energies

Supplementary Materials

Bioelectrosynthetic conversion of CO_2 using different redox mediators: electron and carbon balances in a bioelectrochemical system

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Table S1. Estimation of electron recovered into biomass according to each cycle of headspace gas replacement.

Cycle	Control	NR	HNQ	HQ
2	3.00%	1.03%	2.93%	1.43%
4	3.34%	3.20%	4.53%	3.80%
6	4.38%	3.92%	5.76%	3.26%
8	3.28%	3.05%	4.37%	3.19%
10	2.89%	2.83%	3.44%	2.21%
12	2.67%	2.63%	2.69%	2.43%
14	2.54%	2.33%	2.46%	2.49%
16	2.28%	2.04%	2.38%	2.31%

Table S2. Estimation of carbon recovered into biomass according to each cycle of headspace gas replacement.

Cycle (days)	Control	NR	HNQ	HQ
2	-*	-*	-*	-*
4	0.34%	0.09%	0.34%	0.19%
6	1.52%	1.03%	1.47%	1.31%
8	2.92%	2.96%	3.98%	2.21%
10	3.30%	2.84%	2.97%	2.46%
12	4.17%	3.49%	2.83%	2.42%
14	4.11%	4.78%	3.60%	4.19%
16	3.20%	4.00%	4.73%	4.06%

* The carbon balance of the first cycle (i.e. first 2 day), was not estimated because the initially contained buffer component, $NaHCO_3$ (4g/L) might be counted for the carbon balance. However, this effect might decrease through the gas replenishment proceeded in the following cycles.

**The cell synthesis was estimated by the following equation [43, 44]

$$\frac{1}{5}CO_2 + \frac{1}{20}NH_4^+ + \frac{1}{20}HCO_3^- + H^+ + e^- = \frac{1}{20}C_5H_7O_2N + \frac{9}{20}H_2O$$

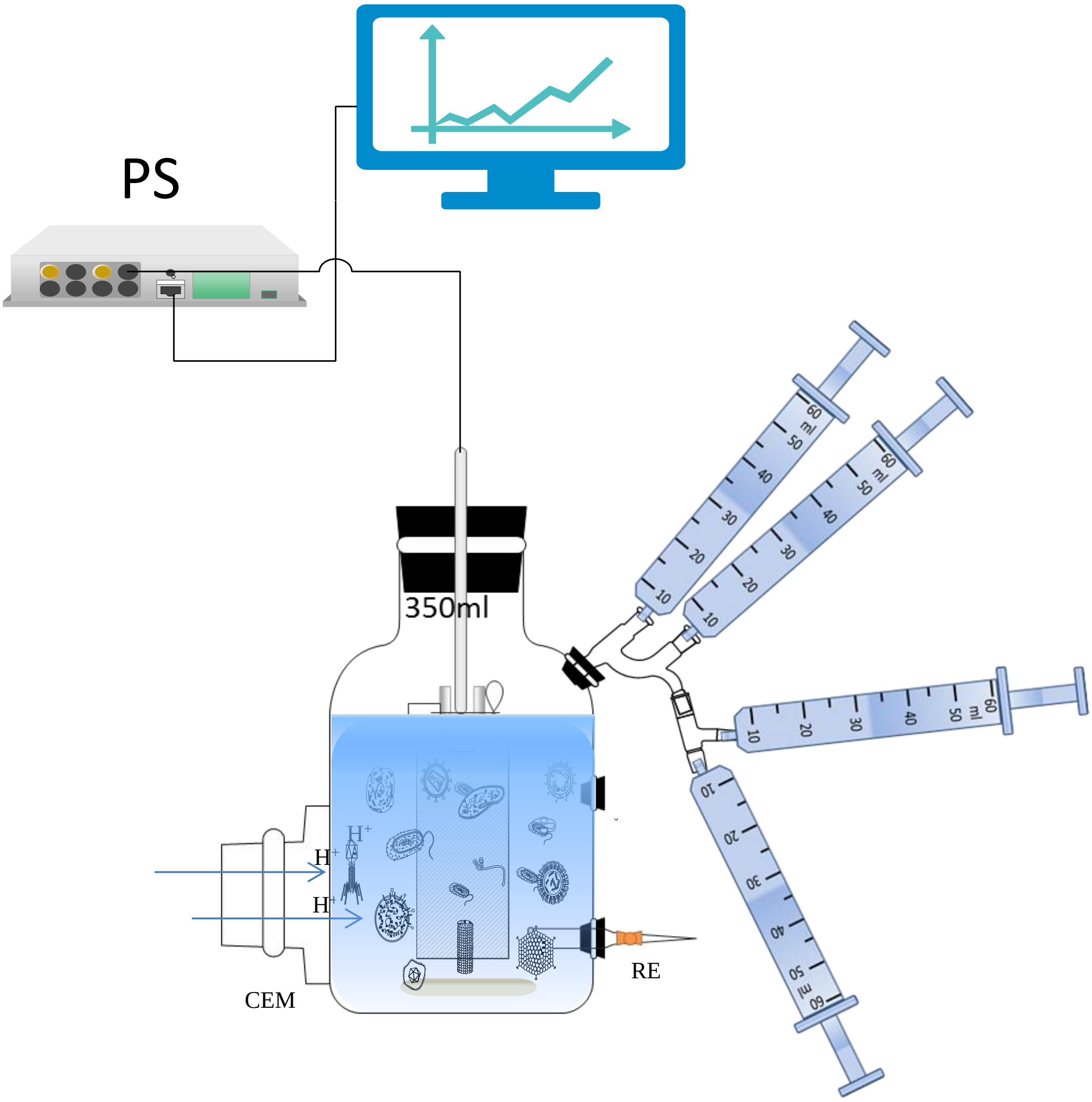

Figure S1. Schematic diagram of microbial electrosynthesis reactor. Four syringes were implemented to compensate the pressure change in the headspace, and simultaneously provide CO_2 for electrosynthesis.

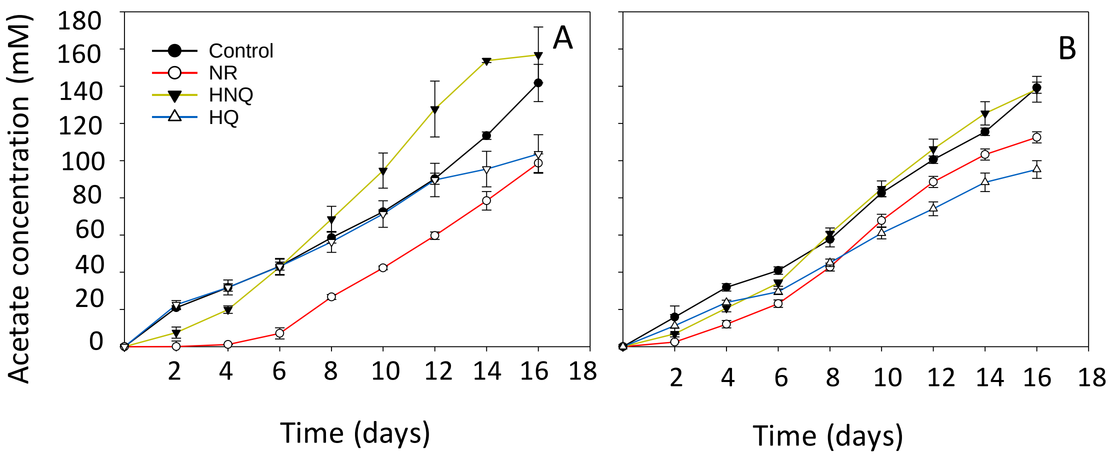

Figure S2. Acetate production at 0.5 mM (A) and 1 mM (B) of redox mediators during 8 cycles. Increase of mediator concentration resulted in negative effect on acetate concentration probably due to the toxic and inhibitory effect on electron transport.

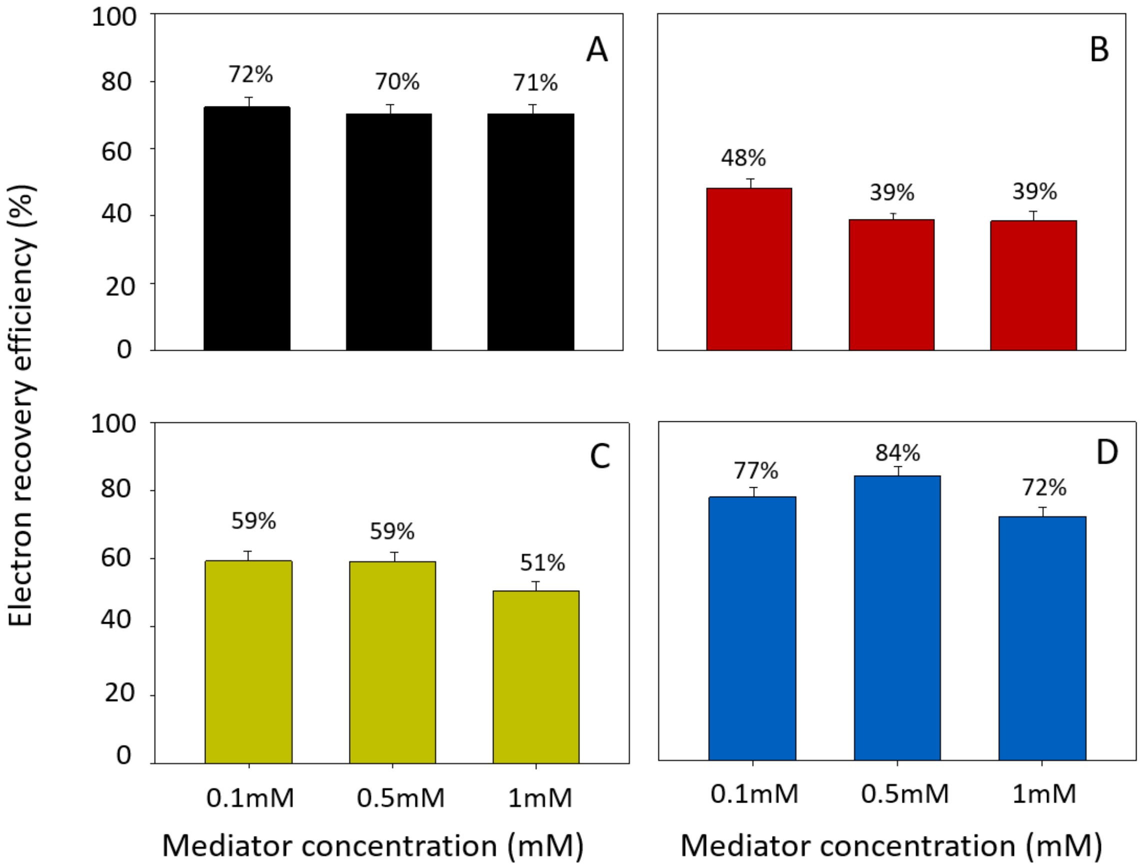

Figure S3. Average electron recovery into acetate with 0.1mM, 0.5mM, 1mM of redox mediators during 8 cycles. (A), Control; (B), NR; (C), HNQ; (D), HQ.

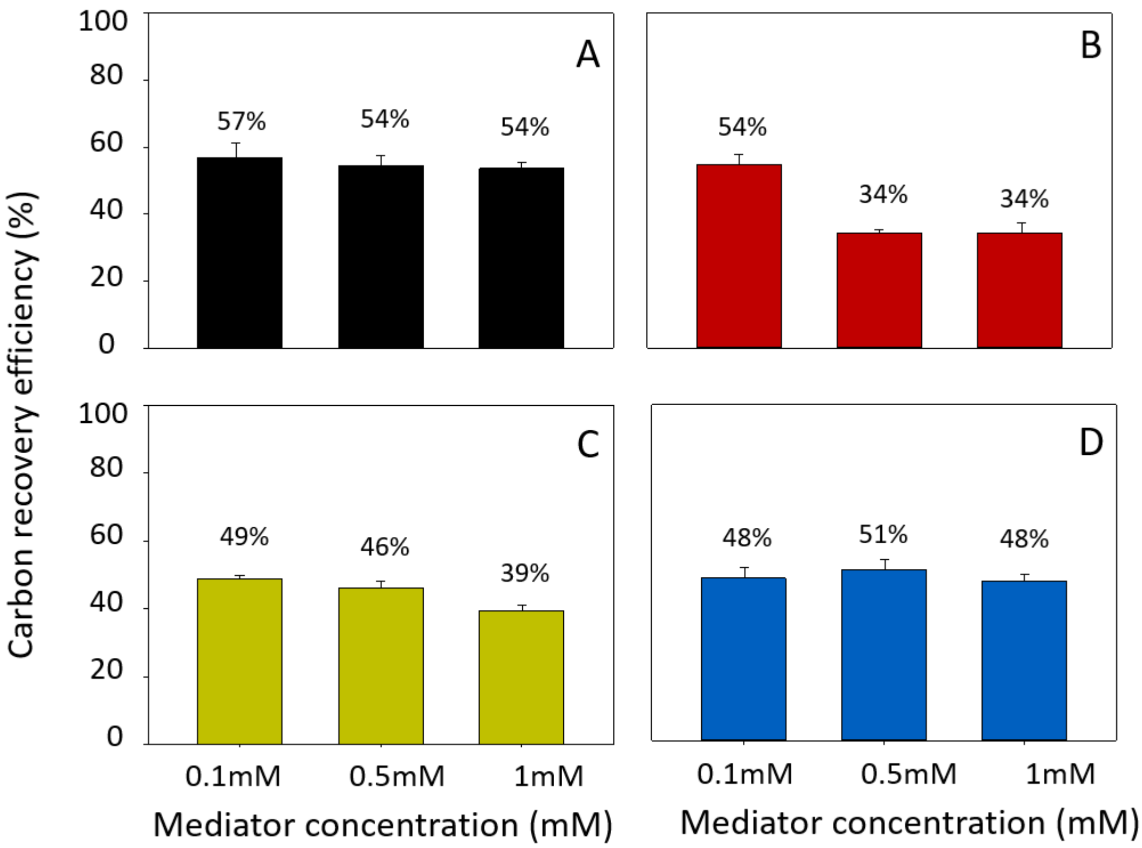

Figure S4. Average carbon recovery into acetate at 0.1mM, 0.5mM, 1mM of redox mediators during 8 cycles, (A), Control; (B), NR; (C), HNQ; (D), HQ.

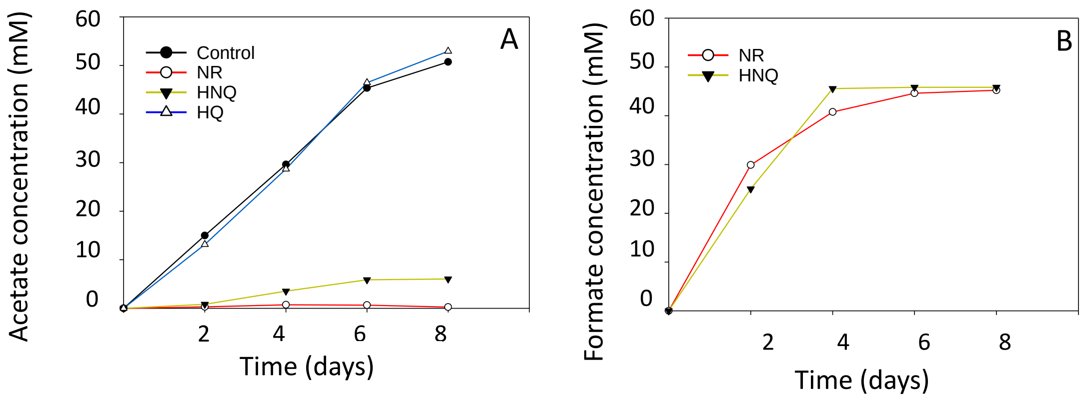

Figure S5. Acetate production with 7mM of redox mediators. (A), acetate; (B), formate production.

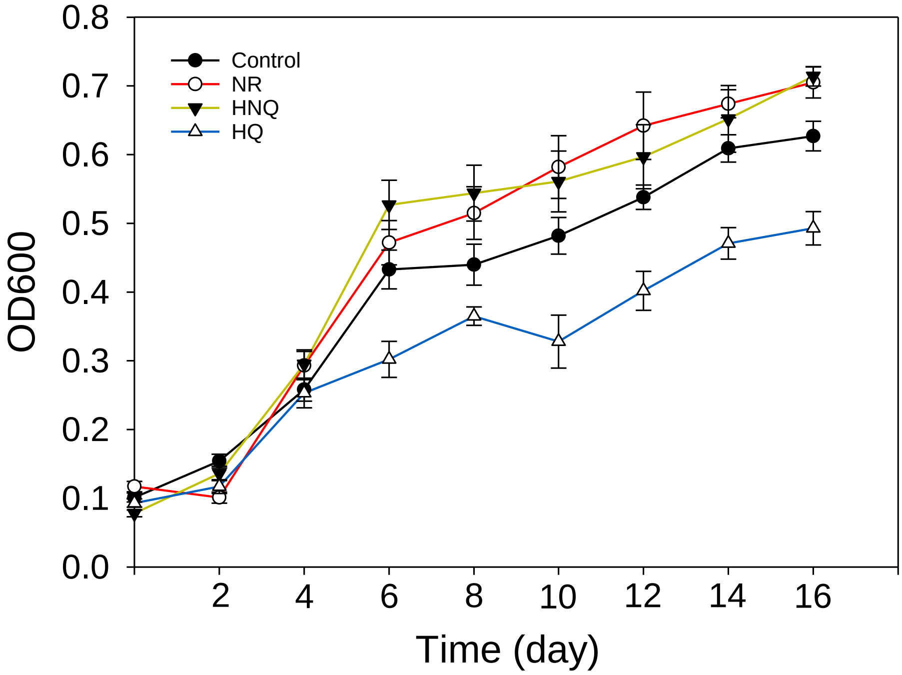

Figure S6. Cell growth (OD_{600}) profiles during electrosynthesis on each 8 cycles for 16 days.

References

1. Dudley, B. BP Statistical Review of World Energy Statistical Review of World. *BP Statistical Review*, London, UK 2019. Available online: https://www.bp.com/content/dam/bp/business sites/en/global/corporate/pdfs/energy-economics/statistical-review/bp-stats-review-2019-full-report.pdf. (accessed on 10 October 2019).
2. Woo, W.T.; Song, L. *China's Dilemma: Economic Growth, the Environment and Climate Change*; ANU Press: Canberra, Australia, 2008..
3. Zhou, J.; Zhang, F.; Meng, H.; Zhang, Y.; Li, Y. Introducing extra NADPH consumption ability significantly increases the photosynthetic efficiency and biomass production of cyanobacteria. *Metab. Eng.* **2016**, *38*, 217–227, doi:10.1016/j.ymben.2016.08.002.
4. Gong, F.; Zhu, H.; Zhang, Y.; Li, Y. Biological carbon fixation: From natural to synthetic. *J. CO_2 Util.* **2018**, *28*, 221–227, doi:10.1016/j.jcou.2018.09.014.
5. Berg, I.A.; Kockelkorn, D.; Buckel, W.; Fuchs, G. A 3-Hydroxypropionate/4-Hydroxybutyrate Autotrophic Carbon Dioxide Assimilation Pathway in Archaea. *Science* **2007**, *318*, 1782–1786, doi:10.1126/science.1149976.
6. Huber, H.; Gallenberger, M.; Jahn, U.; Eylert, E.; Berg, I.A.; Kockelkorn, D.; Eisenreich, W.; Fuchs, G. A dicarboxylate/4-hydroxybutyrate autotrophic carbon assimilation cycle in the hyperthermophilic Archaeum Ignicoccus hospitalis. *Natl. Acad. Sci. USA* **2008**, *105*, 7851–7856.
7. Medina-Ramos, J.; Pupillo, R.C.; Keane, T.P.; DiMeglio, J.L.; Rosenthal, J.J.J. Efficient conversion of CO2 to CO using tin and other inexpensive and easily prepared post-transition metal catalysts. *J. Am. Chem. Soc.* **2015,** 137, 5021–5027.
8. Wang, H.; Matios, E.; Wang, C.; Luo, J.; Lu, X.; Hu, X.; Li, W.J.N.L. Rapid and Scalable Synthesis of Cuprous Halide-Derived Copper Nano-Architectures for Selective Electrochemical Reduction of Carbon Dioxide. *Am. Chem. Soc.* **2019,** 19, 3925–3932.
9. Varela, A.S.; Ranjbar Sahraie, N.; Steinberg, J.; Ju, W.; Oh, H.S.; Strasser, P.J.A.C.I.E. Metal-doped nitrogenated carbon as an efficient catalyst for direct CO2 electroreduction to CO and hydrocarbons. *Angew. Commun.* **2015,** 54, 10758–10762.
10. Scott, K.; Yu, E.H. *Microbial Electrochemical and Fuel Cells: Fundamentals and Applications*; Woodhead Publishing: Cambridge, UK, 2015.
11. Rabaey, K.; Rozendal, R.A. Microbial electrosynthesis—revisiting the electrical route for microbial production. *Nat. Rev. Microbiol.* **2010,** 8, 706.

12. Im, C.H.; Kim, C.; Song, Y.E.; Oh, S.-E.; Jeon, B.-H.; Kim, J.R. Electrochemically enhanced microbial CO conversion to volatile fatty acids using neutral red as an electron mediator. *Chemosphere* **2018,** 191, 166–173.
13. Ter Heijne, A.; Geppert, F.; Sleutels, T.H.; Batlle-Vilanova, P.; Liu, D.; Puig, S. Mixed culture biocathodes for production of hydrogen, methane, and carboxylates. In *Bioelectrosynthesis*; Springer: Berlin/Heidelberg, Germany, 2017; pp. 203–229.
14. Hamelers, H.V.; Ter Heijne, A.; Sleutels, T.H.; Jeremiasse, A.W.; Strik, D.P.; Buisman, C.J. New applications and performance of bioelectrochemical systems. *Appl. Microbiol. Biotechnol.* **2010,** 85, 1673–1685.
15. Ganigué, R.; Puig, S.; Batlle-Vilanova, P.; Balaguer, M.D.; Colprim, J. Microbial electrosynthesis of butyrate from carbon dioxide. *Chem. Commun.* **2015,** 51, 3235–3238.
16. Bajracharya, S.; Yuliasni, R.; Vanbroekhoven, K.; Buisman, C.J.; Strik, D.P.; Pant, D. Long-term operation of microbial electrosynthesis cell reducing CO2 to multi-carbon chemicals with a mixed culture avoiding methanogenesis. *Bioelectrochemistry* **2017,** 113, 26–34.
17. ZHANG, Y.; ZHANG, W.; JIANG, Y.; Su, M.; Tao, Y.; Li, D. Simultaneous microbial electrosynthesis of acetate and butyrate from carbon dioxide in bioelectrochemical systems. *Chin. J. Apply Environ. Biol* **2014,** 20, 174–178.
18. Kim, C.; Kim, M.Y.; Michie, I.; Jeon, B.-H.; Premier, G.C.; Park, S.; Kim, J.R. Anodic electro-fermentation of 3-hydroxypropionic acid from glycerol by recombinant Klebsiella pneumoniae L17 in a bioelectrochemical system. *Biotechnol Biofuels* **2017,** 10, 199–199.
19. Rosenbaum, M.; Aulenta, F.; Villano, M.; Angenent, L.T. Cathodes as electron donors for microbial metabolism: which extracellular electron transfer mechanisms are involved? *Bioresour. Technol.* **2011,** 102, 324–333.
20. Jiang, Y.; Su, M.; Zhang, Y.; Zhan, G.; Tao, Y.; Li, D. Bioelectrochemical systems for simultaneously production of methane and acetate from carbon dioxide at relatively high rate. *Int. J. Hydrog. Energy* **2013,** 38, 3497–3502.
21. Pan, X.; Angelidaki, I.; Alvarado-Morales, M.; Liu, H.; Liu, Y.; Huang, X.; Zhu, G. Methane production from formate, acetate and H2/CO2; focusing on kinetics and microbial characterization. *Bioresource Technol.* **2016,** 218, 796–806.
22. Su, M.; Jiang, Y.; Li, D. Production of acetate from carbon dioxide in bioelectrochemical systems based on autotrophic mixed culture. *J. Microbiol. Biotechnol.* **2013,** 23, 1140–1146.
23. Arends, J.B.; Patil, S.A.; Roume, H.; Rabaey, K. Continuous long-term electricity-driven bioproduction of carboxylates and isopropanol from CO2 with a mixed microbial community. *J. CO_2 Util.* **2017,** 20, 141–149.
24. Kracke, F.; Vassilev, I.; Krömer, J.O. Microbial electron transport and energy conservation–the foundation for optimizing bioelectrochemical systems. *Front. Microbiol.* **2015,** 6, 575.
25. Steinbusch, K.J.; Hamelers, H.V.; Schaap, J.D.; Kampman, C.; Buisman, C.J. Bioelectrochemical ethanol production through mediated acetate reduction by mixed cultures. *Environ. Sci. Technol.* **2009,** 44, 513–517.
26. Harrington, T.D.; Tran, V.N.; Mohamed, A.; Renslow, R.; Biria, S.; Orfe, L.; Call, D.R.; Beyenal, H. The mechanism of neutral red-mediated microbial electrosynthesis in Escherichia coli: menaquinone reduction. *Bioresour. Technol.* **2015,** 192, 689–695.
27. Aulenta, F.; Di Maio, V.; Ferri, T.; Majone, M. The humic acid analogue antraquinone-2, 6-disulfonate (AQDS) serves as an electron shuttle in the electricity-driven microbial dechlorination of trichloroethene to cis-dichloroethene. *Bioresource Technol.* **2010,** 101, 9728–9733.
28. Sund, C.J.; McMasters, S.; Crittenden, S.R.; Harrell, L.E.; Sumner, J.J. Effect of electron mediators on current generation and fermentation in a microbial fuel cell. *Applied microbiology and biotechnology* **2007,** 76, 561–568.
29. Kim, M.Y.; Kim, C.; Ainala, S.K.; Bae, H.; Jeon, B.-H.; Park, S.; Kim, J.R. Metabolic shift of Klebsiella pneumoniae L17 by electrode-based electron transfer using glycerol in a microbial fuel cell. *Bioelectrochemistry* **2019,** 125, 1–7.
30. Leclerc, M.; Elfoul-Bensaid, L.; Bernalier, A.J.C. m. Effect of yeast extract on growth and metabolism of H 2-utilizing acetogenic bacteria from the human colon. *Curr. Microbiol.* **1998,** 37, 166–171.
31. Parker, V.D. The hydroquinone–quinone redox behaviour in acetonitrile. *J. Chem. Soc. D Chem. Commun.* **1969**, 13, 716-717.
32. Tschörtner, J.; Lai, B.; Krömer, J.O. Biophotovoltaics: Green power generation from sunlight and water. *Front. Microbiol.* **2019,** 10, 866.
33. Wardman, P. Reduction potentials of one-electron couples involving free radicals in aqueous solution. *J. Phys. Chem. Ref. Data* **1989,** 18, 1637–1755.

34. Taran, O. Electron transfer between electrically conductive minerals and quinones. *Front. Chem.* **2017,** 5, 49.
35. Hijji, Y.M.; Barare, B.; Zhang, Y. Lawsone (2-hydroxy-1, 4-naphthoquinone) as a sensitive cyanide and acetate sensor. *Sens. Actuators B Chem.* **2012,** 169, 106–112.
36. Watanabe, K.; Manefield, M.; Lee, M.; Kouzuma, A. Electron shuttles in biotechnology. *Curr. Opin. Biotechnol.* **2009,** 20, 633–641.
37. Miroliaei, M.R.; Samimi, A.; Mohebbi-Kalhori, D.; Khorram, M. Kinetics investigation of diversity cultures of E. coli and Shewanella sp. and their combined effect with mediator on MFC performance. *J. Ind. Eng. Chem.* **2015,** 25, 42–50.
38. Penteado, E.D.; Fernandez-Marchante, C.M.; Zaiat, M.; Gonzalez, E.R.; Rodrigo, M.A. On the effects of ferricyanide as cathodic mediator on the performance of microbial fuel cells. *Electrocatalysis* **2017,** 8, 59–66.
39. Im, C.H.; Song, Y.E.; Jeon, B.-H.; Kim, J.R.J.C.L. Biologically activated graphite fiber electrode for autotrophic acetate production from CO2 in a bioelectrochemical system. *Carbon Lett.* **2016,** 20, 76–80.
40. Popov, A.L.; Kim, J.R.; Dinsdale, R.M.; Esteves, S.R.; Guwy, A.J.; Premier, G.C. The effect of physico-chemically immobilized methylene blue and neutral red on the anode of microbial fuel cell. *Biotechnol. Bioprocess. Eng.* **2012,** 17, 361–370.
41. Rudnicka, M.; Ludynia, M.; Karcz, W. Effects of Naphthazarin (DHNQ) Combined with Lawsone (NQ-2-OH) or 1, 4-Naphthoquinone (NQ) on the Auxin-Induced Growth of Zea mays L. Coleoptile Segments. *Int. J. Mol. Sci.* **2019,** 20, 1788.
42. Ma, C.; He, N.; Zhao, Y.; Xia, D.; Wei, J.; Kang, W. Antimicrobial mechanism of hydroquinone. *Appl. Biochem. Biotechnol.* **2019,** 189, 1291–1303.
43. Stephanopoulos, G.; Aristidou, A.A.; Nielsen, J. *Metabolic Engineering: Principles and Methodologies*; Elsevier: Amsterdam, The Netherlands, 1998.
44. Rittmann, B.E.; McCarty, P.L. *Environmental Biotechnology: Principles and Applications*; Tata McGraw-Hill Education: New York, NY, USA, 2012.
45. Seelajaroen, H.; Haberbauer, M.; Hemmelmair, C.; Aljabour, A.; Dumitru, L.M.; Hassel, A.W.; Sariciftci, N.S.J.C. Enhanced Bio-Electrochemical Reduction of Carbon Dioxide by Using Neutral Red as a Redox Mediator. *Chembiochem* **2019,** 20, 1196–1205.