# Semi-quantitative performance and mechanism evaluation of carbon nanomaterials as cathode coatings for microbial fouling reduction

## Supplemental Information

Qiaoying Zhang, Joanne Nghiem, Gregory J. Silverberg, Chad D. Vecitis*

School of Engineering and Applied Sciences, Harvard University, Cambridge, MA 02138

*Corresponding author: Chad D. Vecitis,

Email: vecitis@seas.harvard.edu, Phone: (617) 496-1458.

Address: School of Engineering and Applied Sciences, Harvard University, Cambridge, MA 02138

**Table S1. Water quality in some representative aqueous environments.**

| | pH | Conductivity (mS/cm) | Total organic carbon (TOC, mg $L^{-1}$) |
|---|---|---|---|
| 1/8 TSB in 0.9% NaCl[a] | 7.48 | 17.55 | ~1100 |
| Charles River water (1) | 7.29 | 0.55 | 8.3 |
| Untreated domestic wastewater (2) | NA | NA | 160 |
| Baker's yeast wastewater (3) | 7.80 | 19.20 | 1061 |
| Wine distillery effluent (4) | NA | NA | 8300 |

a: Measured in lab with a pH probe (Thermo Scientific Orion™ 9810BN), conductivity meter (Oakton CON 150), and a TOC analyzer (Shimadzu TOC-Vw).

**Table S2. Cell recovery by vortexing.**

| | Control | Vortex 0.5 min | Vortex 1 min | Vortex 2 min |
|---|---|---|---|---|
| Recovery (%) | | 43% | 87% | 96% |
| Dead ratio (%) | 15% | 4% | 10% | 18% |

The experiments were carried out by depositing the *P. fluorescens* on a CNT membrane with vacuum filtration (2 psi pressure drop) and the control was completed by directly observing the CNT surface. Inactive (non-motile) cells may have been vacuum filtered more strongly onto the CNT membrane and thus were less effectively removed by vortexing.

**Table S3. The Welch's t-test summary of the total bacterial adhesion on the control coatings.**

| P-value | CNT | OA-CNT | O-CNT | CB |
|---|---|---|---|---|
| CNT | | | | |
| OA-CNT | **0.005** | | | |
| O-CNT | **0.000** | 0.112 | | |
| CB | **0.000** | **0.049** | 0.308 | |
| rGO | **0.000** | **0.034** | 0.119 | 0.737 |

a: The average bacterial density on the control coatings ranged from high to low is CNT > OA-CNT > O-CNT > CB > rGO.

b: The P-values lower than 0.05 are in **bold**.

As a comparison, the bacteria adhesion on a polished Ti substrate was $(0.95 \pm 0.20) \times 10^6$ $cm^{-2}$ (lower than CNT and greater than other CNM) and the dead ratio was 23% (slightly greater than CB and lower than other CNM).

**Table S4. The Welch's t-test summary of the total bacterial adhesion on a CNT coating as a function of voltage and incubation time.**

| | P-value to the control | | | |
|---|---|---|---|---|
| | 0.5 h | 3 h | 6 h | 12 h |
| −1.0 V | 0.323 | **0.017** | 0.187 | **0.028** |
| −2.0 V | 0.132 | **0.008** | 0.224 | **0.010** |

a: The P-values lower than 0.05 are in **bold**.

b: The 6 h data showed less significance (higher P-value) due to relative greater variations on the control.

**Table S5. Culturability of *P. fluorescens* on a CNT coating compared to the DAPI/PI staining results after 3 h incubation at 0, −1.0, and −2.0 V total voltage.**

| | Colony forming unit ($10^6$ $cm^{-2}$) | DAPI ($10^6$ $cm^{-2}$) | DAPI−PI ($10^6$ $cm^{-2}$) |
|---|---|---|---|
| Control | 0.78 ± 0.13 | 1.36 ± 0.20 | 0.77 ± 0.15 |
| −1.0 V | 0.08 ± 0.00 | 0.70 ± 0.15 | 0.17 ± 0.15 |
| −2.0 V | 0.07 ± 0.01 | 0.26 ± 0.07 | 0.09 ± 0.06 |

Analysis is completed after 3 h incubation at 30 ºC in 0.9% NaCl saline solution in 1/8 TSB. The samples were treated in the same way as described in the methods section of the manuscript using vortexed solutions of bacteria cells from the sample surfaces. The solutions were serially diluted with saline solution and the plate counting was completed using Bacto™ tryptic soy agar (Becton Dickinson) after incubation at 30 ºC.

From the table, the culturable bacteria density on the control well corresponds to the staining results by subtracting the PI stained cells from the DAPI stained ones. However, for the −1.0 V cathode, the culturable bacteria density ($0.08 \pm 0.00 \times 10^6$ $cm^{-2}$) was much smaller than the DAPI−PI ($0.17 \pm 0.15 \times 10^6$ $cm^{-2}$), while that for the −2.0 V cathode was similar. The results indicated that the CNT coating at −1.0 V caused more bacterial culturability loss apart from membrane rupture (i.e., some cells still have intact membranes, non-permeable to PI, but they lose the ability to reproduce), and at −2.0 V, the membrane integrity loss percent was similar to the culturability loss.

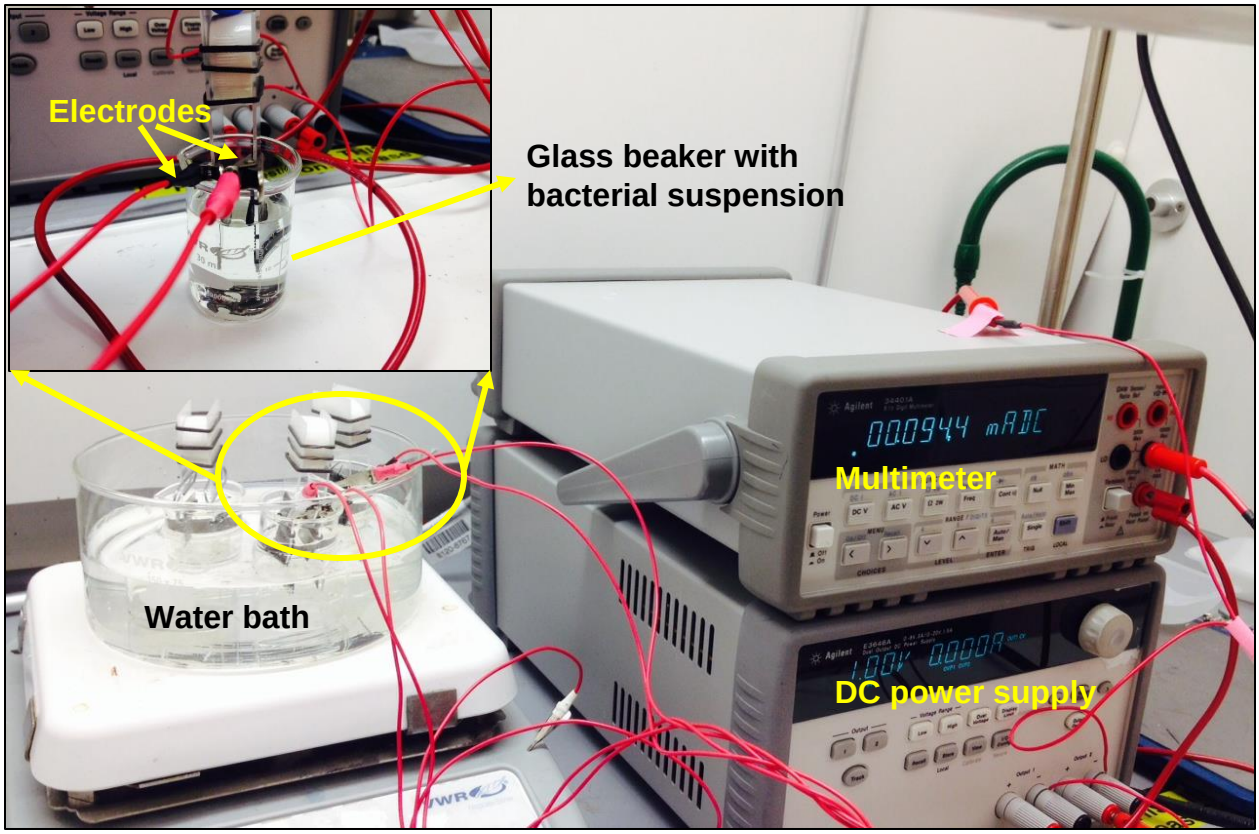


**Figure S1. An image of the bacteria experimental setup.**

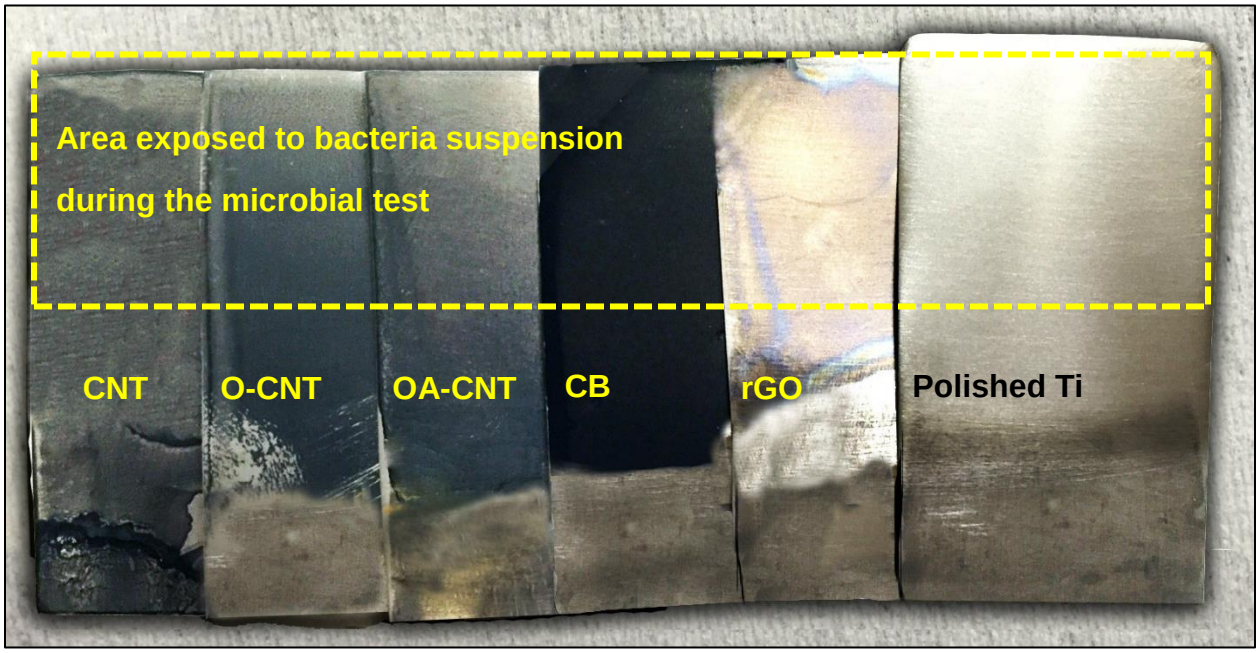


**Figure S2. Image of the polished Ti coupons with and without CNM coatings.** From left to right is CNT, O-CNT, OA-CNT, CB, rGO, and bare Ti surface, respectively.

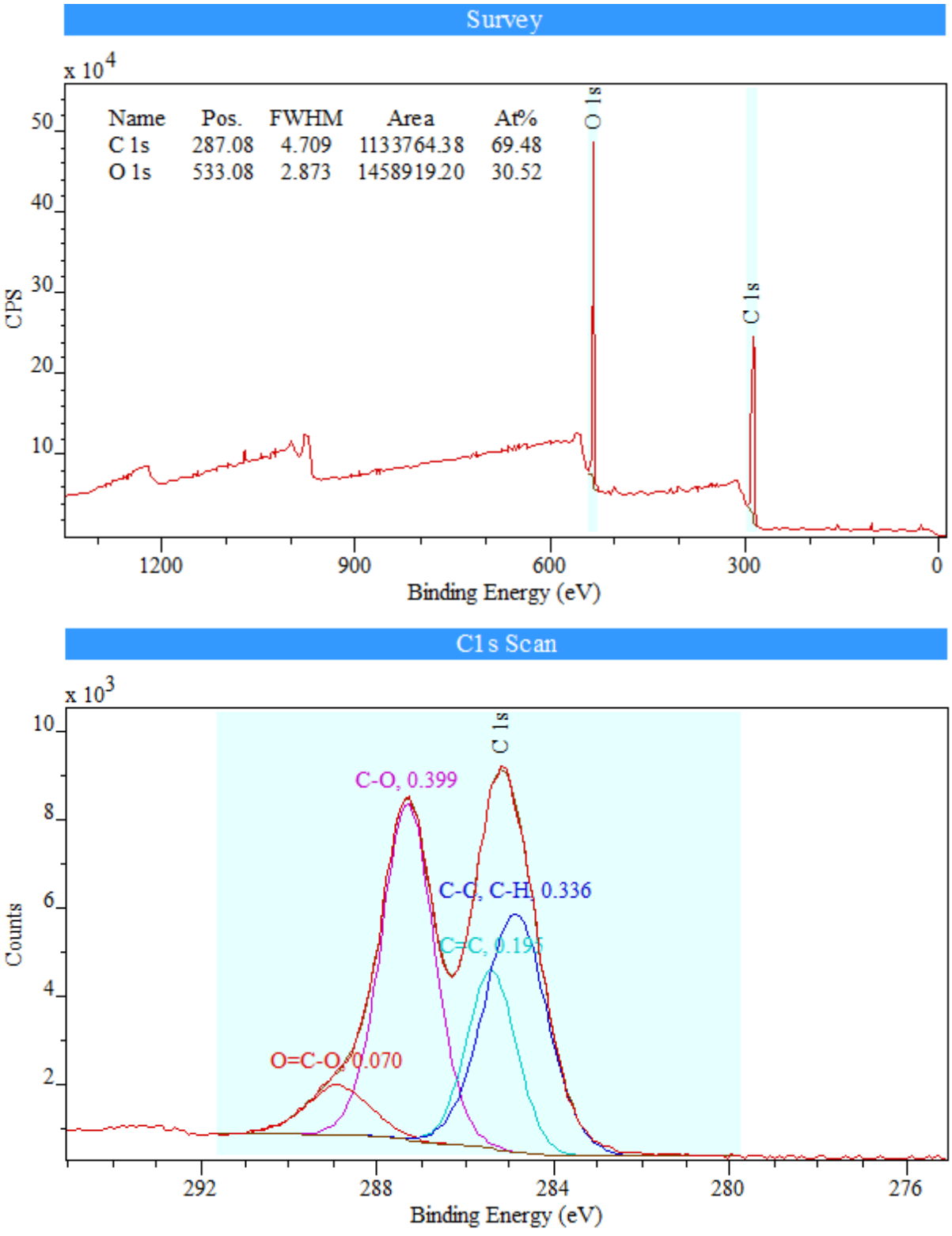


**Figure S3. The XPS files for the survey and C(1s) scan of the rGO sample without Nafion.**

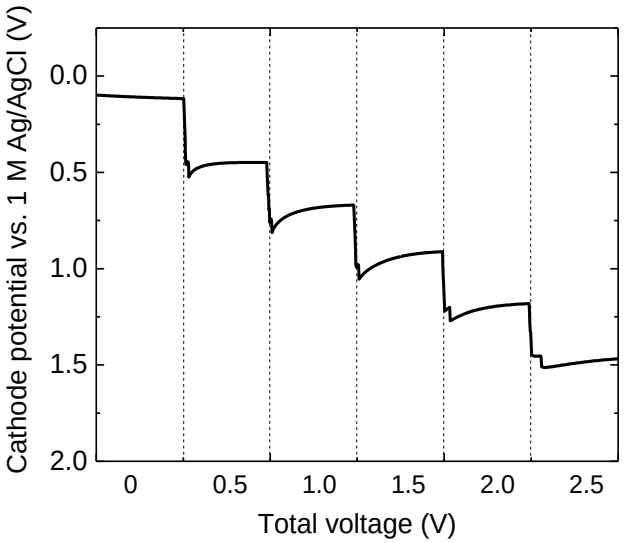


**Figure S4. Cathode potential of the CNT coating as a function of total voltage.** Analysis was completed in 0.9% NaCl saline solution with 1/8 TSB.

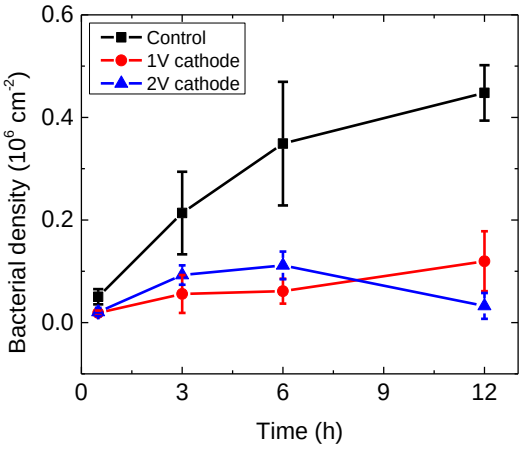


**Figure S5. *Escherichia coli* (*E. Coli*, ATCC® 700830TM) growth on the CNT surface as a function of total voltage and incubation time.** Total bacterial adhesion on the CNT surface with a total voltage of 0, −1.0, and −2.0 V after 0.5, 3, 6, and 12 h incubation at 30 ºC in 0.9% NaCl saline solution with 1/8 TSB.

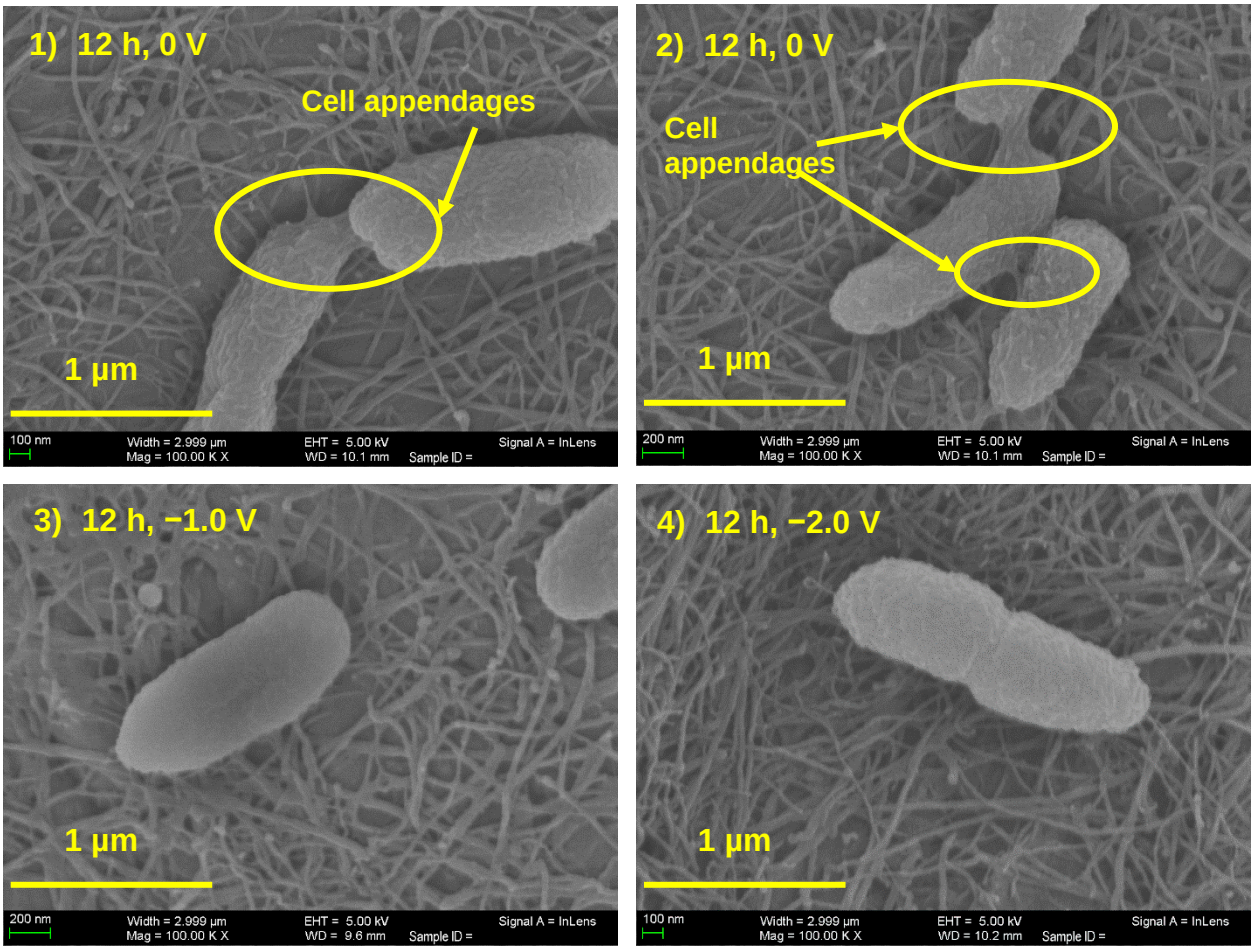


**Figure S6. SEM images of bacterial morphology on CNT cathode after 12 h incubation, a & b)** in the absence of voltage, **c)** at −1.0 V, and **d)** at −2.0 V at 30 ºC in 0.9% NaCl saline solution with 1/8 TSB.

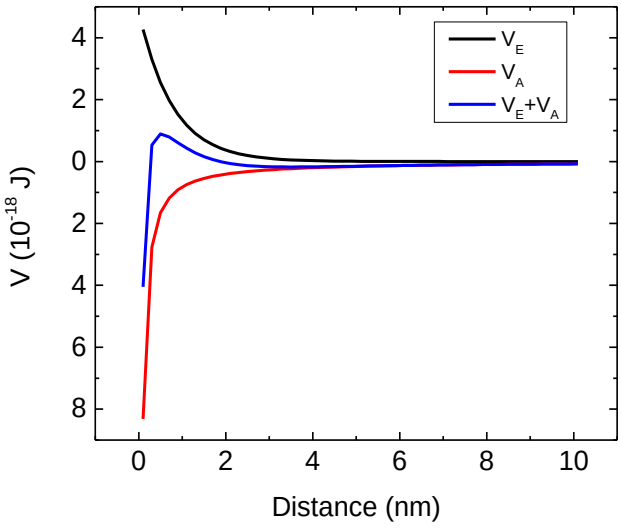

**Figure S7. DLVO energy profile for the electrode-bacteria interaction at a cathode potential of −0.4 V.**

The DLVO energy was calculated according to previously reported method (5). Briefly, it is assumed that the bacteria cells are spherical particles, the CNT coating cathode is a flat plate particle collector with a total area of the measured BET surface area, and that there are no direct interactions between the bacteria and coated surface. Thus, two equations are selected to quantify the van der Waals, Eq. S1, and electrostatic, Eq. S2, interactions:

$$V_E = \frac{128\pi a n_\infty kT}{\kappa^2}\gamma_1\gamma_2 exp(-\kappa h) \quad \text{(S1)}$$

$$V_A = -\frac{A}{6}[\frac{a}{h} + \frac{a}{h+2a} + ln(\frac{h}{h+2a})] \quad \text{(S2)}$$

Where $V_E$ and $V_A$ (J) are the potential energies for the electrostatic and van der Waals interactions, respectively, a (m) is the particle radius (assumed to be $1\times10^{-6}$ for bacteria cells), h (m) is the particle to surface separation distance, $n_\infty$ (# m-3) is the bulk number density of ions (# m-3), $\gamma_1$ and $\gamma_2$ denote reduced surface potential (tanh(zeφ/kT)) for the particle and the plate (dimensionless), κ ($m^{-1}$) represents the reciprocal Debye length, k ($m^2$ kg $s^{-2}$ $K^{-1}$) is the Boltzmann constant, A (J) denotes the Hamaker constant, and T (K) is the solution temperature and is assumed to be $10^{-20}$ J. The zeta potential of the *P. fluorescens* cells is -10.8 mV and is used as the surface potential directly. The potential of the electrode surface is calculated according to the following equations.

$$\varphi = -\frac{\mathrm{Ne}}{\kappa\lambda\varepsilon_0}\exp(-\mathrm{h}\kappa) \quad \text{(S3)}$$

$$\frac{1}{\kappa} = \sqrt{\frac{\lambda\varepsilon_0 kT}{n_\infty \nu^+(\nu^+ + \nu^-)e^2}} \quad \text{(S4)}$$

Where $\lambda$ (dimensionless) denotes the dielectric constant (80 for water at room temperature), $\varepsilon_0$ ($F\cdot m^{-1}$) is for the dielectric permittivity of vacuum, and $\nu^+$ and $\nu^-$ (dimensionless) denote valence of the electrolyte ions. The *Ne* (C $m^{-2}$) is the surface charge density measured by cyclic voltammetry method.


1. **U.S. Environmental Protection Agency Region I NE.** 1997. Charles River sediment/water quality analysis project report.
2. **Nazaroff WW, Alvarez-Cohen L.** 2001. Environmental engineering science. Wiley.
3. **Kobya M, Delipinar S.** 2008. Treatment of the baker's yeast wastewater by electrocoagulation. J Hazard Mater **154:**1133-1140.
4. **Bernet N, Habouzit F, Moletta R.** 1996. Use of an industrial effluent as a carbon source for denitrification of a high-strength wastewater. Appl Microbiol Biotechnol **46:**92-97.
5. **Zhang Q, Vecitis CD.** 2014. Conductive CNT-PVDF membrane for capacitive organic fouling reduction. J Membr Sci **459:**143-156.