Supporting information:

13 pages, 12 figures included in this document

A Systematic Approach to In-depth Understand Photoelectrocatalytic Bacterial Inactivation Mechanisms by Tracking the Decomposed Building Blocks

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The preparation method of highly oriented TiO_2 nanotube anode. The Ti foil was pre-anodized for 4 h at 30 V in a mixture of ethylene glycol, 0.5 M HAc, and 0.2 M NH_4F, sonicated in deionized water and anodized for another 24 h at 30 V in the same electrolyte. After washed with deionized water, the anodized Ti foil was finally annealed at 500 ºC for 2 h with a heating and cooling rate of 2 ºC min^{-1} to obtain the TiO_2 nanotubes. The average inner-diameter and length of the TiO_2 nanotubes (anatase) were 70 nm and ~6.9 μm, respectively. With linear sweep voltammetry assay, in 0.2 M $NaNO_3$ under illumination of 365 nm and 10 $mWcm^{-2}$, saturated photocurrent density of 1.75 mA cm^{-2} was obtained at +1 V. For more information of the TiO_2 nanotube anode, please refer to our previous report.[1]

Methods for $[H_2O_2]$ and [•OH]ss determination. The H_2O_2 concentration in PEC system under different pH conditions was measured based on the decolorization of Methyl Orange (MO) caused by •OH oxidation generated from Fenton reaction.[2] To be specific, 1.8 mL of samples were taken at regular time and mixed with 0.2 mL 100 mg L^{-1} MO. After the pH of the mixture was adjusted to 2.5 with 0.1 M H_2SO_4, twenty microliters of 0.05 M $FeSO_4$ was added to initialize the reaction. The absorbance at 507 nm ($OD_{507\ nm}$) was recorded at 0 and 2 min of the reaction, respectively. The decrease of $OD_{507\ nm}$ was proportional to the concentration of H_2O_2.

p-chlorobenzoic acid (*p*CBA) was used as probe for steady state •OH concentration ([•OH]ss) determination considering that it reacts with •OH at high rate and selectivity,[3,4] The concentration of the probe was monitored at 240 nm by HPLC equipped with a DIKMA C18 column (250 mm × 4.6 mm × 5 μm), and the mobile phase consisted of 55% acetonitrile and 45% water containing 0.1% phosphoric acid at 0.8 mL min^{-1}. The initial concentration of

*p*CBA ($c_{0,pCBA}$) was 20 μM.

Procedures for intracellular ROSs level assays with fluorescent probe DCFH-DA. 5 ml of cell suspension inactivated for regular time was withdrawn from the 10^7 CFU mL^{-1} disinfection system and 5 μl 1 mM DCFH-DA was added immediately. The mixture was vortexed and incubated at 37 °C for 20 min in dark place. Cell suspension was further concentrated to achieve stronger fluorescent signals with tubular ultrafiltration modules (Amicon® Ultra-4 10K，Millipore) to a final volume of 200 μL and pipetted into a microplate for fluorescent measurement at 525 nm with excitation at 488 nm.

Catalase (CAT) and superoxide dismutase (SOD) activity analysis. Eight mL of *E. coli* suspension (1.1×10^7 CFU mL^{-1}) after different treatment (PEC/PC/EC) was filtered through 0.22 μm filters (SCBB-207, Shanghai ANPEL Scientific Instrument Co., Ltd., China) to harvest the cells. One hundred microliters of B-PER® Bacterial Protein Extraction Reagent (Pierce Biotechnology, USA) was mixed with the cells thoroughly and incubated for 15 min at room temperature. Lysozyme and DNase I was added into the B-PER reagent prior to the extraction at final concentration of 0.1 mg mL^{-1} and 5 U mL^{-1}, respectively, as well as Protease Inhibitor Cocktail (BS380, Sangon Biotech, Shanghai, China) at 1/20 dilution. The lysate was centrifuged at 10,000 rpm for 1 min, and the supernatant was ready for CAT and SOD activity assays using Catalase Assay Kit (S0051, Beyotime Institute of Biotechnology, China) and Superoxide Dismutase Assay Kit (Item No. 706002, Cayman Chemical, USA), respectively. One unit of catalase was defined as the amount of enzyme needed to catalyse the decomposition of 1 μM H_2O_2 in 1 min at 25 ºC when pH = 7, and one unit of SOD was defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical

when the assay was performed following the instruction of the kit.

β-D-galactosidase activity assay. Aliquots of 0.5 mL samples were taken at regular time of PEC, PC or EC treatment, and mixed with 0.5 mL 2×PBS. 0.2 mL of 5 mM *o*-nitrophenyl-β-D-galactopyranoside (ONPG) was added to start reaction at 37 °C with vibration for 60 min, and the absorbance at 420 and 550 nm was measured after the reaction was ended with 0.5 mL Na_2CO_3 (1 M). The β-D-galactosidase enzyme activity was calculated according to Eq. S1.

$$\text{ONPG hydrolysis rate} = \frac{OD_{420} - 1.75OD_{550}}{T \times \varepsilon_{ONP}} \times \frac{1.7mL}{0.5mL} \quad \text{(S1)}$$

ONPG hydrolysis rate (nmol min^{-1} mL^{-1}); OD_{420} and OD_{550} are the absorbance at 420 and 550 nm, respectively; ε_{ONP} represents the molar absorption coefficient of *o*-nitrophenol (ONP, OD_{420} nM^{-1}) and T is the total enzymatic reaction time (min). For cell permeabilization, one drop of 0.1% sodium dodecyl sulfate (SDS) and two drops of chloroform was pipetted into the mixture of 0.5 mL cells and 0.5 mL 2×PBS. The mixture was votexed vigorously and incubated at 37 °C for 2 min before the addition of ONPG.

SEM sample preparation protocol. The cover slides of 5 mm×5 mm were soaked with 0.5 M H_2SO_4 for 24 h and washed with water before coated with 0.1% polylysine. Bacterial cells were harvested by centrifugation and prefixed with 2.5% glutaraldehyde for 4 h at room temperature. Then the cells were attached on the coated glass slides and immersed into 0.1% phosphate-buffered saline for 20 min, and this step was repeated for six times. Dehydration was performed with a graded series of ethanol (30% for 15 min,50% for 15 min, 70% for 12 h, 90% for 15 min and then 100% for15 min 3 times). The solvent was finally replaced with

100% butyl alcohol three times each for 15 min, and the specimens were freeze dried and gold sputter coated before visualized with FESEM.

Protein SDS-PAGE. Ten mL of 10^8 CFU mL^{-1} bacterial suspension was collected from the disinfection systems at regular intervals, and the bacteria was harvested by ultrafiltration and centrifugation. The protein content of the harvested bacteria was extracted with 45 μL B-Per reagent. Thirty microliters of the protein extracted was mixed with 10 uL 4×Protein SDS PAGE loading buffer (Takara) and boiled for 3 min. 30 μL boiled protein samples were loaded into the wells of the gel (Br-Pregel, 10% Ttis-HCl, 10 wells, Sangon Biotech, Shanghai, China). SDS-PAGE was performed at 100 V, 20 mA for 85 min in Tris-Glycine SDS running buffer. Protein was stained with Coomassie R-250 before graph capture.

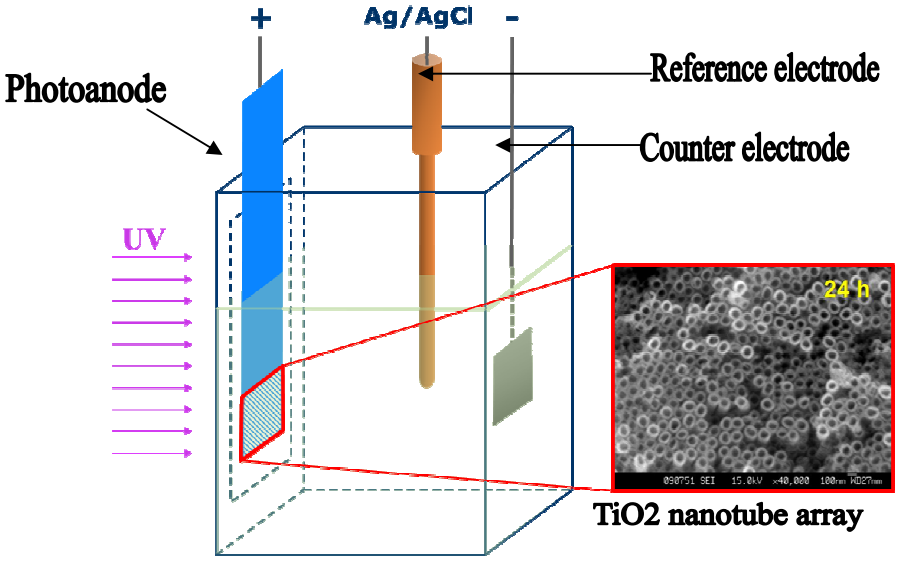

Figure S1 Schematic diagram of the bulk apparatus for PEC, PC and EC experiments.

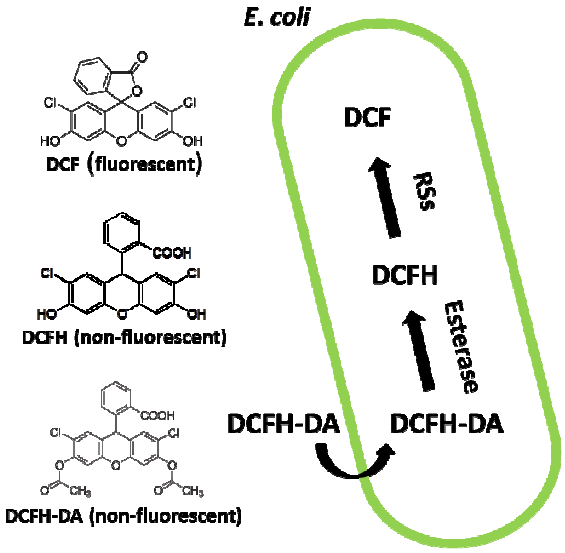

Figure S2 Mechanism of the fluorescent probe DCFH-DA for intracellular ROSs levels detection.[5] (DCF: 2′, 7′-dichlorofluorescein; DCFH: 2′, 7′-dichlorodihydrofluorescein; DCFH-DA: 2',7'-dichlorodihydrofluorescein diacetate)

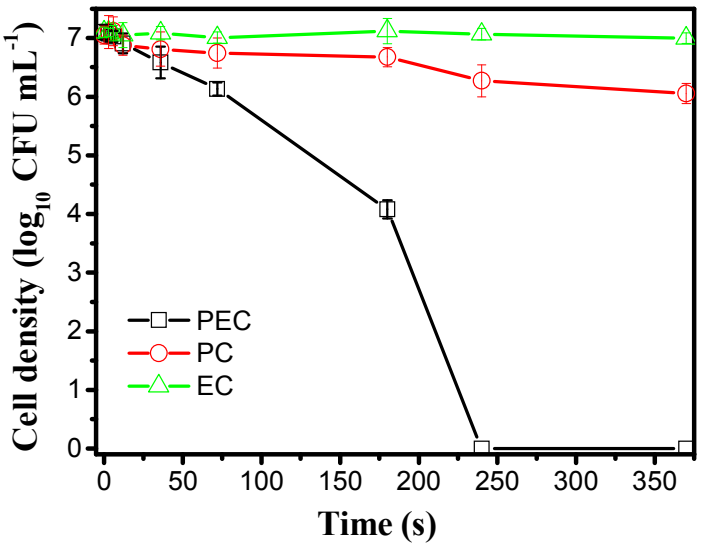

Figure S3 Cell viability during PEC, PC and EC inactivation of *E. coli* K-12 (1.10×10^7 CFU mL^{-1}) in thin-layer reactor, where the same experimental conditions as in the bulk PEC apparatus including photoanode, UV intensity and bias potential were adopted.

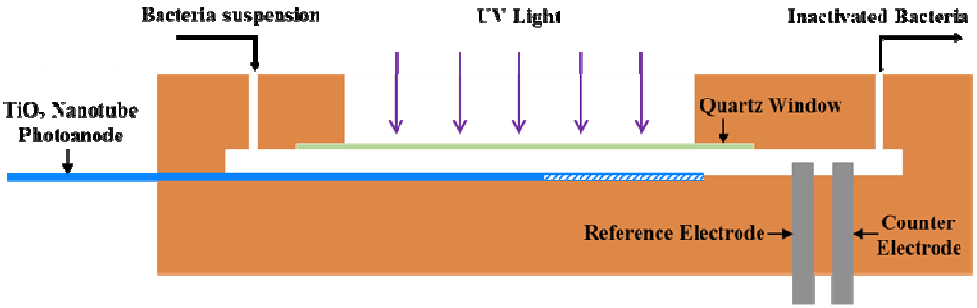

Figure S4 Schematic diagram of thin-layer reactor.

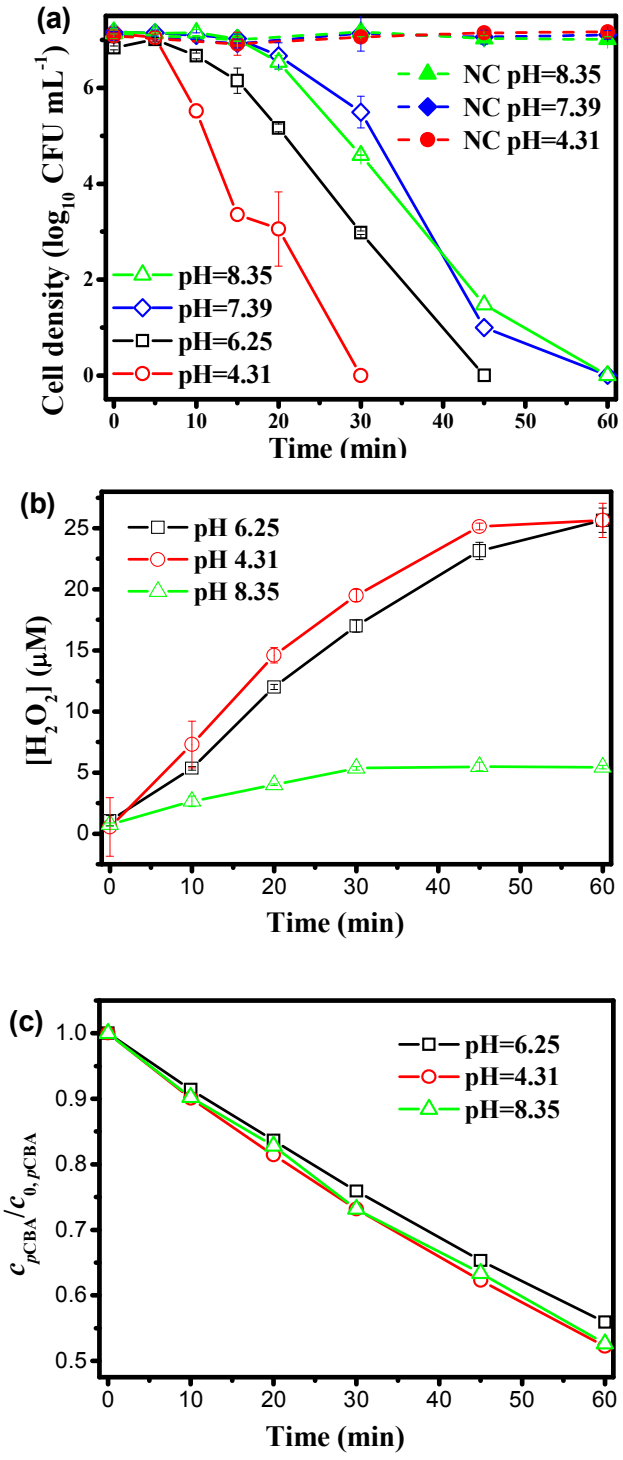

Figure S5 (a) The influence of pH on PEC inactivation of *E. coli* K-12, and Measurement of (b) H_2O_2 (c) $^{\bullet}OH$ concentration in PEC system at variable pH conditions.

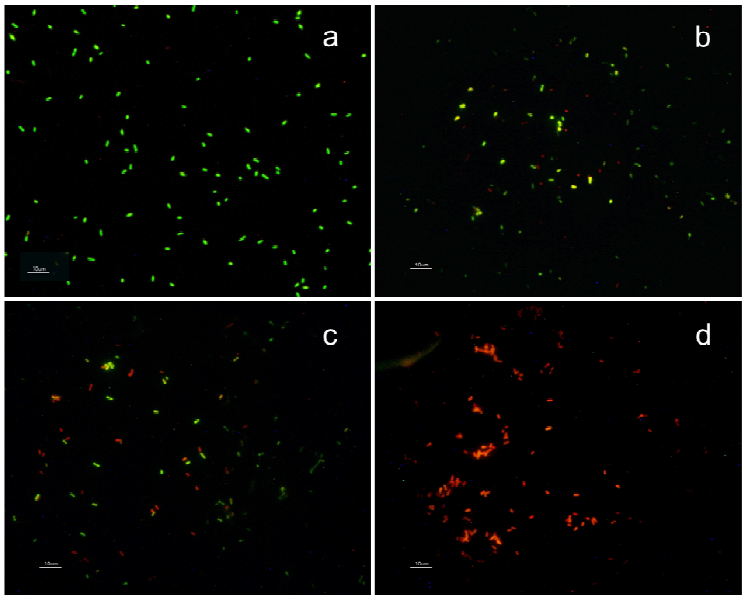

Figure S6 Fluorescent microscope images of PEC inactivated *E. coli* K-12 suspension stained with Live/Dead BacLight Bacteria Viability Kit. a: PEC 0 min; b: PEC 10 min; c: PEC 30 min; d: PEC 60 min;

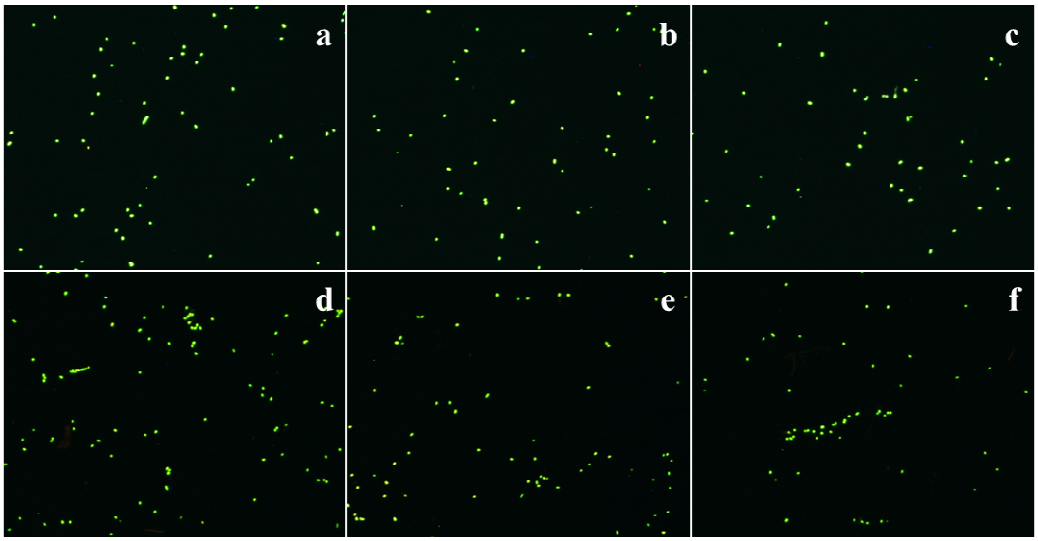

Figure S7 Fluorescent microscopic images of *E. coli* K-12 cells stained by Live/Dead BacLight Bacterial Viability Kit during PC and EC inactivation. a: EC 0 min; b: EC 30 min; c: EC 60 min; d: PC 0 min; e: PC 30 min; f: PC 60 min;

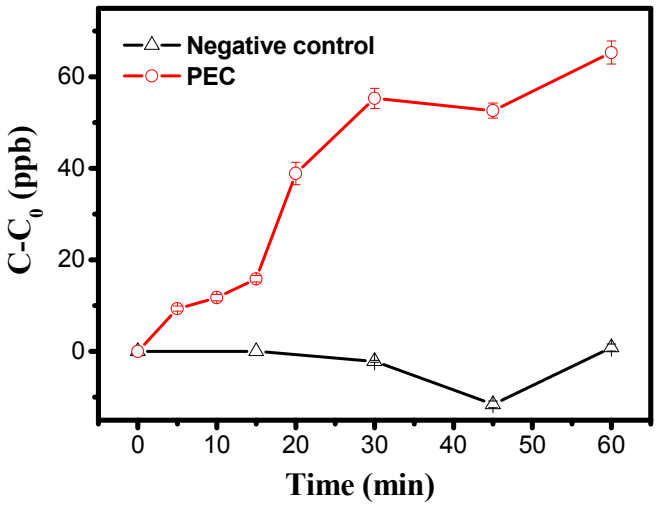

Figure S8. Potassium ion (K^+) leakage from *E. coli* K-12 cells during PEC inactivation of 1.10×10^7 CFU mL^{-1} suspension. The negative control was conducted without UV illumination or bias potential.

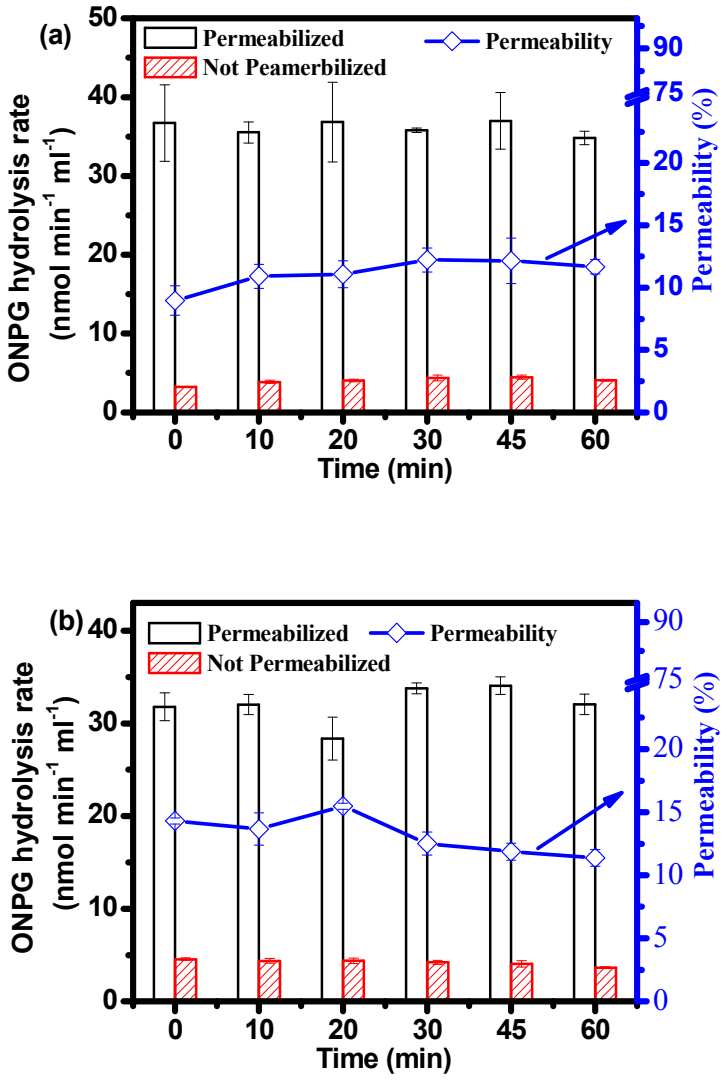

Figure S9 Cell membrane permeability of *E coli* K-12 cells in (a) EC and (b) PC disinfection processes determined by ONPG hydrolysis assay.

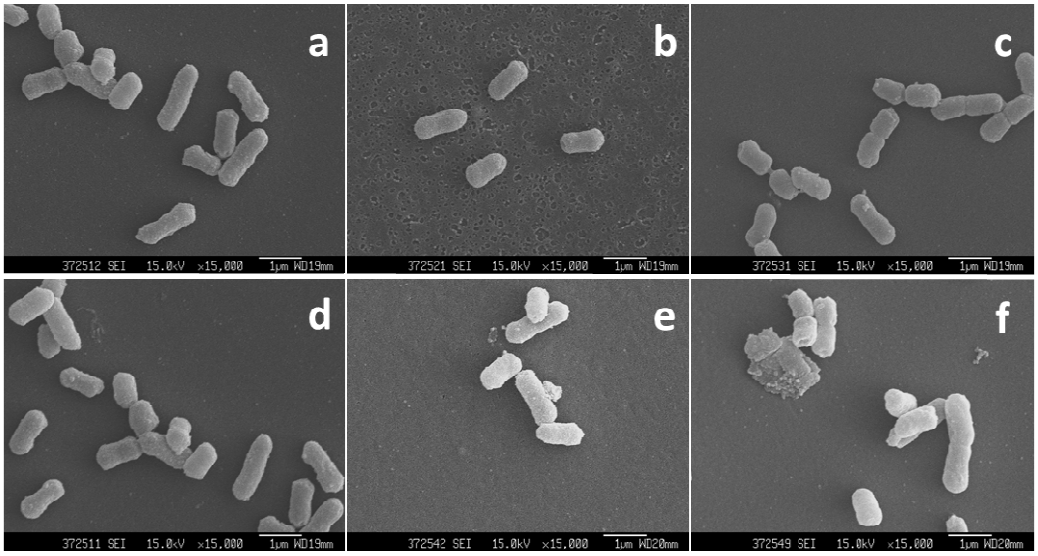

Figure S10 SEM images of *E. coli* K-12 cells treated by EC and PC systems. a: EC 0 min; b: EC 60 min; c: EC 120 min; d: PC 0 min; e: PC 60 min; f: PC 120 min.

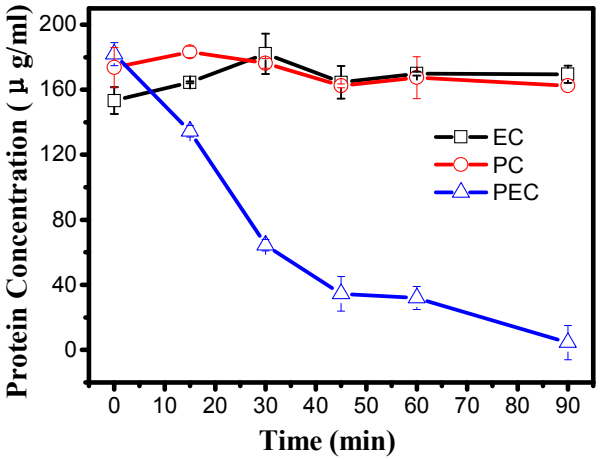

Figure S11 Protein concentration in 40 μL B-PER extraction from 8 mL *E. coli* K-12 cells (1.1×10^7 CFU mL^{-1}) during 90 min of PEC, PC and EC treatment.

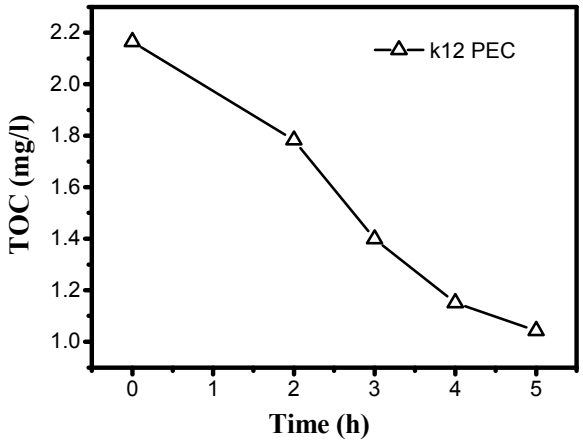

Figure S12 Monitoring of total organic carbon (TOC) of the *E. coli* K-12 cell suspension inactivated by PEC for 5 h (1.1×10^7 CFU mL^{-1} suspension).

References:

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