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Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells

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Abundant energy, stored primarily in the form of carbohydrates, can be found in waste biomass from agricultural, municipal and industrial sources as well as in dedicated energy crops, such as corn and other grains[1–4]. Potential strategies for deriving useful forms of energy from carbohydrates include production of ethanol[4–6] and conversion to hydrogen[7–10], but these approaches face technical and economic hurdles. An alternative strategy is direct conversion of sugars to electrical power. Existing transition metal–catalyzed fuel cells cannot be used to generate electric power from carbohydrates[11]. Alternatively, biofuel cells in which whole cells or isolated redox enzymes catalyze the oxidation of the sugar have been developed[12–19], but their applicability has been limited by several factors, including (i) the need to add electron-shuttling compounds that mediate electron transfer from the cell to the anode, (ii) incomplete oxidation of the sugars and (iii) lack of long-term stability of the fuel cells. Here we report on a novel microorganism, *Rhodoferax ferrireducens*, that can oxidize glucose to CO_2 and quantitatively transfer electrons to graphite electrodes without the need for an electron-shuttling mediator. Growth is supported by energy derived from the electron transfer process itself and results in stable, long-term power production.

R. ferrireducens was isolated from anoxic subsurface sediments of Oyster Bay, Virginia, USA, as a dissimilatory Fe(III)-reducing microorganism[20]. It is able to grow at temperatures of 4 °C to 30 °C, with an optimum at 25 °C. Although its substrate range was originally reported to be limited to organic acids, further evaluation demonstrated that it could oxidize glucose to carbon dioxide with Fe(III) serving as the sole electron acceptor (**Fig. 1a**) and could conserve energy to support growth from this metabolism (**Fig. 1b**). *R. ferrireducens* did not grow on glucose in the absence of Fe(III) and glucose did not react with Fe(III) in the absence of the bacterium. The stoichiometry of glucose utilization and Fe(III) reduction was consistent with the reaction:

$$C_6H_{12}O_6 + 6\,H_2O + 24\,Fe(III) \rightarrow 6\,CO_2 + 24\,Fe(II) + 24\,H^+$$

Fructose was oxidized in a similar manner. This is the first time that complete oxidation of sugars coupled to Fe(III) reduction has been observed in an organism capable of growing at circumneutral pH.

Recent studies have demonstrated that Fe(III)-reducing microorganisms in the family *Geobacteraceae* can also directly transfer electrons onto electrodes[21–23]. However, the range of electron donors that these organisms can use is limited primarily to simple organic acids, such as acetate. Thus, they rely on fermentative microorganisms to produce their required electron donors from sugars and other more complex organic compounds. To determine whether *R. ferrireducens* might transfer electrons to an electrode with glucose as the electron donor, the bacterium was inoculated into the anode compartment of an anaerobic two-chambered vessel with each side containing a graphite electrode and the two chambers connected by a cation-selective membrane[21,23]. The anode was poised at +200 mV against an Ag/AgCl reference electrode. Glucose (2 mM) in the anode medium was consumed with the production of current and the growth of *R. ferrireducens*. Once the current generation plateaued, a 10% inoculum from the anode chamber was transferred to a new chamber. Current continued to be produced as glucose was consumed (**Fig. 2a**). Current production was associated with cell growth, as demonstrated by an increase in microbial cell protein in the medium over time (**Fig. 2b**). There was a nearly equivalent amount of growth on the anode surface, as 1.96 mg ± 0.24 mg (mean ± s.d.; $n = 3$) of cell protein could be extracted from the anode with NaOH. There was negligible current generation and glucose consumption in the absence of cells (**Fig. 2a**) and *R. ferrireducens* was unable to grow in the chamber if the anode was not provided as an electron acceptor (data not shown).

The coulombic yields, expressed as total coulombs (amperes × seconds) passed through the fuel cell, calculated from current versus time plots with and without cells (**Fig. 2a**), were 751.0 C and 9.0 C, respectively, providing a net coulombic yield of 742.0 C from glucose oxidation. This power production was accompanied by the consumption of 1.85 mM glucose (**Fig. 2a**) in 210 ml in the anode chamber or 389 μmol of glucose. The oxidation of glucose to carbon dioxide yields 24 electrons:

$$C_6H_{12}O_6 + 6\,H_2O \rightarrow 6\,CO_2 + 24\,H^+ + 24\,e^-$$

Therefore, complete oxidation of all of the glucose that *R. ferrireducens* consumed would yield 900 C (1 mol of electrons = 96,500 C; 1 mol of glucose = 96,500 × 24 C or 1 μmol of glucose = 2.316 C. So, 389 μmol of glucose = 2.316 × 389 = 900 C). Thus, recovery of electrons from

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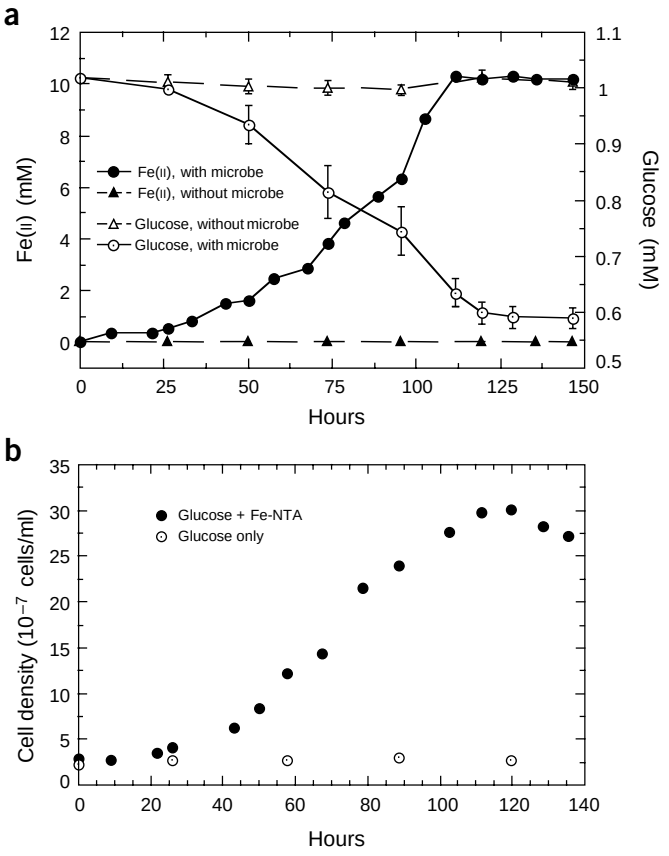

Figure 1 Growth of *R. ferrireducens* at 25 °C with glucose as electron donor and Fe(III) as the electron acceptor. (**a**) Glucose consumption and Fe(III) reduction. (**b**) Cell growth.

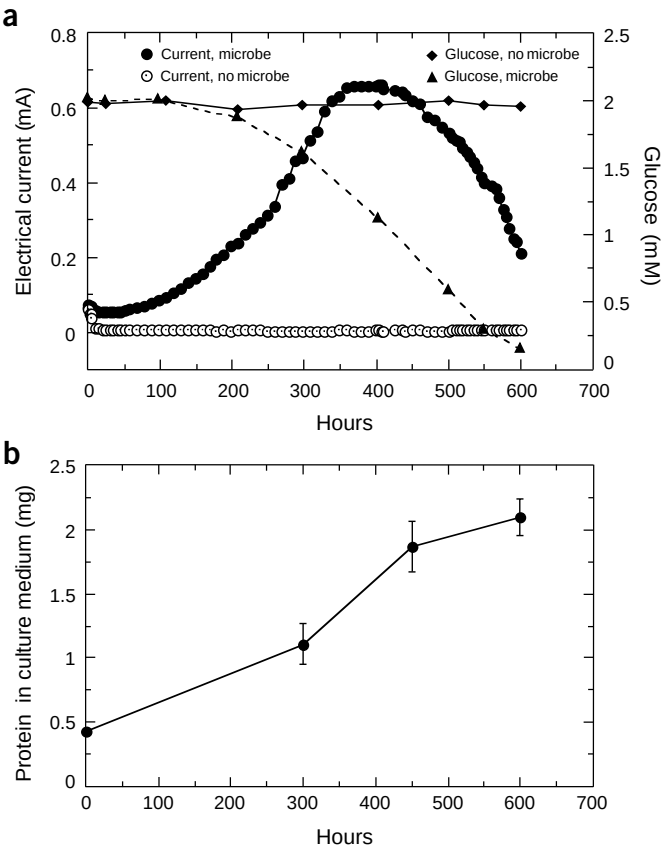

Figure 2 Current generation by and growth of *R. ferrireducens* at 25 °C in two-chambered fuel cells under poised-potential conditions using graphite rod electrodes. (**a**) Current production and glucose consumption. (**b**) Increase in planktonic cell protein.

glucose oxidation was 83% (742/900) of that theoretically available from glucose oxidation. When one considers that this study was conducted under growth conditions and that *R. ferrireducens* needed to assimilate some of the glucose to generate cell carbon, it is clear that *R. ferrireducens* was very effectively transferring electrons derived from glucose oxidation to the electrode.

Current production from glucose was further examined in two-chambered vessels in a true fuel-cell mode in which the anode chamber with the culture was anaerobic, but the sterile cathode chamber was aerobic with potassium ferricyanide to enhance oxygen reduction by the cathode. Excess glucose (10 mM) was provided to avoid electron donor limitation. Under these conditions *R. ferrireducens* sustained a steady current density of about 31 mA/m^2 (0.20 mA; 265 mV) when 1,000 Ω load resistance was applied to control electron flow from the anode to the cathode (**Fig. 3a**). The efficiency of electron transfer in fuel-cell mode was similar (81%) to that observed when the anode was poised at +200 mV. When the medium in the anode chamber was removed and replaced with fresh glucose medium, current production rapidly returned to the levels observed before replacement of the medium (**Fig. 3a**). This exchange of medium with immediate resumption in current production was repeated five times with similar results (data not shown). This suggests that microbial cells attached to the electrode surface were primarily responsible for the current production.

The finding that electron transfer to the anode provided energy to support growth led to the question of whether the cells would still be functional after periods in which there was no current flow. Therefore, the electrical connection between the anode and the cathode was eliminated for 36 h (**Fig. 3a**). When the connection was re-established, power production resumed at the previously observed levels.

These results indicate that this microbial fuel cell exhibits many of the desirable features of secondary storage batteries, including (i) the ability to be recharged to its nearly original charged state following discharge; (ii) no severe capacity fading on charge/discharge cycling; (iii) the ability to accept fast recharge; (iv) reasonable cycle life; and (v) low capacity loss under open circuit conditions as well as in prolonged storage under idle conditions[24,25].

Increasing the surface area of graphite available for microbial colonization increased power output. For example, when the anode and cathode were constructed of graphite felt, rather than graphite rods, approximately threefold more (~0.57 mA; 620 mV) current was produced (**Fig. 3b**). This reflected the higher surface area of the felt, as the current and cell densities (28.0 mA/m^2; 0.047 mg protein per cm^2) on the felt were essentially equivalent to those on the rods (31.0 mA/m^2; 0.032 mg protein per cm^2). Porous graphite foam electrodes, having almost the same geometric surface area as that of graphite rods, produced ~2.4-fold more current (74 mA/m^2; 445 mV) with glucose as the fuel than did the graphite rods (**Fig. 3b**). This could be attributed to the higher concentration of cells (0.086 mg protein per cm^2) attached to the foam electrodes than to the graphite rods (0.032 mg protein per cm^2). These results demonstrate that simply optimizing the surface area of the anode may substantially increase power production.

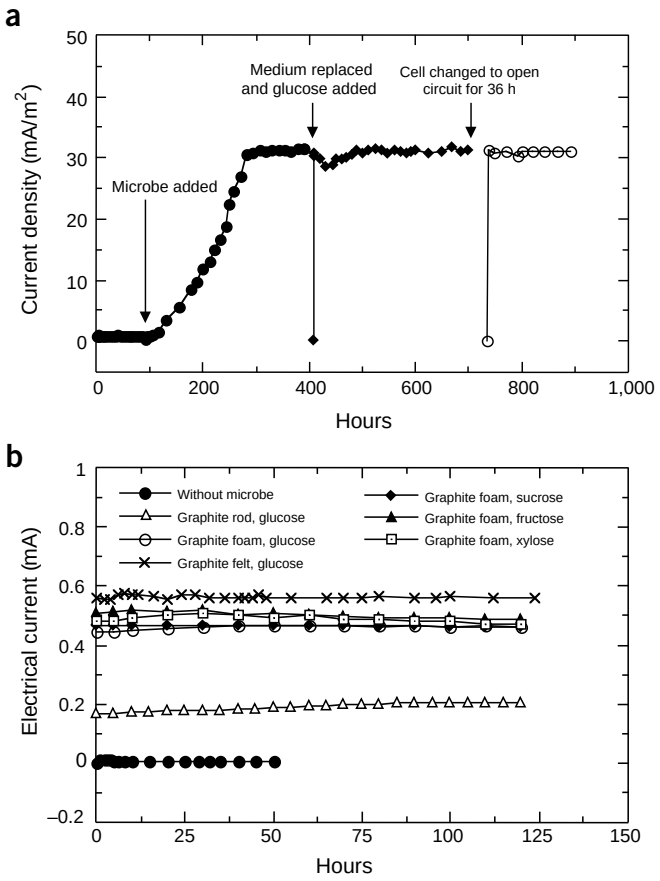

Figure 3 Current generation in fuel cell mode at 25 °C. (**a**) Effect of various manipulations on current production with glucose as the electron donor. (**b**) Effect of different graphite electrode materials and sugars on electricity generation. Initial sugar concentrations: glucose, 10 mM; fructose, 10 mM; sucrose, 5 mM; xylose, 15 mM. Total accessible geometric surface area of the electrodes: graphite rod, 6.5×10^{-3} m^2; graphite foam, 6.1×10^{-3} m^2; graphite felt, 20.0×10^{-3} m^2.

In addition to glucose, *R. ferrireducens* was also capable of effectively producing current from other sugars, such as fructose, sucrose and xylose (**Fig. 3b**). The amount of current produced was comparable to that for glucose and current production remained steady for days.

These results represent an important advancement in the microbial conversion of sugar to electricity. For example, in a previous study with a sugar-based mediatorless fuel cell, a *Clostridium* strain transferred no more than 0.04% of the electrons available in glucose to the anode[16]. In contrast, *R. ferrireducens* converted over 80% of the glucose electrons to current. Even microbial fuel cells that have used electron shuttling mediators have only had coulombic yields of 1–50% when using sugars as fuel[12–15] . The requirement for electron-shuttling mediators in these systems is a major drawback because these mediators are generally unstable and not effective for long-term performance of fuel cells[26]. Some biofuel cells[17–19], using immobilized enzymes, that have been developed for biomedical applications have the potential to provide current densities as high as ~8,300 mA/m^2. However, these systems are very inefficient in converting glucose to power, as glucose is only incompletely oxidized to gluconic acid and thus only 2 of the 24 electrons that are available per glucose molecule are harvested as electricity. Furthermore, the amount of power produced is small, on the order of 3.65×10^{-3} mA at 520 mV (ref. 19). Thus, the *R. ferrireducens* system described here shows much more promise for harvesting power from carbohydrates.

Clearly, further studies to improve fuel cell design, identify better electrode materials and optimize other process parameters are required to enhance current density for practical application of this sugar-based microbial fuel cell. For example, recent studies have suggested that incorporating manganese into graphite anodes may substantially enhance power production[27]. However, the ability to consistently produce power from glucose and other sugars in a long-term stable manner with *R. ferrireducens* suggests that efficient conversion of carbohydrate-containing waste materials and biomass to electricity is feasible.

METHODS

Source of the bacterium, growth medium and culture conditions. *R. ferrireducens* strain T118^T (American Type Culture Collection BAA-621; German Collection of Microorganisms and Cell Cultures (DSM) 15,236) was from our laboratory culture collection. Cells were grown under strict anaerobic conditions in a previously described bicarbonate-buffered, defined medium[28] under N_2/CO_2 (80:20) at 25 °C. For growth on Fe(III), 10 mM Fe(III) chelated with nitrilotriacetic acid (Fe(III)-NTA) was provided.

Fuel cell design. Studies with electrodes as the electron acceptor were conducted in a two-chambered glass vessel connected with a cation-selective membrane (Nafion-117) as previously described[21,23] with either graphite rod (grade G10; Graphite Engineering and Sales), graphite foam (16.4 g, Poco foam; Poco Graphite) or fine woven graphite felt (5.1 g, grade GF-S6; Electrosynthesis) as the electrodes. The total accessible geometrical surface areas of the electrodes were: graphite rod, 6.5×10^{-3} m^2; graphite foam, 6.1×10^{-3} m^2; and graphite felt, 20.0×10^{-3} m^2. The electrodes were cleaned with 1.0 M NaOH followed by 1.0 M HCl after each experiment and stored in distilled water before use. Watertight electrical connections were made as previously described[23]. The liquid volume in each chamber was approximately 210 ml with a headspace of about 160 ml. The anode chamber was continuously flushed with N_2/CO_2 (80:20) to maintain anaerobic conditions and maintain the pH balance of the growth medium. The cathode chamber contained growth medium under N_2/CO_2 for the poised-potential studies. The potential at the anode was maintained at +200 mV against a Ag/AgCl reference electrode with a potentiostat (Model 2053; AMEL Instruments). In the fuel cell studies, the cathode chamber contained 30 mM Tris buffer (pH 7.0), which was continuously flushed with sterile, water-saturated air. The chambers were stirred with a magnetic stir bar at 300 r.p.m. The inoculum for the electrode studies was a stationary phase culture that had been grown with glucose (2 mM) as an electron donor and fumarate (20 mM) as an electron acceptor.

Analytical techniques. Cell current and voltage were measured with a precision multimeter (Model 2000; Keithley Instruments). Cell numbers were determined with acridine orange staining and epifluorescent microscopy[29]. To measure planktonic cell protein, cells in 5 ml of culture were collected with centrifugation and the cell pellet was digested in 0.2 M NaOH, at 100 °C for 20 min. Protein attached to electrodes was extracted with 3–5 ml of 0.2 M NaOH for 1 h at room temperature (23–25 °C) at least six times. Soluble protein in the extracts was measured with the bicinchoninic acid method with a bovine serum albumin protein as the standard (Sigma Chemical Company). Glucose was separated on an organic acid analysis column (Aminex Ion-Exclusion HPX-87H; Bio-Rad) with high-pressure liquid chromatography and quantified with a refractive-index detector (Model SP8430; Spectra-Physics). Fe(II) was assayed with ferrozine[30].

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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1. Bjerre, A.B., Olesen, A.B., Fernqvist, T., Plöger, A. & Schmidt. A.S. Pretreatment of wheat straw using combined wet oxidation and alkaline hydrolysis resulting in convertible cellulose and hemicellulose. *Biotechnol. Bioeng.* **49**, 568–577 (1996).
2. Kim, K.H., Tucker, M.P. & Nguyen, Q.A. Effect of pressing lignocellulosic biomass on sugar yield in two-stage dilute-acid hydrolysis process. *Biotechnol. Prog.* **18**, 489–494 (2002).
3. van Wyk, J.P.H. Biotechnology and the utilization of biowaste as a resource for bioproduct development. *Trends Biotechnol.* **19**, 172–177 (2001).
4. DiPardo, J. Outlook for biomass ethanol production and demand. *EIA Forecasts* September 13, 2003. http://www.eia.doe.gov/oiaf/analysispaper/biomass.html.
5. Sheehan, J. & Himmel, M. Enzymes, energy, and environment: a strategic perspective on the U.S. Department of Energy's research and development activities for bioethanol. *Biotechnol. Prog.* **15**, 817–827 (1999).
6. Martin, C., Galbe, M., Wahlbom, C.F., Hahn-Hägerdal, B. & Jönsson, L.J. Ethanol production from enzymatic hydrolysis of sugarcane bagasse using recombinant xylose-utilising *Saccharomyces cerevisiae*. *Enzyme Microb. Technol.* **31**, 274–282 (2002).
7. Woodward, J., Orr, M., Cordray, K. & Greenbaum, E. Enzymatic production of biohydrogen. *Nature* **405**, 1014–1015 (2000).
8. Cortright, R.D., Davda, R.R. & Dumesic, J.A. Hydrogen from catalytic reforming of biomass-derived hydrocarbons in liquid water. *Nature* **418**, 964–967 (2002).
9. Czernik, S., French, R., Feik, C. & Chornet, E. Hydrogen by catalytic steam reforming of liquid byproducts from biomass thermoconversion processes. *Ind. Eng. Chem. Res.* **41**, 4209–4215 (2002).
10. Hao, X.H., Guo, L.J., Mao, X., Zhang, X.M. & Chen, X.J. Hydrogen production from glucose used as model compound of biomass gasified in supercritical water. *Int. J. Hydrogen Energy* **28**, 55–64 (2003).
11. Tayhas, G., Palmore, R. & Whitesides, G.M. in *Enzymatic Conversion of Biomass for Fuels Production* (eds. Himmel, M.E., Baker, J.O. & Overend, R.P.) 271–290 (American Chemical Society, Washington, DC, 1994).
12. Roller, S.D. *et al.* Electron-transfer coupling in microbial fuel cells:1. Comparison of redox-mediator reduction rates and respiratory rates of bacteria. *J. Chem. Technol. Biotechnol.* **34B**, 3–12 (1984).
13. Delaney, G.M. *et al.* Electron-transfer coupling in microbial fuel cells: 2. Performance of fuel cells containing selected microorganism-mediator-substrate combinations. *J. Chem. Technol. Biotechnol.* **34B**, 13–27 (1984).
14. Thurston, C.F. *et al.* Glucose metabolism in a microbial fuel cell. Stoichiometry of product formation in a thionine-mediated *Proteus vulgaris* fuel cell and its relation to coulombic yields. *J. Gen. Microbiol.* **131**, 1393–1401 (1985).
15. Park, D.H. & Zeikus, J.G. Electricity generation in microbial fuel cells using neutral red as an electronophore. *Appl. Environ. Microbiol.* **66**, 1292–1297 (2000).
16. Park, H.S. *et al.* A novel electrochemically active and Fe(III)-reducing bacterium phylogenetically related to *Clostridium butyricum* isolated from a microbial fuel cell. *Anaerobe* **7**, 297–306 (2001).
17. Katz, E., Shipway, A.N. & Willner, I. in *Handbook of Fuel Cells—Fundamentals, Technology, Applications* (eds. Vielstich, W., Gasteiger, A. & Lamm, A.) vol. 1(4), Ch. 21, 355–381 (John Wiley & Sons, Chichester, 2003).
18. Mano, N., Mao, F. & Heller, A. A miniature biofuel cell operating in physiological buffer. *J. Am. Chem. Soc.* **124**, 12962–12963 (2002).
19. Mano, N., Mao, F. & Heller, A. Characteristics of a miniature compartment-less glucose-O_2 biofuel cell and its operation in a living plant. *J. Am. Chem. Soc.* **125**, 6588–6594 (2003).
20. Finneran, K.T., Johnsen, C.V. & Lovley, D.R. *Rhodoferax ferrireducens* gen. nov., sp. nov.; a psychrotolerant, facultatively anaerobic bacterium that oxidizes acetate with the reduction of Fe(III). *Int. J. Syst. Evol. Microbiol.* **53**, 669–673 (2003).
21. Bond, D.R., Holmes, D.E., Tender, L.M. & Lovley, D.R. Electrode-reducing microorganisms that harvest energy from marine sediments. *Science* **295**, 483–485 (2002).
22. Tender, L.M. *et al.* Harnessing microbially generated power on the seafloor. *Nat. Biotechnol.* **20**, 821–825 (2002).
23. Bond, D.R. & Lovley, D.R. Electricity production by *Geobacter sulfurreducens* attached to electrodes. *Appl. Environ. Microbiol.* **69**, 1548–1555 (2003).
24. Aurbach, D. *et al.* Attempts to improve the behavior of Li electrodes in rechargeable lithium batteries. *J. Electrochem. Soc.* **149**, A1267–A1277 (2002).
25. Yamane, H. *et al.* Cycle performance in each state-of-charge in $LiMn_2O_4$. *J. Electrochem. Soc.* **149**, A1514–A1520 (2002).
26. Allen, R.M. & Bennetto, H.P. Microbial fuel cells: electricity production from carbohydrates. *Appl. Biochem. Biotechnol.* **39/40**, 27–40 (1993).
27. Park, D.H. & Zeikus, J.G. Improved fuel cell and electrode designs for producing electricity from microbial degradation. *Biotechnol. Bioeng.* **81**, 348–355 (2003).
28. Lovley, D.R. & Phillips, E.J.P. Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. *Appl. Environ. Microbiol.* **54**, 1472–1480 (1988).
29. Hobbie, J.E., Daley, R.J. & Jasper, S. Use of nucleopore filters for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.* **33**, 1225–1228 (1977).
30. Lovley, D.R. & Phillips, E.J.P. Organic matter mineralization with reduction of ferric iron in anaerobic sediments. *Appl. Environ. Microbiol.* **51**, 683–689 (1986).