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A lipid membrane intercalating conjugated oligoelectrolyte enables electrode driven succinate production in *Shewanella*†

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An amphiphilic conjugated oligoelectrolyte (COE) that spontaneously intercalates into lipid membranes enables *Shewanella oneidensis* to use a graphite electrode as the sole electron donor for succinate production. Current consumed in a poised electrochemical system by *Shewanella* with micromolar concentrations of COE correlates well with the succinate produced *via* fumarate reduction as determined by HPLC analysis. Confocal microscopy confirms incorporation of the COE into the microbes on the electrode surface. This work presents a unique strategy to induce favorable bioelectronic interactions for the production of reduced microbial metabolites.

Broader context

The use of microorganisms to catalyze electrically driven chemical reactions has applications in bioremediation and is a promising strategy to produce biofuels. Microbial electrosynthesis, for example, relies on bacteria to catalyze the reduction of CO_2 to multicarbon compounds with electrons provided by an electrode. Expanding on this, a photosynthetic process can be achieved with water supplying the electrons and solar energy being used to power a microbial electrosynthesis device. The success of such technologies ultimately relies on electrons transferring from an electrode into bacteria across insulating lipid membranes: an uncommon feat at the very least. The microbe–electrode interface thus becomes a key area of focus for improving the performance and expanding the scope of biocatalyzed, electrode driven reactions. In this report we describe a strategy to modify this interface with a synthetic molecule in order to achieve electrode driven production of succinate by *Shewanella*.

Conjugated oligoelectrolytes (COEs) contain an electronically delocalized, π-conjugated molecular framework and pendant groups bearing ionic functionalities. These oligomers, like their polymeric counterparts, embody light harvesting optical and semiconducting electronic properties that are useful for various applications, such as biosensors and optoelectronic devices.[1] The amphiphilicity of COEs also leads to solubility in aqueous media and in some instances spontaneous supramolecular organizations that are driven by hydrophobic interactions.[2] There is a tendency of certain COEs, such as 4,4′-bis(4′-(*N*,*N*-bis(6′′-(*N*′,*N*′,*N*′-trimethylammonium)hexyl)amino)-styryl)stilbene tetraiodide (**DSSN+**), to intercalate within lipid bilayer membranes in an ordered orientation, wherein the long axis of the conjugated region spans the membrane (Fig. 1).[3] Moreover, their integration into yeast microbial fuel cells (MFCs) leads to a five-fold increase in the maximum generated current. Comparable improvement is also observed in wastewater MFCs.[4] Presumably **DSSN+** facilitates a more intimate electronic interaction between microorganisms and electrodes by enabling a

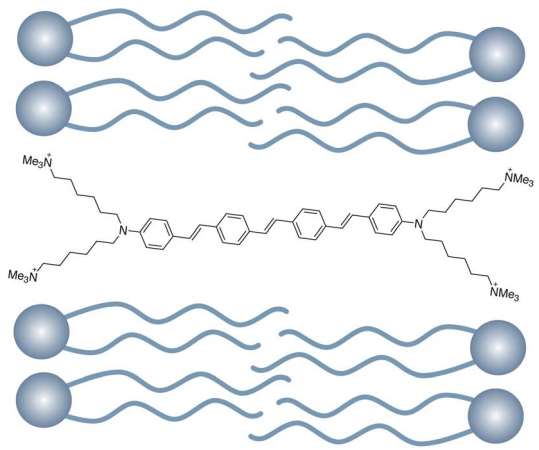

Fig. 1 Structure of 4,4′-bis(4′-(*N*,*N*-bis(6′′-(*N*,*N*,*N*-trimethylammonium)hexyl)amino)-styryl)stilbene tetraiodide (**DSSN+**), and a cartoon representation of the proposed ordered orientation within a lipid bilayer.

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transmembrane extracellular charge transfer mechanism. A similar effect has been achieved *via* membrane modification of *Desulfovibrio desulfuricans* with Pd nanoparticles that improve extracellular electron transfer.[5] Electrical wiring of microorganisms with electrodes *via* osmium redox polyelectrolytes has also been demonstrated.[6]

Microorganisms that possess extracellular electron transfer mechanisms have found utility in a variety of bioelectronics devices due to their intrinsic ability to interact with electrodes. A notable example is *Geobacter sulfurreducens*, due to its relevance to MFC applications.[7] It is worth pointing out that the majority of the work regarding microbe–electrode interactions has focused on processes in which electrons are transferred from microbe to electrode, such as the anode localized redox reactions in MFCs.[8] Microbial systems that function on the basis of electron transfer from electrodes to microbes have received more recent attention.[9] In such systems an applied negative potential is used to inject electrons into microorganisms and drive microbial enzymatic reactions.[10] An example of such a system describes the consumption of CO_2 and production of acetate by *Sporomusa ovata*.[11]

Examples of cellular growth, metabolism, and enzymatic processes that can be influenced or driven by electron donation from electrodes to microorganisms have been achieved by multiple approaches. One early example describes the influence of an applied negative potential on *Clostridium acetobutylicum* fermentation products when using methyl viologen as an electron shuttle.[12] Neutral red has also been used in a similar manner to drive the growth and metabolism of *Actinobacillus succinogens*.[13] The diffusion based mediators[14] employed in these cases, however, may exhibit drawbacks when applied broadly, such as redox incompatibility,[15] cellular uptake that does not result in electron transfer, possible toxicity or solubility problems,[16] and diffusion limited kinetics.[17] Genetic engineering[18] and adaptive evolution[19] have also been examined for modulating electronic interactions between microorganisms and electrodes. It is also worth noting that the mechanisms of electron transfer into and out of microorganisms have been shown to be physically and energetically different in some instances.[20]

Shewanella oneidensis MR-1 is well known for its extracellular electron transfer to external acceptors[21] but its ability to accept electrons is an emerging area of research. A study that proposes a mechanism of electron transfer into *S. oneidensis* has emerged that examines electron transfer on a timescale of one hour.[22] In this contribution, we describe days-long electrode driven succinate production by *S. oneidensis* facilitated by addition of the membrane-intercalating conjugated oligoelectrolyte **DSSN+**. An illustration of the "H-cell" apparatus[23] employed in this study that highlights the key electrochemical and bio-electrochemical processes is shown in Fig. 2b.

We first describe the effect of various **DSSN+** concentrations on the current injected into *S. oneidensis* initially grown anaerobically in freshwater media[24] with a lactate electron donor and fumarate electron acceptor. *S. oneidensis* was cultured to stationary phase at an optical density (600 nm) of ~0.5, isolated *via* centrifuge and re-suspended in sterile and oxygen-free freshwater media. H-cells containing deoxygenated freshwater media devoid of amino acids and vitamins and supplemented with 40 mM fumarate (cathode chamber only) operating under an applied bias of −300 mV *vs.* SHE were inoculated with the concentrated *S. oneidensis* suspension on day 0 to an optical density of ~0.5 and the current was monitored over time (Fig. 2a, top). The cumulative number of electrons transferred by the cathodes was calculated, *via* integration of the current *vs.* time traces; these results are shown at the bottom of Fig. 2a (solid lines) and are correlated with the number of electrons appearing in the reduced product succinate (dotted lines), which was analysed by periodic HPLC samples. Negligible current response is observed for a sterile control in which **DSSN+** was added to a concentration of 5 μM (Fig. S1†) and an *S. oneidensis* control to which no **DSSN+** was added (Fig. 2a, blue). These control experiments confirm the difficulty of injecting electrons into *S. oneidensis* and that **DSSN+** does not participate in electrochemical processes under these experimental conditions. An immediate increase in current was observed upon **DSSN+** addition to final concentrations of 1 μM and 10 μM that increased over a period of ~12 hours, ultimately reaching maxima of −16 μA cm^{-2} and −150 μA cm^{-2}, respectively. An approximately ten-fold increase in maximum current and over four-fold increase in cumulative electrons injected is observed between 1 and 10 μM **DSSN+**. The succinate produced in all cases correlates well with the number of electrons injected. In the 0 μM and 1 μM experiments there is slightly more succinate produced than current injected which may, in part, be explained by the ability of *S. oneidensis* to store excess electrons during growth.[25] This set of experiments demonstrates the ability of **DSSN+** to facilitate electron injection over a long timescale into an organism that is otherwise incapable of utilizing an electrode as a sole electron source under these experimental conditions.

A subsequent experiment was run to confirm the effect of **DSSN+** on the current injected into *S. oneidensis* and to probe the influence of planktonic cells (Fig. 3). *S. oneidensis* was again initially grown on lactate and fumarate, centrifuged, resuspended in fresh media and injected into the cathode chamber of an H-cell (day 0) with 40 mM fumarate operated at −300 mV *vs.* SHE. There is little to no current observed until day 3 when **DSSN+** is added to the cathode chamber to a final concentration of 5 μM and the current steadily increases, reaching a maximum of −13 μA cm^{-2} near day 6. On day 7 the media was exchanged to remove planktonic cells and fumarate was replenished, after which the current returns to levels near the maximum but begins to decline rapidly. Planktonic cells were centrifuged and resuspended in fresh media and replaced back into the cathode chamber on day 8 after which the current increases steadily until day 9 when it begins to level off around −18 μA cm^{-2}. This response is most reasonably attributed to electrons accepted by the planktonic cells, presumably upon collision with the electrode.

Confocal laser scanning microscopy of the cathode after day 10 from the experiment corresponding to Fig. 3 was carried out utilizing the emission response of **DSSN+** following direct excitation at 488 nm (no additional fluorescent dye was added).

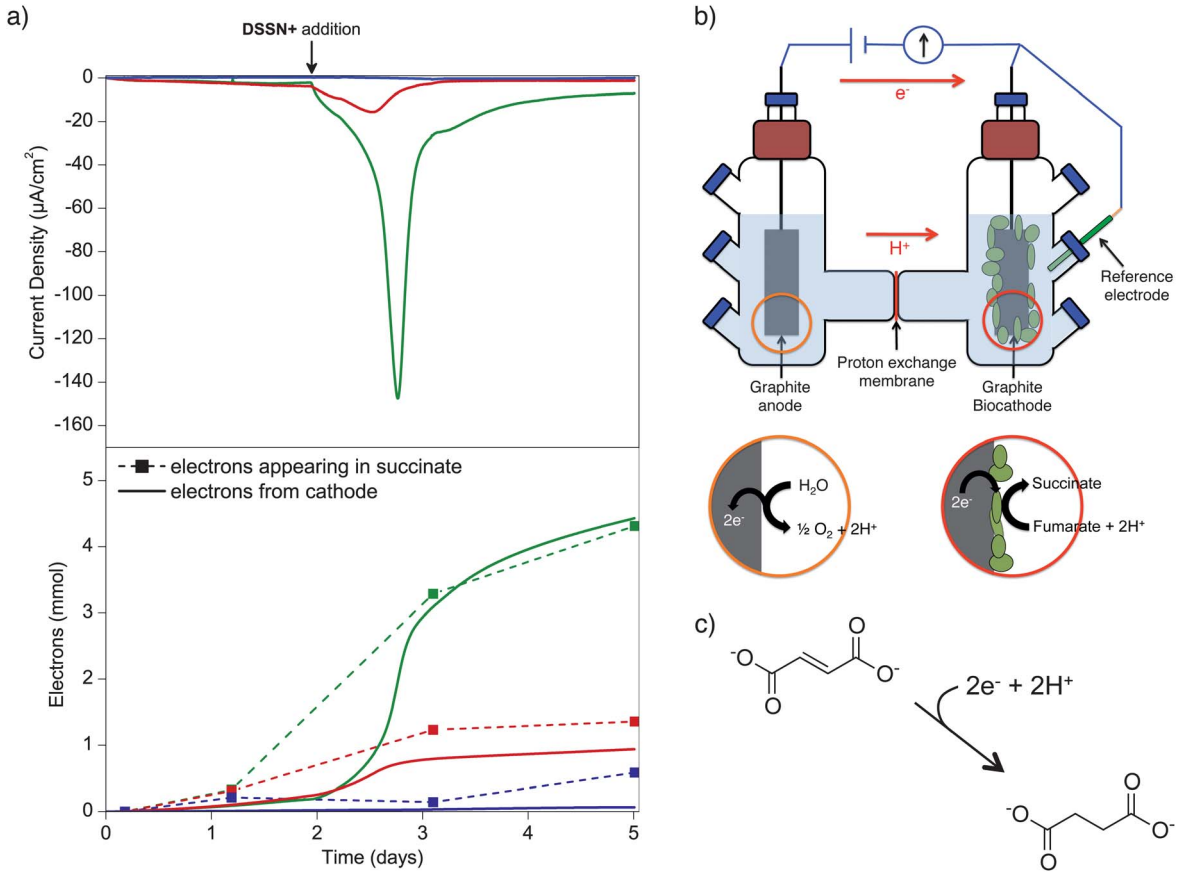

Fig. 2 (a, top) Current *vs.* time traces of H-cells inoculated with concentrated *S. oneidensis* on day 0 and run in the presence of 0 M (blue), 1 μM (red), and 10 μM (green) **DSSN+**. **DSSN+** addition (not including blue trace) has been indicated. Steady current was established for an hour before the addition of cells and this baseline was subtracted from all curves. (bottom) The corresponding cumulative number of electrons transferred as a function of time determined by integration of the current *vs.* time traces (solid) and number of electrons appearing in succinate determined by HPLC analysis of aliquots from cathode chambers (dashed). (b) The 'H-cell' apparatus used in this study. A constant bias is applied to the cathode (−300 mV *vs.* SHE) and current is monitored *via* potentiostat. *S. oneidensis* modified with **DSSN+** accepts electrons from the cathode and subsequently converts fumarate to succinate. Charge balance is maintained by the oxidation of water at the anode. (c) The reduction of fumarate to succinate.

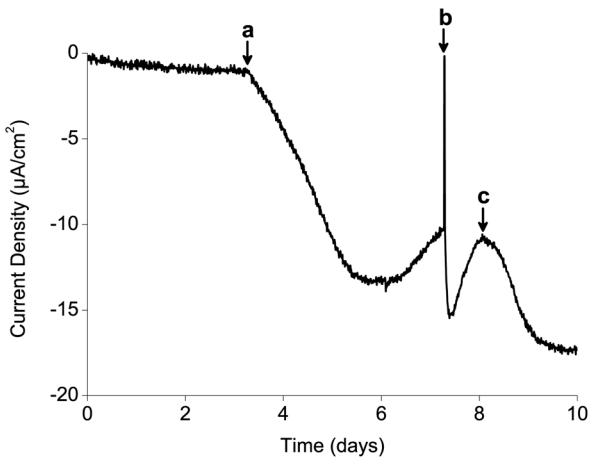

Fig. 3 Current *vs.* time of an H-cell run employing *S. oneidensis* poised at −300 mV *vs.* SHE in the presence of fumarate (initially 40 mM). (a) Addition of **DSSN+** to a final concentration of 5 μM. (b) Medium exchange with removal of planktonic cells and addition of fumarate to 40 mM. (c) Replacement of planktonic cells.

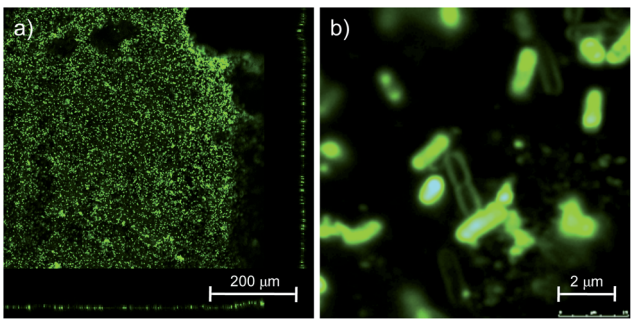

Fig. 4 Confocal images of a graphite biocathode populated by **DSSN+** modified *S. oneidensis* following H-cell operation. The fluorescence response is obtained *via* direct excitation of **DSSN+** at 488 nm. (a) *Z*-stack image of the electrode surface with bottom and right margins displaying side views showing incomplete surface coverage by a monolayer of cells. Vertical and horizontal scales are equal. (b) Single slice image showing individual *S. oneidensis* cells on the electrode surface.

The results shown in Fig. 4 indicate the presence of a sparse biofilm composed of a mostly incomplete monolayer of cells with **DSSN+** successfully incorporated throughout. Additionally, the incomplete coverage leaves open the possibility of planktonic cells interacting with exposed electrode surface. Fig. 4b shows individual intact cells with emission profiles illustrating accumulation of **DSSN+**. It is reasonable to assume that **DSSN+** has accumulated within the lipid membranes in the proposed orientation (Fig. 1) based on previous results,[3] but it should be noted that no direct evidence of this is presented here, nor is it ruled out that **DSSN+** is accumulating inside the cells.

In summary, we have shown that the membrane-intercalating conjugated oligoelectrolyte **DSSN+** enables *S. oneidensis* to use a graphite electrode as the sole electron donor for the reduction of fumarate. Addition of **DSSN+** to operating H-cells results in an immediate and long-term current response with the number of electrons injected by the electrode showing good correlation with the electrons appearing in the reduced metabolic product succinate. The maximum current and cumulative number of electrons injected depends on the concentration of **DSSN+**, consistent with the direct role of this molecule in facilitating charge transfer. Confocal microscopy analysis of electrodes following H-cell operation shows that **DSSN+** reaches and remains in *S. oneidensis* throughout operation. It should be mentioned that **DSSN+** at low micromolar concentrations has neither a negative effect on the growth of *S. oneidensis* (Fig. S2†) nor does it appear to be lysing cells under non-growth conditions (Fig. S3†). Furthermore, a surfactant that causes significant lysis of *S. oneidensis* (Fig. S3†) added in current consumption experiments analogous to those presented here does not elicit an appreciable response (Fig. S4†). These findings along with the fact that **DSSN+** is not acting as a traditional electron shuttle because it does not undergo reversible redox,[3] support a mechanism wherein the conjugated oligoelectrolyte facilitates direct transmembrane electron transport from the electrode into charge acceptor sites within the outer membrane or periplasm. Due to the large number of redox sites in and around the *S. oneidensis* membranes, including the well-studied Mtr respiratory pathway,[26] and localization of fumarate reductase in the periplasm,[27] it is reasonable to propose that electrons may be transferred to such sites. Such a process would not require **DSSN+** to span both cell membranes. It is worth noting that in the aforementioned study implicating the Mtr respiratory pathway in electrode driven reductive metabolism in *S. oneidensis*,[22] biofilms were pre-grown under oxidizing potential during which redox active flavins are secreted by the cells which tend to stick to graphite electrodes even after media exchange.[28] Under our non-growth experimental conditions, *S. oneidensis* alone could not accept electrons from the electrode, even when poised at a more negative potential of −360 mV *vs.* SHE (Fig. S5†) equivalent to the previous report, and thus it is inferred that **DSSN+** is directly responsible for facilitating a novel microbe–electrode interaction, the mechanism of which is under investigation. In conclusion, that **DSSN+** spontaneously intercalates into cell membranes and facilitates bioelectronic communication with electrodes gives this synthetic membrane modification strategy the potential to increase the performance of existing bioelectronic systems, increase the number of applicable organisms, and act as an alternative to more complex strategies to induce favorable bioelectronic interactions.

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References

1 (*a*) A. Herland, K. P. R. Nilsson, J. D. M. Olsson, P. Hammarström, P. Konradsson and O. Inganäs, *J. Am. Chem. Soc.*, 2005, **127**, 2317–2323; (*b*) Y. Chen, H. Bai, Q. Chen, C. Li and G. Shi, *Sens. Actuators, B*, 2009, **138**, 563–571; (*c*) R. Yang, Y. Xu, X.-D. Dang, T.-Q. Nguyen, Y. Cao and G. C. Bazan, *J. Am. Chem. Soc.*, 2008, **130**, 3282–3283; (*d*) Y. Xu, R. Yang, J. Peng, A. A. Mikhailovsky, Y. Cao, T.-Q. Nguyen and G. C. Bazan, *Adv. Mater.*, 2009, **21**, 584–588.

2 (*a*) F. J. M. Hoeben, P. Jonkheijm, E. W. Meijer and A. P. H. J. Schenning, *Chem. Rev.*, 2005, **105**, 1491–1546; (*b*) H. Jiang, P. Taranekar, J. R. Reynolds and K. S. Schanze, *Angew. Chem., Int. Ed.*, 2009, **48**, 4300–4316; (*c*) J. W. Hong, H. Benmansour and G. C. Bazan, *Chem.–Eur. J.*, 2003, **9**, 3186–3192; (*d*) J. H. Ortony, T. Chatterjee, L. E. Garner, A. Chworos, A. Mikhailovsky, E. J. Kramer and G. C. Bazan, *J. Am. Chem. Soc.*, 2011, **133**, 8380–8387.

3 L. E. Garner, J. Park, S. Dyar, A. Chworos, J. Sumner and G. Bazan, *J. Am. Chem. Soc.*, 2010, **132**, 10042–10052.

4 L. E. Garner, A. W. Thomas, J. J. Sumner, S. P. Harvey and G. C. Bazan, *Energy Environ. Sci.*, 2012, **5**, 9449–9552.

5 X. Wu, F. Zhao, N. Rahunen, J. R. Varcoe, C. Avignone-Rossa, A. E. Thumser and R. C. T. Slade, *Angew. Chem., Int. Ed.*, 2011, **50**, 427–430.

6 (*a*) V. Coman, T. Gustavsson, A. Finkelsteinas, C. von Wachenfeldt, C. Hagerhall and L. Gorton, *J. Am. Chem. Soc.*, 2009, **131**, 16171–16176; (*b*) I. Vostiar, E. E. Ferapontova and L. Gorton, *Electrochem. Commun.*, 2004, **6**, 621–626.

7 (*a*) K. P. Nevin, H. Richter, S. F. Covalla, J. P. Johnson, T. L. Woodard, A. L. Orloff, H. Jia, M. Zhang and D. R. Lovley, *Environ. Microbiol.*, 2008, **10**, 2505–2514; (*b*) D. R. Lovley, T. Ueki, T. Zhang, N. S. Malvankar, P. M. Shrestha, K. A. Flanagan, M. Aklujkar, J. E. Butler, L. Giloteaux and A.-E. Rotaru, *et al.* in *Advances in Microbial Physiology*, ed. R. K. Poole, Elsevier, 2011, vol. 59, pp. 1–100.

8 (*a*) B. E. Logan, *Nat. Rev. Microbiol.*, 2009, **7**, 375–381; (*b*) O. Schaetzle, F. Barriere and K. Baronian, *Energy Environ. Sci.*, 2008, **1**, 607–620.

9 (*a*) J. C. Thrash and J. D. Coates, *Environ. Sci. Technol.*, 2008, **42**, 3921–3931; (*b*) D. R. Lovley, *Environ. Microbiol. Rep.*, 2011, **3**, 27–35; (*c*) K. B. Gregory, D. R. Bond and D. R. Lovley, *Environ. Microbiol.*, 2004, **6**, 596–604.

10 (*a*) K. Rabaey and R. A. Rozendal, *Nat. Rev. Microbiol.*, 2010, **8**, 706–716; (*b*) D. H. Park and J. G. Zeikus, *J. Bacteriol.*, 1999, **181**, 2403–2410.

11 (*a*) K. P. Nevin, T. L. Woodard, A. E. Franks, Z. M. Summers and D. R. Lovley, *mBio*, 2010, **1**, e00103–e00110; (*b*) K. P. Nevin, S. A. Hensley, A. E. Franks, Z. M. Summers, J. Ou, T. L. Woodard, O. L. Snoeyenbos-West and D. R. Lovley, *Appl. Environ. Microbiol.*, 2011, **77**, 2882–2886.

12 T. S. Kim and B. H. Kim, *Biotechnol. Lett.*, 1988, **10**, 123–128.

13 D. H. Park, M. Laivenieks, M. V. Guettler, M. K. Jain and J. G. Zeikus, *Appl. Environ. Microbiol.*, 1999, **65**, 2912–2917.

14 K. Watanabe, M. Manefield, M. Lee and A. Kouzuma, *Curr. Opin. Biotechnol.*, 2009, **20**, 633–641.

15 (*a*) S. Wilkinson, J. Klar and S. Applegarth, *Electroanalysis*, 2006, **18**, 2001–2007; (*b*) D. H. Park and J. G. Zeikus, *Appl. Environ. Microbiol.*, 2000, **66**, 1292–1297.

16 R. A. Bullen, T. C. Arnot, J. B. Lakeman and F. C. Walsh, *Biosens. Bioelectron.*, 2006, **21**, 2015–2045.

17 C. I. Torres, A. K. Marcus, H.-S. Lee, P. Parameswaran, R. Krajmalnik-Brown and B. E. Rittmann, *FEMS Microbiol. Rev.*, 2010, **34**, 3–17.

18 (*a*) H. M. Jensen, A. E. Albers, K. R. Malley, Y. Y. Londer, B. E. Cohen, B. A. Helms, P. Weigele, J. T. Groves and C. M. Ajo-Franklin, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 19213–19218; (*b*) N. S. Malvankar, M. Vargas, K. P. Nevin, A. E. Franks, C. Leang, B.-C. Kim, K. Inoue, T. Mester, S. F. Covalla, J. P. Johnson, *et al.*, *Nat. Nanotechnol.*, 2011, **6**, 573–579; (*c*) N. S. Malvankar, T. Mester, M. T. Tuominen and D. R. Lovley, *ChemPhysChem*, 2012, **13**, 463–468.

19 H. Yi, K. P. Nevin, B.-C. Kim, A. E. Franks, A. Klimes, L. M. Tender and D. R. Lovley, *Biosens. Bioelectron.*, 2009, **24**, 3498–3503.

20 (*a*) M. Rosenbaum, F. Aulenta, M. Villano and L. T. Angenent, *Bioresour. Technol.*, 2011, **102**, 324–333; (*b*) S. M. Strycharz, R. H. Glaven, M. V. Coppi, S. M. Gannon, L. A. Perpetua, A. Liu, K. P. Nevin and D. R. Lovley, *Bioelectrochemistry*, 2011, **80**, 142–150.

21 (*a*) R. S. Hartshorne, C. L. Reardon, D. Ross, J. Nuester, T. A. Clarke, A. J. Gates, P. C. Mills, J. K. Fredrickson, J. M. Zachara, L. Shi, A. S. Beliaev, M. J. Marshall, M. Tien, S. Brantley, J. N. Butt and D. J. Richardson, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 22169–22174; (*b*) H. von Canstein, J. Ogawa, S. Shimizu and J. R. Lloyd, *Appl. Environ. Microbiol.*, 2008, **74**, 615–623.

22 D. E. Ross, J. M. Flynn, D. B. Baron, J. A. Gralnick and D. R. Bond, *PLoS One*, 2011, **6**, e16649.

23 D. R. Bond and D. R. Lovley, *Appl. Environ. Microbiol.*, 2003, **69**, 1548–1555.

24 D. R. Lovley, S. J. Giovannoni, D. C. White, J. E. Champine, E. J. P. Phillips, Y. A. Gorby and S. Goodwin, *Arch. Microbiol.*, 1993, **159**, 336–344.

25 (*a*) N. Uría, X. Muñoz Berbel, O. Sánchez, F. X. Muñoz and J. Mas, *Environ. Sci. Technol.*, 2011, **45**, 10250–10256; (*b*) B. Schuetz, M. Schicklberger, J. Kuermann, A. M. Spormann and J. Gescher, *Appl. Environ. Microbiol.*, 2009, **75**, 7789–7796.

26 (*a*) D. Coursolle and J. A. Gralnick, *Mol. Microbiol.*, 2010, **77**, 995–1008; (*b*) D. Coursolle, D. B. Baron, D. R. Bond and J. A. Gralnick, *J. Bacteriol.*, 2009, **192**, 467–474; (*c*) R. Hartshorne, C. Reardon, D. Ross, J. Nuester, T. Clarke, A. Gates, P. Mills, J. Fredrickson, J. Zachara and L. Shi, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 22169–22174; (*d*) B. Schuetz, M. Schicklberger, J. Kuermann, A. M. Spormann and J. Gescher, *Appl. Environ. Microbiol.*, 2009, **75**, 7789–7796.

27 C. R. Myers and J. M. Meyers, *FEMS Microbiol. Lett.*, 1992, **98**, 13–20.

28 E. Marsili, D. B. Baron, I. D. Shikhare, D. Coursolle, J. A. Gralnick and D. R. Bond, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 3968–3973.