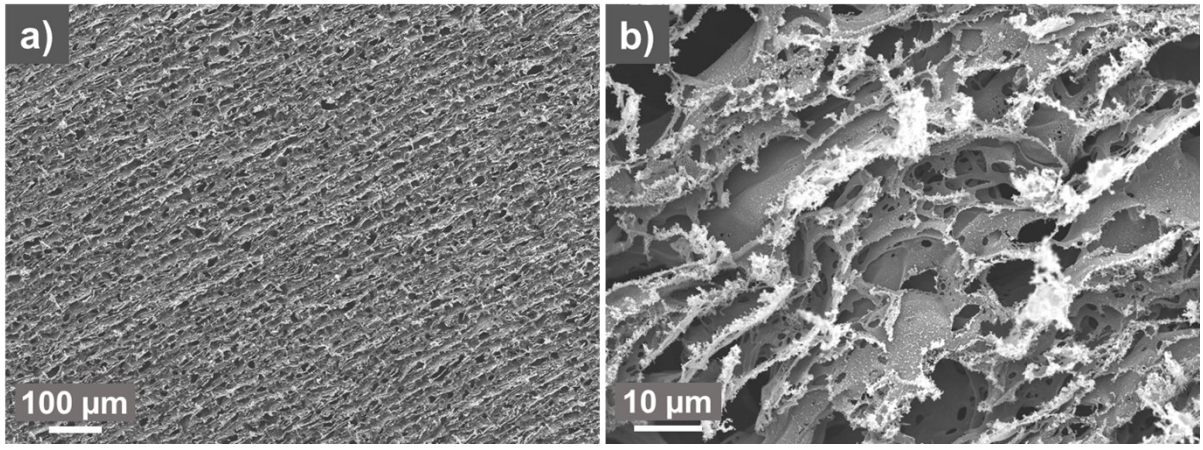

Figure S16. SEM image of LIG filters at (a) low resolution (b) high-resolution

Scheme S4: Electrochemical Disinfection of Viruses with Surface Water

The surface water was collected from Powai Lake, Mumbai. The water sample was initially filtered using 8 µM Whatman filter paper and then a 0.2 µM filter to remove the suspended particles and microorganisms. For the disinfection experiment, the water was spiked with ~10^6 PFU mL^{-1} of MS2 bacteriophage, and 100 mM NaCl was added to improve the solution's conductivity as the lake water had low conductivity of ~285 µS/cm. Further, a vacuum pump passed the solution through the filters at a constant flow rate of ~500 $Lm^{-2}h^{-1}$ at various voltages.

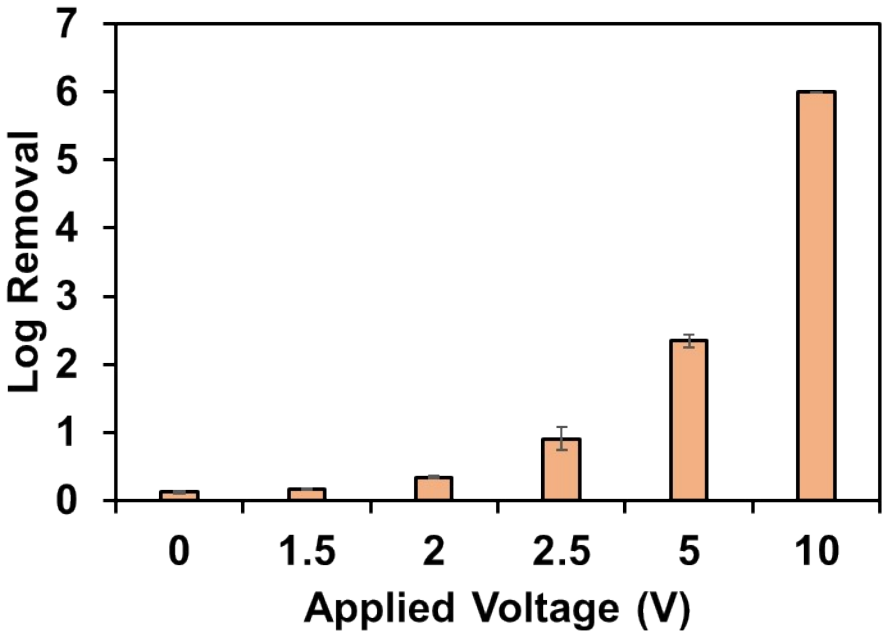

Figure S17: Inactivation of bacteriophage MS2 in surface water at a constant flow rate of 500 $Lm^{-2}h^{-1}$.

Scheme S5: Inactivation of MS2 bacteriophage by electrosorption

The MS2 bacteriophage solution was passed through the filters to allow for the viruses to get adsorbed at 0 V and 1.5 V. At 0 V, the top and bottom filters, and at 1.5 V, both anode and cathode adsorbed with viruses were desorbed by washing them with 20 mL of 0.9% NaCl solution and sonication for 2 min to remove the viruses from the filter surface. The total MS2 electrosorbed is calculated from the difference between the desorbed virus at 1.5 V and 0 V. In order to avoid the interference of other inactivation mechanisms, such as electrooxidation, electrosorption was not checked at voltages beyond 1.5 V. The culturable viruses were enumerated using the plaque assay. The percent inhibition of viruses was calculated which exhibited merely ~0.25 % removal of viruses, depicting that electrosorption's was insignificant.

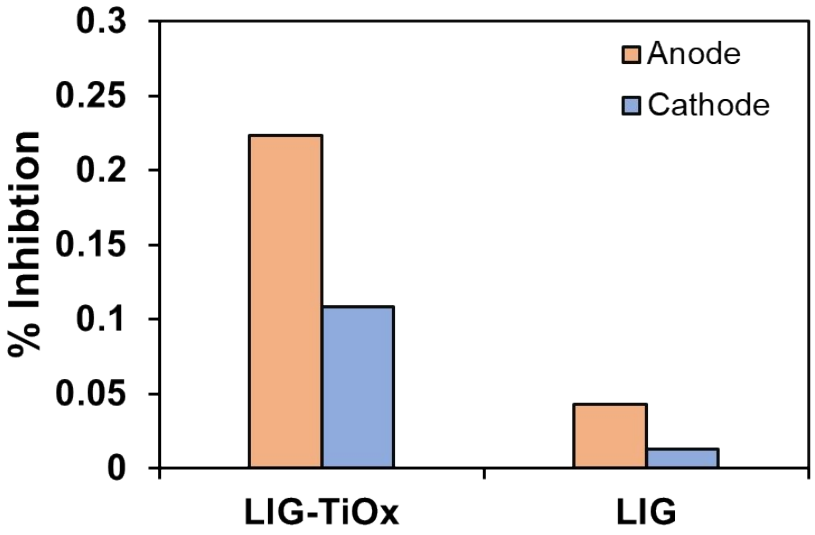

Figure S18: Percent inhibition of MS2 bacteriophage due to electrosorption on the surface of LIG-TiO_x and LIG filters.

Scheme S5. Generation of oxidants at anode and cathode.

Indirect oxidation

At anode:[5–7]

$$A + Cl^- \rightarrow A(\bullet Cl_{ads}) + e \quad (1)$$

$$A(\bullet Cl_{ads}) \rightarrow 2A + Cl_2 \quad (2)$$

$$Cl_2 + H_2O \rightarrow HClO + HCl \quad (3)$$

$$HClO \rightarrow ClO^- + H^+ \quad (4)$$

At cathode:[8]

$$O_2 + 2H_2O + 2e^- \rightarrow H_2O_2 + 2OH^- \qquad E° = 0.695 \text{ V vs SHE} \quad (5)$$

References

1. Kropinski AM, Mazzocco A, Waddell TE, Lingohr E, Johnson RP. Enumeration of bacteriophages by double agar overlay plaque assay. Methods Mol Biol. 2009;501:69–76.

2. Kumar A, Barbhuiya NH, Jashrapuria K, Dixit N, Arnusch CJ, Singh SP. Magnéli-Phase Ti 4 O 7 -Doped Laser-Induced Graphene Surfaces and Filters for Pollutant Degradation and Microorganism Removal . ACS Appl Mater Interfaces. 2022 Nov 23;14:52448–58.

3. Barbhuiya NH, Singh SP, Makovitzki A, Narkhede P, Oren Z, Adar Y, et al. Virus Inactivation in Water Using Laser-Induced Graphene Filters. Materials. 2021;14(12):3179.

4. Yang X, Li W, Ai T, Bao W, Dong H, Jiang P, et al. An efficient hydrogen evolution by self-supported nickel sulfur-based hybrid nanoplate electrocatalyst. Ionics (Kiel). 2022 Jan 1;28(1):353–60.

5. Jeong J, Kim C, Yoon J. The effect of electrode material on the generation of oxidants and microbial inactivation in the electrochemical disinfection processes. Water Res. 2009;43(4):895–901.

6. Rajkumar D, Kim JG. Oxidation of various reactive dyes with in situ electro-generated active chlorine for textile dyeing industry wastewater treatment. J Hazard Mater. 2006 Aug 21;136(2):203–12.

7. Sun M, Wang X, Winter LR, Zhao Y, Ma W, Hedtke T, et al. Electrified Membranes for Water Treatment Applications. ACS ES&T Engineering. 2021 Apr 9;1(4):725–52.

8. Mase K, Ohkubo K, Fukuzumi S. Efficient two-electron reduction of dioxygen to hydrogen peroxide with one-electron reductants with a small overpotential catalyzed by a cobalt chlorin complex. J Am Chem Soc. 2013 Feb 20;135(7):2800–8.