APPLIED AND INDUSTRIAL MICROBIOLOGY

Microbial electrosynthesis — revisiting the electrical route for microbial production

Korneel Rabaey and René A. Rozendal

Abstract | Microbial electrocatalysis relies on microorganisms as catalysts for reactions occurring at electrodes. Microbial fuel cells and microbial electrolysis cells are well known in this context; both use microorganisms to oxidize organic or inorganic matter at an anode to generate electrical power or H_2, respectively. The discovery that electrical current can also drive microbial metabolism has recently lead to a plethora of other applications in bioremediation and in the production of fuels and chemicals. Notably, the microbial production of chemicals, called microbial electrosynthesis, provides a highly attractive, novel route for the generation of valuable products from electricity or even wastewater. This Review addresses the principles, challenges and opportunities of microbial electrosynthesis, an exciting new discipline at the nexus of microbiology and electrochemistry.

Biocatalyst
A catalyst of biological origin, which can be an enzyme, an organelle or even a whole cell.

Bioelectrosynthesis
The use of biocatalysts to achieve electricity-driven synthesis.

Overpotential
The difference between the thermodynamically determined potential and the experimentally observed potential of a half reaction; in an electrolytic cell, this corresponds to an energy loss, such that more energy is required to carry out the reaction than is expected.

Advanced Water Management Centre, Gehrmann Building, The University of Queensland, Brisbane, Queensland 4072, Australia.
Correspondence to K.R.
e-mail: k.rabaey@uq.edu.au
doi:10.1038/nrmicro2422

Electrical power can now be produced in a sustainable way by, for example, wind turbines and photovoltaic cells. This prospect of sustainable energy production has made electrical energy the key for our future transportation[1] and chemical production needs (using battery-driven vehicles and electrosynthesis, respectively). For electrosynthesis, adequate electrocatalysts are needed to catalyse the electrode-driven chemical reactions. Owing to their higher specificity and versatility relative to existing chemical catalysts, biocatalysts are increasingly being considered for these electrosynthetic processes. Bioelectrosynthesis relies on the interaction between biocatalysts and electrodes[2,3] and mainly uses enzymes or organelles that are physically immobilized on the electrode surface. However, although enzymes and organelles can provide a high reaction specificity and controllability, the use of whole microorganisms in bioelectrosynthetic processes has several advantages, including self-regeneration of the catalyst, adaptation of the catalyst quantity to the required conversion activity, flexibility in substrate use and higher versatility than enzymes or organelles for product formation or conversion pathways. The disadvantage of microorganisms is that they consume part of the substrate or donor for growth — albeit possibly only intermittently — and, as such, they are not true catalysts. However, like true catalysts, whole microorganisms have been shown to decrease the overpotentials at both anodes[4] and cathodes[5], resulting in improved performance. Recently, the term microbial electrosynthesis was used to describe the electricity-driven reduction of CO_2 (REF. 6) using whole microorganisms as electrocatalysts. In line with the definition of conventional (that is, non-microbial) electrosynthesis, we expand microbial electrosynthesis here to mean 'the microbially catalysed synthesis of chemical compounds in an electrochemical cell', which, in addition to the electricity-driven reduction of CO_2, also includes the electricity-driven reduction or oxidation of other organic feedstocks. In this Review, we describe the known pathways of extracellular electron transfer (EET) in bacteria and discuss the opportunities for microbial electrosynthesis.

Bioelectrochemical systems: the basics

Microbial electrosynthetic processes are conducted in so-called bioelectrochemical systems (BESs), which consist of an anode, a cathode and, typically, a membrane separating the two (FIG. 1). An oxidation process occurs at the anode (for example, acetate oxidation or water oxidation), whereas a reduction process occurs at the cathode (for example, O_2 reduction or H_2 evolution). The electrodes are surrounded by an electrolyte — the fluid around the electrode containing the reactants and/or products — which is generally an aqueous solution or wastewater (as a feed source). BESs can be operated in 'microbial fuel cell' mode, in which they deliver power[7], in short-circuit mode, in which the anode and cathode are connected without a resistor, or

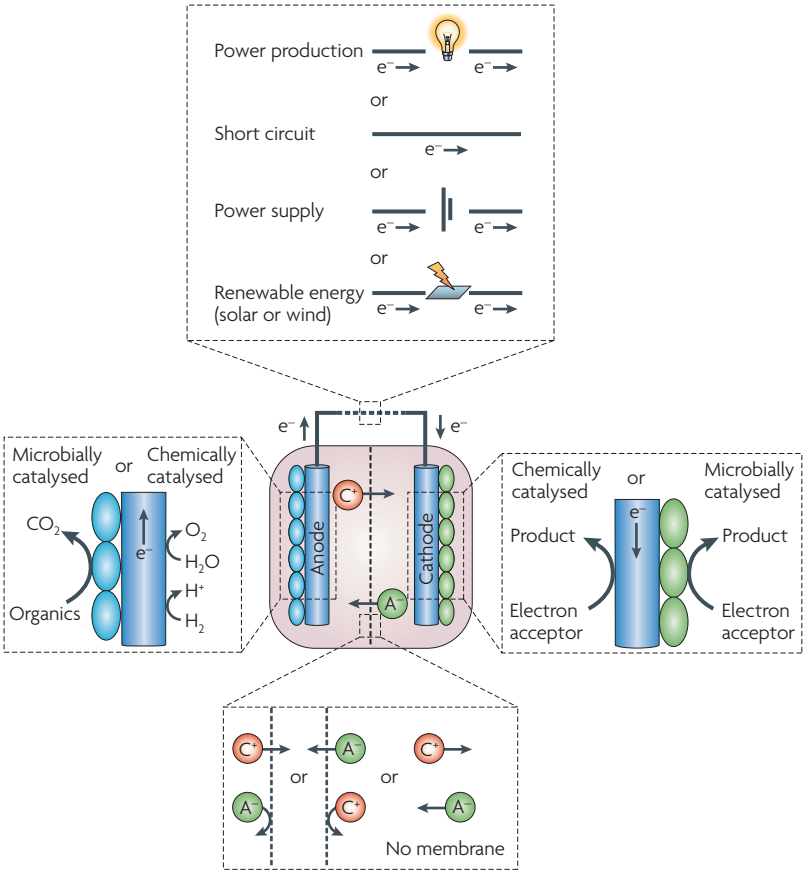


Figure 1 | **A high-level overview of the concepts associated with bioelectrochemical systems.** A plethora of choices can be made regarding the membrane, the nature of the catalysts at both the anode and the cathode, and the source of the reducing power. This leads to a highly versatile technology that can carry out a diverse range of processes.

in 'microbial electrolysis cell' mode, in which power is invested to increase the kinetics of the reactions and/or to drive thermodynamically unfavourable reactions[8]. In theory, much energy could be derived from microbial conversion reactions and limited energy would need to be invested to drive a microbial electrolysis process (BOX 1), but in reality the energy gained or invested is considerably less or more, respectively. To understand this, one needs to consider the losses in the BESs (for an in depth discussion, see REFS 9–12). First, the oxidation or reduction reaction at the electrode will incur so-called activation overpotential, causing a voltage loss due to imperfect catalysis at the electrode. The addition of a chemical or biological catalyst decreases this activation overpotential but will never eliminate it. Second, when electrons flow through an electrical circuit, ions simultaneously need to move through the electrolyte to restore the charge balance between anode and cathode. The electrolyte has a certain conductivity (for wastewater, typically 1–10 millisiemens per cm)[13] and this, together with losses in the electrodes and the electrical circuit, will lead to an ohmic loss. Notably this aspect is crucial for successful scaling up of the technology[13]. Last, at higher current densities (or low mixing) the supply of substrate to the electrode or the discharge of protons or hydroxyl ions may become diffusion limited[14]. This also leads to a decrease in the power output or an increase in the power requirement.

Humic substance
Recalcitrant organic compound that is formed during the decomposition of plant, animal and microbial cells.

Drawing electrons from microorganisms

In 1910, M. C. Potter wrote that "The disintegration of organic compounds by microorganisms is accompanied by the liberation of electrical energy" (REF. 15). This finding, made using *Saccharomyces cerevisiae*, was perhaps the first observation of what we now know as EET, the process by which microorganisms can transport electrons into and out of the cell from or towards an insoluble electron donor or acceptor. The primary focus of most research on EET has been (and still is) the transfer from organic electron donors towards minerals and electrodes. Community analyses of microbial fuel cell anodes reveal a high species diversity, including both Gram-positive and Gram-negative organisms[16,17]. However, the current models for EET are built around only Gram-negative isolates, as most Gram-positive isolates have not shown a strong capacity for EET thus far[18–20]. Two key mechanisms for electron transfer can be discerned: these are direct and indirect transfer. Based on the innate capabilities of organisms isolated from microbial fuel cells, it seems that in microbial populations multiple strategies are in operation simultaneously[21], maximizing the use of available resources.

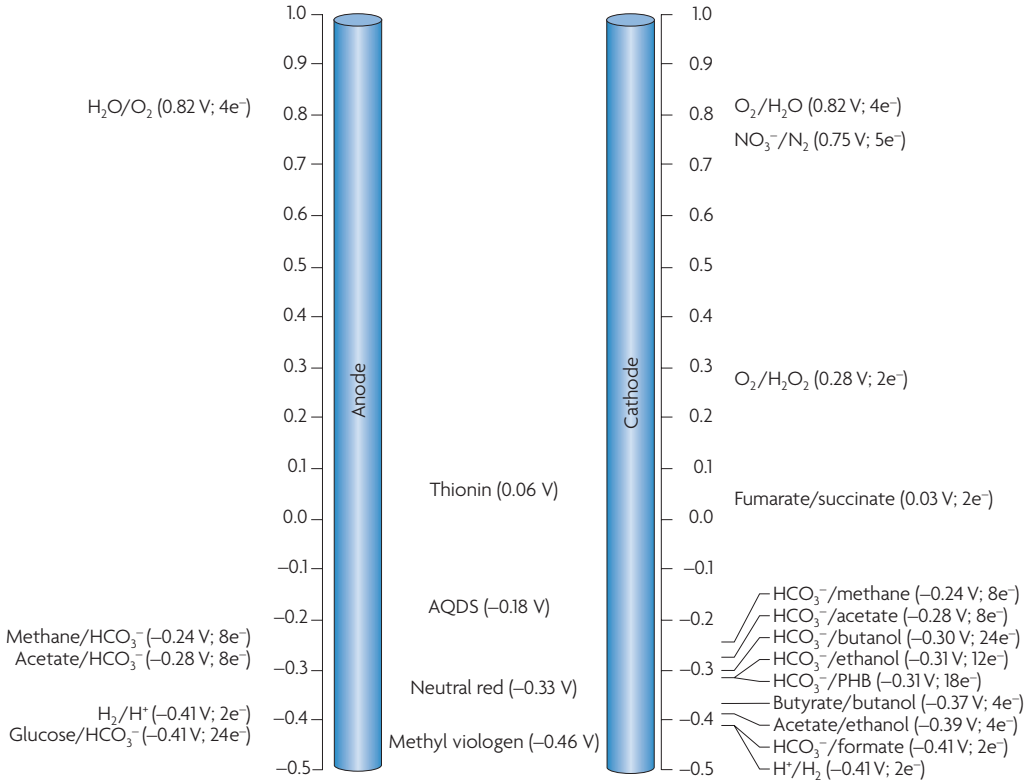
Here we define direct EET as 'not requiring the diffusion of a mobile component to and from the cell for electron transport'. Direct transfer has been widely studied in *Geobacter sulfurreducens*[22,23] and *Shewanella oneidensis* str. MR-1 (REF. 24), and there are several excellent reviews regarding the putative mechanisms of direct transfer in these species[17,25–27]. Briefly, direct transfer typically involves at least a series of periplasmic and outer-membrane complexes. For *S. oneidensis*, the apparent terminal cell-bound complex is MtrC, a decahaem cytochrome located on the outside of the membrane and capable of donating electrons in a broad potential range[28]. Electrons are transported from the periplasm to MtrC through a transmembrane electron transfer module consisting of MtrA, the transporting protein, incorporated inside MtrB, a sheath protein[28]. For *G. sulfurreducens*, a similar dependency on membrane-bound cytochromes has been well documented[29]. In recent years the involvement of pili or pilus-like appendages (called nanowires in this context) was established[30]. These seem to be essential for high levels of current production in *G. sulfurreducens*[31], in conjunction with OmcZ, a matrix-located cytochrome[32]. It has been suggested that nanowires also establish electron transport between different microorganisms in a community[33].

The second, indirect method for EET involves the production or use of so-called electron shuttles, which transport the electrons from the cell to the electrode. Examples of electron shuttles produced as secondary metabolites by organisms in BESs are phenazines[19,21] and flavins[34,35], whereas humic substances are electron shuttles that are not produced by the cell[36]. In addition, primary metabolites of bacteria such as sulphur species[37–39] and H_2 (REFS 2,40) can convey electrons towards iron oxides

Box 1 | **Theoretical cell voltages**

Bioelectrochemical systems (BESs) can produce power (when they are known as microbial fuel cells) or require an input of power (when they are known as microbial electrolysis cells), depending on the reactions taking place at the electrodes (see the figure*). In a microbial fuel cell, oxidation of an electron donor at the anode (for example, the oxidation of acetate to HCO_3^-; standard electrode potential at pH7 (E'_0) = –0.28 V versus the standard hydrogen electrode (SHE)) is coupled to the reduction of an electron acceptor with a higher electrode potential at the cathode (for example, the reduction of O_2 to water; E'_0 = 0.82 V versus SHE). The resulting cell voltage (cathode potential minus anode potential; 1.10 V in this example) is positive and, thus, power is produced. Conversely, in a microbial electrolysis cell, the oxidation of an electron donor at the anode (for example, acetate/HCO_3^-; E'_0 = –0.28 V versus SHE) is coupled to the reduction of an electron acceptor with a lower electrode potential at the cathode (for example, H^+/H_2; E'_0 = –0.41 V versus SHE). As the resulting cell voltage is negative (–0.13 V), an input of power is required. If water is the electron donor (that is, H_2O/O_2; E'_0 = 0.82 V versus SHE), high energy inputs are required. This illustrates the advantage of bioanodes, which can reduce energy input.

In a BES, microbial reactions that do not proceed through direct electron transfer mechanisms can be catalysed by the use of electron mediators, such as thionin, neutral red and methyl viologen. These compounds can shuttle electrons between electrode surfaces and microorganisms.



PHB, poly-β-hydroxybutyrate. *The electrode potentials of all electron donor and acceptor couples are calculated from Gibbs free energy data, from REFS 99,117, according to the methods described in REF. 13. The E'_0 values of the electron mediators are from REFS 83,118.

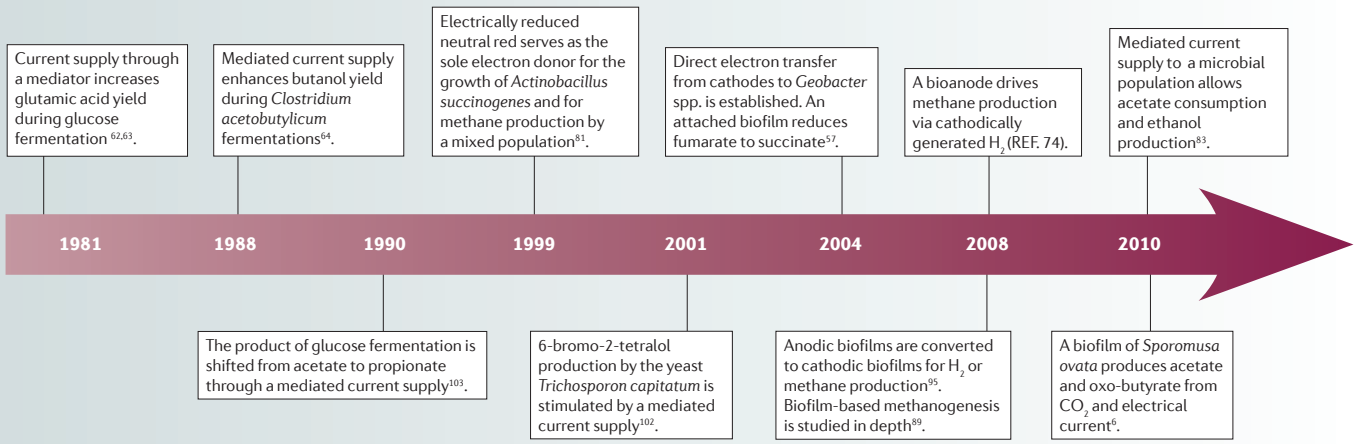
and electrodes, respectively. For *Pelobacter carbinolicus*, sulphur species are essential for EET towards minerals during the oxidation of ethanol[41]; whether this is also the case for EET towards electrodes remains to be investigated.

Light can be used as additional driver of anode catalysis; anodic H_2 production from malate by *Rhodospirillum rubrum* was linked to cathodic O_2 generation by a blue-green marine alga (tentatively identified as a member of the Oscillatoriales)[2]. Light drove both reactions, and therefore this study is perhaps the first to describe a biocatalysed cathode. This concept was later revived by several independent groups[42–44]. However, the key interest thus far for bioanodes has been in the context of microbial fuel cells for wastewater treatment[45–47], and for power production from renewable feedstocks[48] and sediments[49].

Microbially assisted electrosynthesis

Already, the use of a bioanode in combination with a chemical cathode for electrosynthesis is attracting much attention. H_2 can be produced by adding power to a microbial electrolysis cell with a platinum or otherwise catalysed cathode[50,51]. In addition, the consumption of protons at the cathode, be it for the reduction of O_2 or water, leads to an increasing pH[52,53], which was recently

Timeline | **Major achievements towards microbial electrosynthesis**



exploited for the production of caustic solutions[54]. Likewise, the use of carbon cathodes in BESs leads to the cathodic formation of hydrogen peroxide[55], which can be harvested as a valuable chemical. This hydrogen peroxide can be used for subsequent oxidation reactions, not only for bioproduction but also for bioremediation. Recently, hydrogen peroxide was obtained at a BES cathode and used to degrade *p*-nitrophenol in a Fenton reaction[56]; this is a strong oxidation reaction requiring hydrogen peroxide and ferrous iron (Fe II), typically in acidic conditions. Thus, microbially assisted electrosynthesis can effectively be used for the production of oxidants or disinfectants.

Pumping electrons into microorganisms

Whereas mechanistic information about microbially catalysed electron flow towards electrodes is abundant, information about the reverse process is limited. In recent years, several studies have investigated the communities that develop on cathodes. As found for the anodic communities, there was a high species diversity in the cathodic communities[5,57,58], but more research is needed to establish whether these communities are directly or indirectly catalysing the electrode reaction[59,60]. Several studies of electron transfer towards microorganisms, mainly in the context of bioremediation, have been excellently reviewed recently[61]. In the context of bioproduction, reducing power provided by means of an electrode can either redirect fermentation pathways (sometimes called electro-fermentation) or drive respiration (TIMELINE). Electro-fermentation has been investigated in bioproduction pathways such as the production of L-glutamic acid[62,63]. Within constraints such as maintaining redox homeostasis and product toxicity, a reductive process is expected to drive the NADH pool to a more reduced state, which forces the production of reduced metabolites such as butanol and ethanol to increase[64,65]. In electro-fermentation the cathodic current influences the fluxes in an existing fermentation pathway, whereas in what we could label 'electro-respiration' the cathodic current becomes the true driver of a lithoautotrophic or lithoheterotrophic metabolism. The cathodic current supply for respiration has been investigated mainly for bioremediation purposes, such as perchlorate reduction[66,67], denitrification[57,68,69], reductive dechlorination[70–72] and uranium recovery[73]. The electrode potential at which cathodic electron transfer can occur for respiratory processes has typically been higher than the potentials applied to affect fermentation processes[5,69], which is the logical consequence of the fact that the midpoint potentials of most respiratory acceptors are much higher than those of the fermentation 'acceptors'. Considering these differences in potentials, it is likely that different pathways exist for electron uptake in microorganisms. The following sections discuss the different options for EET towards microorganisms; a schematic representation is given in FIG. 2.

The first means of cathodic EET is through H_2. This gas can readily be produced at cathodes and can serve as a driver for microbial metabolism without an apparent negative effect on microbial integrity[74,75]. This fact and the versatile range of products that can be formed when microbial metabolism is driven by H_2 make this approach a good first stepping stone towards electricity-driven bioproduction of chemicals such as methane[74]. However, H_2 has two shortcomings as a driver of microbial metabolism. First, it has a low solubility, making high local concentrations difficult to achieve unless the microbial environment is pressurized. This may be a particular disadvantage for conversions that require a low redox potential close to the H_2 midpoint potential, as a low redox potential requires high H_2 partial pressures. Second, and perhaps more important, is the fact that H_2 production comes with a high overpotential at non-catalysed electrodes. This means that to achieve notable current densities, even when using a platinum-catalysed cathode, the potential of the cathode will be considerably lower than the theoretical standard electrode potential at pH 7 (E'_0), which is –0.410 V as measured versus the standard hydrogen electrode (SHE)[51] (BOX 1). Thus, effective and safe cathodic bioproduction probably needs to circumvent H_2. Several studies using microbial

Bioremediation
The use of microorganisms or biocatalysts for environmental clean-up.

Microbially assisted electrosynthesis
The use of whole microorganisms as electrode catalysts to drive the chemical synthesis of products at a counter electrode.

Lithoautotrophic
Of a microorganism: using an inorganic electron donor and CO_2 as a carbon source.

Lithoheterotrophic
Of a microorganism: using an inorganic electron donor and an organic compound as carbon source.

Electrode potential
The potential of an electrode relative to a reference electrode.

Standard hydrogen electrode
The universal reference electrode, which has a standard electrode potential (that is, at pH 0) of 0 V.

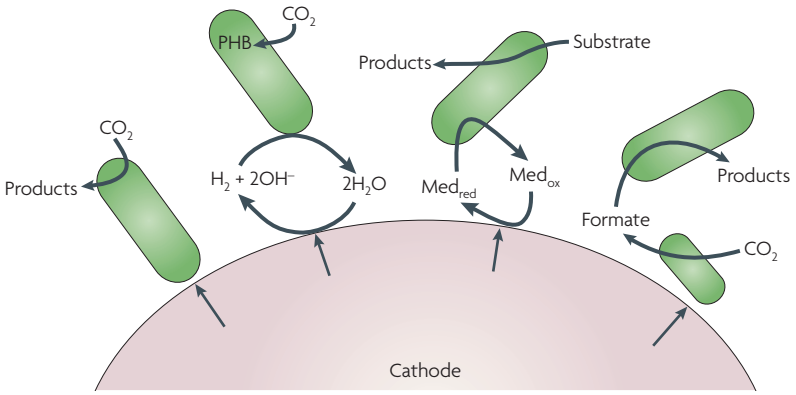


Figure 2 | **Mechanisms for electron transfer from electrodes to microorganisms.** The direct route of electron transfer (far left) seems the most attractive, but it is currently only speculative[57,89]. The production of H_2 for subsequent microbial conversion[74] (middle left) and the use of mediators (Med_{red} and Med_{ox} for reduced and oxidized mediator, respectively; middle right) (for example, methyl viologen)[64] are more established. Finally, the production of intermediate building blocks such as formate (far right) has been shown to be useful for single enzymes[92] and needs to be demonstrated with whole microorganisms. PHB, poly-β-hydroxybutyrate.

populations describe cathode potentials of above 0 V (at pH 7), strongly suggesting that electron transfer does not involve either H_2 or ferredoxin (for example, for ferredoxin, $E'_0 \approx -0.400$ V versus SHE)[76].

The second method of cathodic EET is through electron shuttles. As found for anodic EET, electron shuttles can provide an effective conduit for electrons towards a microorganism during cathodic EET. The advantages of electron shuttles are that they can be dissolved at a higher concentration than H_2, can decrease the overpotential at the electrode, can be chosen for their specific midpoint potential, can be reused many times and can be used in a large reactor (provided that the reactor is sufficiently mixed). The disadvantages of shuttles are their often limited stability, their possible toxic effects on the microorganisms and their loss in flow through systems. Neutral red, methyl viologen and thionin are the most studied compounds in this context[62,64,77,78]. In an early study on butanol fermentation mediated by *Clostridium acetobutylicum*, shuttles like neutral red ($E'_0 = -0.325$ V versus a SHE; BOX 1) were thought to stimulate H_2 uptake[79], but it was later shown *in vitro*, using methyl viologen ($E'_0 = -0.460$ V versus SHE; BOX 1)[80], that electron shuttles can directly drive $NAD(P)^+$ reduction to NAD(P)H using $NAD(P)^+$ ferredoxin oxidoreductase[64]. It was subsequently demonstrated that neutral red can serve as the sole electron donor for growth and succinate production from fumarate by *Actinobacillus succinogenes*[81,82], and its presence in conjunction with electrical current was shown to enhance glucose consumption, cell growth and product formation. Therefore, neutral red can be used as a true driver for microbial conversions and the generation of proton motive force, whereas methyl viologen combines redox effects with (perceived) toxicity effects[83]. It has been suggested that methyl viologen induces a shift from an acidogenic phase to a solventogenic phase in *C. acetobutylicum*[84], thus shifting the output products from fatty acids to the corresponding alcohols. Electron shuttles with higher midpoint potentials, such as anthraquinone-2,6-disulphonate (AQDS) ($E'_0 = -0.184$ V versus SHE; BOX 1), were effective for the cathodic reduction of perchlorate[66]. Again, the midpoint potential of AQDS indicates that EET does not necessarily occur at the NADH/NAD^+ ($E'_0 = -0.320$ V versus SHE) level or even at the H_2 ($E'_0 = -0.410$ V versus SHE; BOX 1) level. The use of iron as an electron shuttle towards microorganisms in acidic conditions has also been investigated[85].

The third and, perhaps, most attractive means of achieving EET from cathodes is through direct biocatalysis. As for anode systems, this decreases overpotentials and, to a certain extent, eliminates the existing diffusional limitations for both H_2 and shuttles. Moreover, from an engineering standpoint, a production process in which the biocatalyst is immobilized in the reactor simplifies the solid–liquid separations. Cathode-driven nitrate reduction was achieved using *Geobacter metallireducens* attached to the cathode[57]. These experiments indicated that *Geobacter* spp. accept electrons directly from the electrode surface. Recently, the same group described the formation of acetate and oxo-butyrate from CO_2 using *Sporomusa ovata*[6]. Direct transfer was assumed to occur, because the microorganisms were attached to an electrode with an applied potential of around –0.400 V versus SHE, which is higher than previously described potentials for H_2 evolution at graphite cathodes[86]. In earlier, bioremediation-based studies, direct electron transfer was also investigated for species including *Anaeromyxobacter dehalogenans* and *Geobacter lovleyi*[72,87]. Recently, several studies using mixed populations at the cathode have recorded high cathode potentials, limited or no H_2 production, and biofilm-based activity, all of which indicate that direct electron transfer also occurs in these microbial populations[67–69,88,89]. In cases in which direct electron transfer or the formation of an electro-active biofilm is not possible, an approach using bacteria that are physically immobilized in, for example, latex can be considered[90].

Last, rather than achieving direct production based on electrical current, an intermediary microorganism or biocatalyst could be used to produce an initial building block, such as formate or acetate, from CO_2. Such building blocks are subsequently used by other microorganisms for the production of larger molecules[91]. For instance, a tungsten-containing formate dehydrogenase enzyme (FDH1) adsorbed to an electrode can convert CO_2 to formate when electrical current is provided[92]. Furthermore, transient formate production has also been achieved using different microbial cultures that were provided with H_2, and a positive relationship between formate yield and H_2 partial pressure was established[93]. The product pattern shifted over time to acetate for *Acetobacterium carbinolicum* and to methane for the tested methanogen, *Methanobacterium formicicum*. Likewise, homoacetogens such as *Clostridium aceticum* produce acetate from CO_2 and H_2 (REF. 94). The capacity for cathodes to deliver reducing power, equivalent to H_2, is an indication of their potential to produce such building-block chemicals.

From electricity to product

Microbial electrosynthesis: starting from CO_2. The reduction of CO_2 can occur in either 'dark' or 'light' conditions. The production of methane has been the most common aim for respiratory bioproduction in dark conditions. Although it is sometimes considered a nuisance byproduct[95], several studies have made the production of methane a key objective[74,81,89]. Advantages of bioelectrochemical methane over conventional biogas are the possibility to store electricity or H_2 as methane[89] and the limited sensitivity of the process to ammonia, which can be present in the feedstock[74] (this relates to the sensitivity of methanogens to ammonia, which is formed at high pH values). The disadvantages are the low value of methane as a product, the energy investment that is required to produce the methane and the cost of pressurizing such a gas for transport. The concept of methane bioproduction was taken further with the development of a BES that produced methane through a biofilm that was immobilized on the cathode[89]. It was suggested that the EET towards the microorganisms was direct and thus did not proceed through H_2. This was also suggested in a recent report[96] detailing the use of electrochemical analyses to examine cathode catalysis. Direct EET enables electron flow without electron shuttle loss, for example, and enables the use of biofilms for methane production, two aspects that are highly attractive from an engineering standpoint. Further research through, for example, pure cultures and biofilm-based systems will unequivocally establish whether EET in these systems is indeed direct.

Conceptually, the above examples can probably be expanded to most (if not all) H_2-based microbial production processes. Examples of H_2-driven reactions include the production of the bioplastic poly-β-hydroxybutyrate by *Cupriavidus necator* (also known as *Alcaligenes eutrophus*)[97] and the production of acetate by homoacetogens, such as *C. aceticum*[94]. The need to establish the energetic favourability of the processes is important for all these conversions, as the reduction of organics proceeds at low redox potentials (examples of energetic-favourability calculations are available in REF. 98, and basic thermodynamic data can be found in REF. 99).

A key disadvantage of CO_2 as an electron acceptor is the large electron requirement for the synthesis of organic compounds. Although the theoretical potentials for the reduction of butyrate to butanol ($E'_0 = -0.37$ V versus SHE; BOX 1) and the reduction of CO_2 to butanol ($E'_0 = -030$ V versus SHE; BOX 1) are similar, the reduction of butyrate to butanol requires only 4 electrons, whereas the reduction of CO_2 to butanol requires 24 electrons, which implies a 6-fold higher current demand and an equivalently large power demand for this reaction. The conversion of CO_2 to butanol will also probably involve multiple synthesis steps, each with certain efficiency losses. Moreover, in most cases CO_2 needs to be obtained from the atmosphere or from waste gas at industrial sites (for example, coal-fired power plants), limiting either the kinetics or the geographical location of the production site. On the upside, CO_2 is ubiquitously available, it is a reasonably good electron acceptor (BOX 1), and its removal from the atmosphere is desirable because of concerns about its increasing concentration. Cathodic processes can be driven at least partially by the introduction of light energy. A biomass production process has been developed whereby light drives cathode catalysis and CO_2 fixation by a phototrophic consortium[100].

Microbial electrosynthesis: starting from organics. Organic compounds, such as acetate, butyrate and lactate, are ubiquitously present in wastewaters and fermenter effluents. Although these compounds are considerably valuable as products, their typically low concentrations make extraction economically unfeasible. Recently, acetate was converted to ethanol using a cathode and a mixed microbial community[83]. The coulombic efficiency of the process was 49% at best, with the highest yield observed in the presence of methyl viologen as an electron shuttle. H_2 was observed in the off gas, indicating that H_2 may have been a key pathway for EET. The methyl viologen depleted rapidly owing to irreversible reduction at the cathode[101], and in its absence high yields of butyrate (an undesired end product) were found. The details of the metabolism involved in ethanol production in this study are currently unclear, as no apparent ATP formation process was identified and excess equivalents were discharged through methanogenesis. In a separate study, the same group used H_2 to reduce butyrate to butanol at low overall alcohol yields[98]; if this could be achieved effectively in the aforementioned set-up converting acetate to ethanol, then the butyrate formation could lead to butanol as a more attractive end product. Conversion of fumarate to succinate has been achieved using *A. succinogenes*, with electrically reduced neutral red as an electron donor[81]. Interestingly, neutral red could also be used to drive respiration and to enhance glucose fermentation coupled to product formation[82]. Moreover, the same process was achieved without the addition of a mediator using *G. sulfurreducens*[57]. Lastly, BESs can also be used for the production of higher-value compounds. For instance, a kinetically enhanced process has been established to convert 6-bromo-2-tetralone to 6-bromo-2-tetralol, which is an intermediate in the synthesis of the potassium channel blocker MK-0499 (a chiral drug candidate)[102]. For such higher-value compounds, for which the resource cost (in energy and chemicals) is typically a small fraction of the production cost, it remains to be seen whether redox control or electron supply with a cathode are sufficiently attractive compared with the existing approaches.

Rerouting the metabolism. The control of fermentation pathways using electrical current — referred to as 'electro-fermentation' or 'an electro-energizing method' in the past — has been investigated for a range of chemicals. The first demonstration of this concept involved providing electrical current through a platinum cathode (using neutral red as an electron shuttle) to increase the yield of L-glutamic acid production from glucose[62]. A 26% increase in butanol formation by *C. acetobutylicum* was also observed during current supply[64]. Butanol production has since been the focus of several studies, mainly

Coulombic efficiency
The efficiency of charge transfer from the electron donor to the anode, or from the cathode to the electron acceptor.

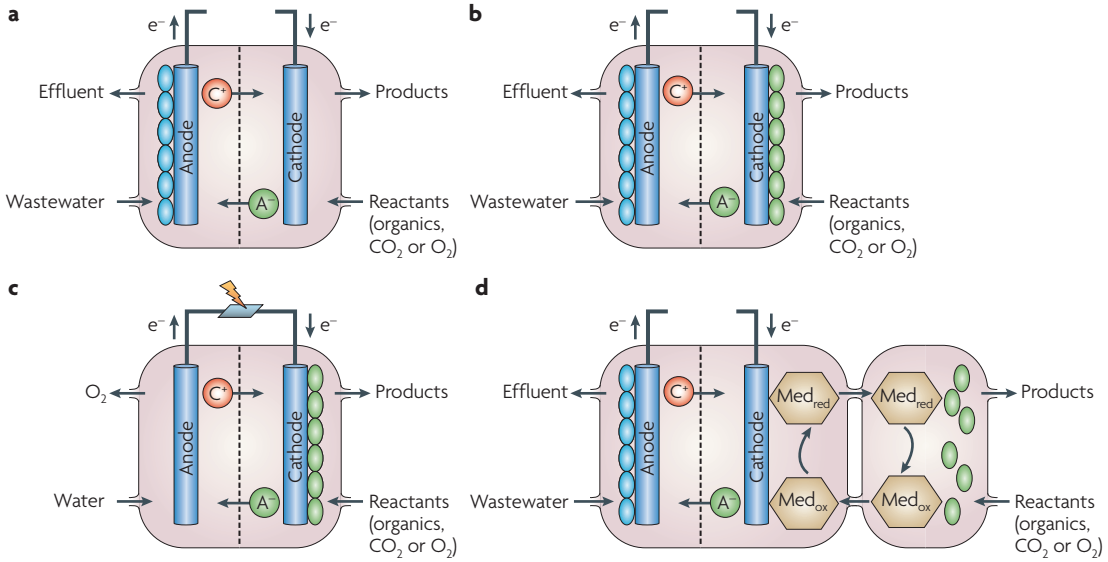


Figure 3 | **Configurations for bioelectrochemical system-based bioproduction. a** | The use of a bioanode in combination with a chemical cathode (example: hydrogen peroxide production from wastewater). **b** | The use of a bioanode in combination with a biocathode (example: bioplastic production from wastewater and CO_2). **c** | The use of a chemical anode in combination with a biocathode (example: solar-driven butanol production from CO_2). **d** | The use of either a chemical or biological anode in combination with a cathode that reduces mediators. The mediators (Med_{ox} and Med_{red}; oxidized and reduced mediator, respectively) can be used either *in situ* or in an external vessel to drive a bioproduction process (example: butanol production from glucose and wastewater).

assisted by methyl viologen as an electron shuttle[79,84], and the recent interest in butanol as a sustainable fuel is sparking renewed interest in this area of research. The impact of electrical current on the fermentative formation of propionate by *Propionibacterium freudenreichii* was also investigated, and it was found that, by virtue of a redox shuttle, reducing equivalents could be transported from a platinum cathode to the cells, leading to a shift in the fermentation end products towards higher propionate yields[103]. In addition to these examples, a wide range of attractive conversions can be envisaged, the energy levels required for which are highlighted in BOX 1. In many cases, the key role for electrical current is as a potentially cheaper source of reducing power than conventional substrates such as glucose.

Towards a production process

Three configurations supporting biocatalysed cathodes can be envisaged (FIG. 3). In the first set-up, both anode and cathode can be biocatalysed (FIG. 3b). A typical example of such a configuration is a BES using organics present in wastewater or sludge hydrolysates to drive the anode and achieve microbial electrosynthesis on the cathode[74]. This setting has been described for methanogenesis[74,89], but it can be applied for any of the microbial electrosynthesis processes at the cathode that are discussed above. BESs in this context can combine a mixed-population anode, tackling the complexity of waste organics, with a pure-culture, high-quality cathodic process aimed at specific product generation. Although one can argue that water is more abundant than organics for current supply, the available quantities of these organics should not be underestimated, and there are some distinct advantages to their use. The bioanode represents an energy saving relative to chemical anodes, as the electrons are generated at a low potential (that is, a high energy level). This is in contrast to anodic water splitting and generating O_2, during which the electrons are generated at a high potential (that is, a low energy level)[104] ($E'_0 = 0.82$ V versus SHE; BOX 1). It is interesting to note that the resulting O_2 also represents a risk towards the cathodic process, as it may diffuse from anode to cathode. In addition, bioanodes are typically made from low-cost carbon materials, in contrast to the costly dimensionally stable anodes (such as titanium-coated electrodes) that are required for water splitting. Furthermore, the oxidation of organics delivers CO_2, which can be reused at the cathode for the electrosynthesis reaction, as well as nutrients such as nitrogen. The supply of both is presently of concern for existing biofuel approaches. In conjunction with peripheral technology, and provided that they perform at larger scales, BESs could become the cornerstone of a wastewater biorefinery, in which solids (for composting), energy (for bioproduction), nutrients (through chemical precipitations) and water are sequentially recovered (FIG. 4). As advances in hydrolysis increasingly allow the use of solid waste or sludge for bioproduction purposes, so these feedstocks will become available for BESs[105].

The second and third configurations for biocatalysed cathodes involve a chemical anode that is linked to either a biocathode or a cathode that indirectly drives a biological reaction (FIG. 3c,d). An example of this would be solar-driven biofuel production, comparable to the recently described acetate production set-up[6]. Solar panels can achieve up to 40% sunlight-to-power efficiency today[106]; here, we assume a future solar panel

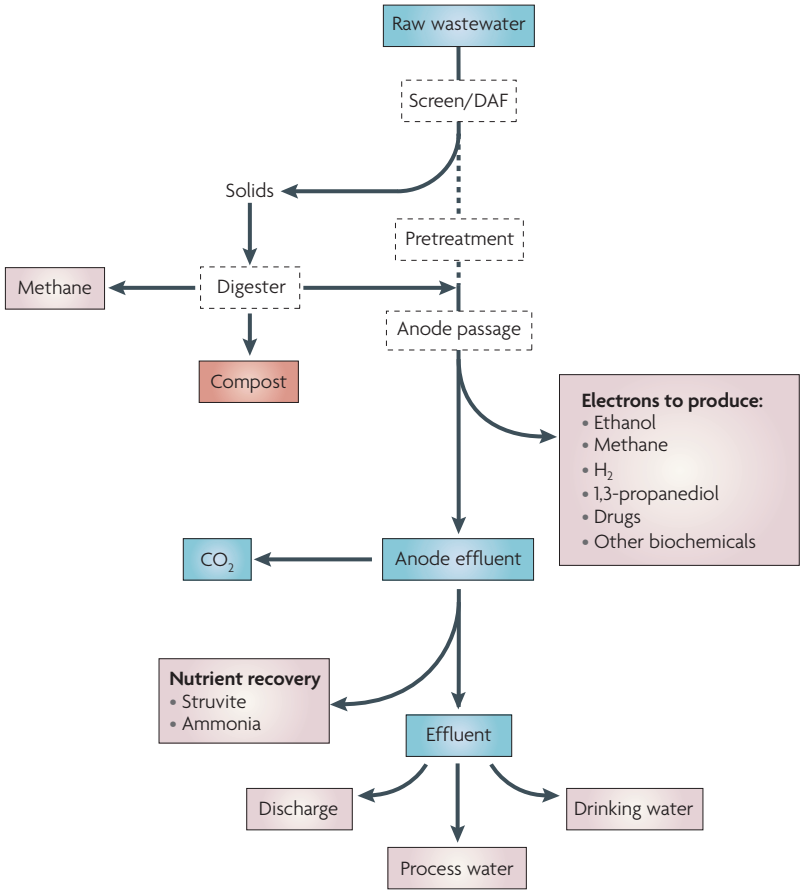


Figure 4 | **A 'bioelectrochemical' refinery.** Wastewater represents a net treatment cost today. Following a biorefinery concept, it is feasible to recover nutrients, energy or products, and water from this resource. The wastewater solids can be separated and used for the production of biogas and composting. The liquid flow can be used as feedstock for a bioelectrochemical system, leading to the recovery of products. Nutrients (notably phosphorus and nitrogen) can be crystallized from the anode effluent, and this prepares the water flow for further polishing by, for example, membrane technology, to generate drinking water (as well as process water and discharge). DAF, dissolved-air flotation.

with 20% efficiency or 200 W per m^2 ground surface for 12 hours per day. If the power derived from these systems is used at 2 V, this implies a current of 100 A per m^2 ground surface. If the electron conversion efficiency of butyrate to butanol is 50%, this implies an annual production of 151 kg butanol per m^2, which is a staggering 1,512 tonnes butanol per hectare. Starting from CO_2 (if such a quantity can be provided), the production at this efficiency would be 252 tonnes butanol per hectare per annum. For a comparison, algae currently produce around 50 tonnes biomass dry weight per hectare per annum, of which only a fraction is diesel or biodiesel[107]. A plethora of compounds could be produced using this approach; the key considerations are the investment cost and which reaction drives anodic oxidation.

Another fuel cell promise? Despite the strong cases outlined above and the promise of these techniques, one cannot ignore the fact that, although electricity-driven metabolism has been studied for several decades, practical applications are yet to be fully realized. In fact, intensive research has only begun in the past year. In December 2009, the US Department of Energy launched the first funding call in this area, under the banner 'electrofuels'. So why this renewed focus?

First, it is only in the past few years that the technological advances in microbial fuel cells and BESs have provided a strong technology platform. Materials science is increasingly capable of providing better materials to interface with the biological world and to function in bioelectrochemical systems[108–111]. Nevertheless, key developments are still needed to create highly conductive, scalable scaffolds with suitable surface properties for microbial attachment and electron exchange. Pilot trials are currently underway and will hopefully deliver the required information on scalability, electrical control and reactor engineering[112].

Second, although considerable progress in understanding EET has been made, the key challenge to making microbial electrosynthesis work will be the microorganism. The pool of available biocatalysts is extremely restricted to date, and the pathways for EET towards microorganisms are not yet known. After mining functional genes in natural and engineered environments, effective metabolic engineering to manipulate electron flows and to establish a catalytic interface between the cathode and the microorganism may be the next step. This will move the field of microbial electrosynthesis into synthetic biology. The opposite to this approach is the use of microbial populations at the cathode, the key impediment to this being selectivity towards a desired end product. Moreover, according to our knowledge, an important issue in most, if not all, known cathode studies is the lack of effective growth of the microorganisms over extended time periods. For pure-culture studies, generally either a biofilm is primed by heterotrophic growth[57] before the cathode operation or a high-density culture is inoculated at the onset of current provision[5]. It was suggested that a lithoheterotrophic metabolism is required for effective cathode growth[5]. This finding is supported in part by the increased longevity of mixed-population cathode systems, in which cross feeding of organics between organisms can occur, and by the observation that supplying the cathode with the anode effluent containing trace organics enhances performance[69,88,113].

Third, the pH of the system is important. It is now well established that anode current densities are restricted by the accumulation of protons at the electrode surface[14,114]. Likewise, the medium in the vicinity of the cathode may become alkaline, rendering reductions less thermodynamically feasible or introducing toxicity caused by, for example, free ammonia[115]. Structural design of the electrodes, manipulating the biofilm structure (if present) and controlling the hydrodynamic profile will be essential for maximizing conversion.

Fourth, and finally, the benchmark for renewables has changed, as legislation is driving the increased development of novel production processes. Renewable electricity is the harbinger of sustainable energy and chemical production. Electrical current can now be

produced almost anywhere, and local use of this electricity for bioproduction will therefore become increasingly attractive in the coming decades. However, detailed life cycle analyses, as recently performed for chemical production[116], will be essential for establishing whether this approach is a good idea, from an environmental and economic perspective.

Conclusion

Microbial electrosynthesis has the potential to become a key process in future bioproduction. The increased knowledge about EET gained over the past few years and the several decades of more empirical use of electrical current and microorganisms are driving rapid development in this area. Fuels and chemicals can be produced from CO_2 or basic organics by either redirecting fermentation pathways to produce more reduced metabolites or driving a respiratory production process. The key challenge will be to turn microorganisms into effective electrocatalysts by understanding how microorganisms deal with supplied reducing power and how they interact with the surface of an electrode.

1. Campbell, J. E., Lobell, D. B. & Field, C. B. Greater transportation energy and GHG offsets from bioelectricity than ethanol. *Science* **324**, 1055–1057 (2009).
A comparison between electricity and fuels in the context of transport and sustainability shows that the low energy yield of combustion makes bioelectricity more favourable.
2. Berk, R. S. & Canfield, J. H. Bioelectrochemical energy conversion. *Appl. Microbiol.* **12**, 10–12 (1964).
3. Hill, H. A. O. & Higgins, I. J. Bioelectrocatalysis. *Philos. Trans. R. Soc. Lond. A* **302**, 267–273 (1981).
4. Lowy, D. A., Tender, L. M., Zeikus, J. G., Park, D. H. & Lovley, D. R. Harvesting energy from the marine sediment–water interface II: kinetic activity of anode materials. *Biosens. Bioelectron.* **21**, 2058–2063 (2006).
5. Rabaey, K. *et al.* Cathodic oxygen reduction catalyzed by bacteria in microbial fuel cells. *ISME J.* **2**, 519–527 (2008).
6. Nevin, K. P., Woodard, T. L., Franks, A. E., Summers, Z. M. & Lovley, D. R. Microbial electrosynthesis: feeding microbes electricity to convert carbon dioxide and water to multicarbon extracellular organic compounds. *MBio* **1**, e00103-10 (2010).
7. Davis, J. B. & Yarbrough, H. F. Preliminary experiments on a microbial fuel cell. *Science* **137**, 615–616 (1962).
Early work on microbial fuel cells, several aspects of which have particular relevance for some of the studies that have been published recently.
8. Logan, B. E. *et al.* Microbial electrolysis cells for high yield hydrogen gas production from organic matter. *Environ. Sci. Technol.* **42**, 8630–8640 (2008).
9. Rabaey, K. *et al.* (eds). *Bioelectrochemical systems: from extracellular electron transfer to biotechnological application* (International Water Association Publishing, London, 2009).
A comprehensive book containing contributions by many key research groups in the field of bioelectrochemical systems, covering methodology, process development and applications.
10. Bard, A. J. & Faulkner, L. R. *Electrochemical methods: fundamentals and applications* 2nd edn (Wiley & Sons, New York, 2001).
This book provides an excellent background regarding electrochemistry and electrochemical analysis and is an essential read for researchers in this field.
11. Logan, B. *et al.* Microbial fuel cells: methodology and technology. *Environ. Sci. Technol.* **40**, 5181–5192 (2006).
12. Rabaey, K., Lissens, G. & Verstraete, W. in *Biofuels for fuel cells: biomass fermentation towards usage in fuel cells* (eds Lens, P. N. *et al.*) (International Water Association Publishing, London, 2005).
13. Rozendal, R. A., Hamelers, H. V. M., Rabaey, K., Keller, J. & Buisman, C. J. N. Towards practical implementation of bioelectrochemical wastewater treatment. *Trends Biotechnol.* **26**, 450–459 (2008).
A recent opinion article that details the importance of not only the microbial aspects but also the technical aspects of bringing bioelectrochemical systems to applications.
14. Torres, C. I., Marcus, A. K. & Rittmann, B. E. Proton transport inside the biofilm limits electrical current generation by anode-respiring bacteria. *Biotechnol. Bioeng.* **100**, 872–881 (2008).
This work shows that proton production during electrochemical oxidation limits microbial activity in biofilms, highlighting the fact that bulk parameters are not the only factors that are important for effective current generation.
15. Potter, M. C. On the difference of potential due to the vital activity of microorganisms. Proc. *Durham Univ. Phil. Soc.* **3**, 245–249 (1910).
16. Logan, B. E. Exoelectrogenic bacteria that power microbial fuel cells. *Nature Rev. Microbiol.* **7**, 375–381 (2009).
17. Lovley, D. R. Bug juice: harvesting electricity with microorganisms. *Nature Rev. Microbiol.* **4**, 497–508 (2006).
18. Milliken, C. E. & May, H. D. Sustained generation of electricity by the spore-forming, Gram-positive, *Desulfitobacterium hafniense* strain DCB2. *Appl. Microbiol. Biotechnol.* **73**, 1180–1189 (2007).
19. Rabaey, K., Boon, N., Höfte, M. & Verstraete, W. Microbial phenazine production enhances electron transfer in biofuel cells. *Environ. Sci. Technol.* **39**, 3401–3408 (2005).
20. Wrighton, K. C. *et al.* A novel ecological role of the Firmicutes identified in thermophilic microbial fuel cells. *ISME J.* **2**, 1146–1156 (2008).
21. Rabaey, K., Boon, N., Siciliano, S. D., Verhaege, M. & Verstraete, W. Biofuel cells select for microbial consortia that self-mediate electron transfer. *Appl. Environ. Microbiol.* **70**, 5373–5382 (2004).
A study demonstrating that an increased current generation can be achieved through enrichment and that microorganisms such as *Pseudomonas* spp. can self-mediate electron transfer.
22. Bond, D. R. & Lovley, D. R. Electricity production by *Geobacter sulfurreducens* attached to electrodes. *Appl. Environ. Microbiol.* **69**, 1548–1555 (2003).
23. Caccavo, F. *et al. Geobacter sulfurreducens* sp. nov., a hydrogen-oxidizing and acetate-oxidizing dissimilatory metal-reducing microorganism. *Appl. Environ. Microbiol.* **60**, 3752–3759 (1994).
24. Venkateswaran, K. *et al.* Polyphasic taxonomy of the genus *Shewanella* and description of *Shewanella oneidensis* sp. nov. *Int. J. Syst. Bacteriol.* **49**, 705–724 (1999).
25. Bretschger, O., Gorby, Y. & Nealson, K. H. in *Bioelectrochemical systems: from extracellular electron transfer to biotechnological application* (eds. Rabaey, K. *et al.*) 81–100 (International Water Association Publishing, London, 2009).
26. Lovley, D. R. The microbe electric: conversion of organic matter to electricity. *Curr. Opin. Biotechnol.* **19**, 564–571 (2008).
27. Torres, C. I. *et al.* A kinetic perspective on extracellular electron transfer by anode-respiring bacteria. *FEMS Microbiol. Rev.* **34**, 3–17 (2010).
28. Hartshorne, R. S. et *al.* Characterization of an electron conduit between bacteria and the extracellular environment. *Proc. Natl Acad. Sci. USA* **106**, 22169–22174 (2009).
This study shows that electron transfer through the *Shewanella oneidensis* outer membrane occurs through the MtrAB complex, the structure and working mechanism of which is described. Similar complexes seem to be ubiquitous in other species.
29. Holmes, D. E. *et al.* Microarray and genetic analysis of electron transfer to electrodes in *Geobacter sulfurreducens*. *Environ. Microbiol.* **8**, 1805–1815 (2006).
30. Reguera, G. *et al.* Extracellular electron transfer via microbial nanowires. *Nature* **435**, 1098–1101 (2005).
31. Reguera, G. *et al.* Biofilm and nanowire production leads to increased current in *Geobacter sulfurreducens* fuel cells. *Appl. Environ. Microbiol.* **72**, 7345–7348 (2006).
This work uses deletion mutants to demonstrate that nanowire production is essential for the formation of effective biofilms on electrodes.
32. Nevin, K. P. *et al.* Anode biofilm transcriptomics reveals outer surface components essential for high density current production in *Geobacter sulfurreducens* fuel cells. *PLoS ONE* **4**, e5628 (2009).
33. Gorby, Y. A. *et al.* Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms. *Proc. Natl Acad. Sci. USA* **103**, 11358–11363 (2006).
A key study on the role of nanowires as possible electron transport media, not only to minerals but also to electrodes and other microorganisms.
34. Marsili, E. *et al. Shewanella* secretes flavins that mediate extracellular electron transfer. *Proc. Natl Acad. Sci. USA* **105**, 3968–3973 (2008).
35. von Canstein, H., Ogawa, J., Shimizu, S. & Lloyd, J. R. Secretion of flavins by *Shewanella species* and their role in extracellular electron transfer. *Appl. Environ. Microbiol.* **74**, 615–623 (2008).
The first demonstration that *Shewanella* spp. may produce flavins as electron shuttles, indicating that *Shewanella* spp. may use different electron transfer strategies.
36. Holmes, D. E., Bond, D. R. & Lovley, D. R. Electron transfer by *Desulfobulbus propionicus* to Fe(III) and graphite electrodes. *Appl. Environ. Microbiol.* **70**, 1234–1237 (2004).
37. Dutta, P. K., Keller, J., Yuan, Z. G., Rozendal, R. A. & Rabaey, K. Role of sulfur during acetate oxidation in biological anodes. *Environ. Sci. Technol.* **43**, 3839–3845 (2009).
38. Nevin, K. P. & Lovley, D. R. Potential for nonenzymatic reduction of Fe(III) via electron shuttling in subsurface sediments. *Environ. Sci. Technol.* **34**, 2472–2478 (2000).
39. Straub, K. L. & Schink, B. Ferrihydrite-dependent growth of *Sulfurospirillum deleyianum* through electron transfer via sulfur cycling. *Appl. Environ. Microbiol.* **70**, 5744–5749 (2004).
This study finds that *Sulfurospirillum deleyianum* uses reduced sulphur species as electron shuttles to ferric iron minerals.
40. Schröder, U., Niessen, J. & Scholz, F. A generation of microbial fuel cells with current outputs boosted by more than one order of magnitude. *Angew. Chem. Int. Ed. Eng.* **42**, 2880–2883 (2003).
41. Haveman, S. A. *et al.* Genome-wide gene expression patterns and growth requirements suggest that *Pelobacter carbinolicus* reduces Fe(III) indirectly via sulfide production. *Appl. Environ. Microbiol.* **74**, 4277–4284 (2008).
42. Rosenbaum, M., Schroder, U. & Scholz, F. Utilizing the green alga *Chlamydomonas reinhardtii* for microbial electricity generation: a living solar cell. *Appl. Microbiol. Biotechnol.* **68**, 753–756 (2005).
43. Yagishita, T., Sawayama, S., Tsukahara, K. & Ogi, T. Photosynthetic bio-fuel cells using cyanobacteria. *Renewable Energy* **9**, 958–961 (1996).
44. Xing, D. F., Zuo, Y., Cheng, S. A., Regan, J. M. & Logan, B. E. Electricity generation by *Rhodopseudomonas palustris* DX-1. *Environ. Sci. Technol.* **42**, 4146–4151 (2008).
45. Habermann, W. & Pommer, E.-H. Biological fuel cells with sulphide storage capacity. *Appl. Microbiol. Biotechnol.* **35**, 128–133 (1991).
An often forgotten manuscript, this is the first to describe microbial fuel cells that use wastewater. The electron transfer is achieved through sulphate reduction and subsequent re-oxidation of the sulphide at the anode.

46. Kim, B. H. *et al.* Enrichment of microbial community generating electricity using a fuel-cell-type electrochemical cell. *Appl. Microbiol. Biotechnol.***63**, 672–681 (2004).
47. Liu, H., Ramnarayanan, R. & Logan, B. E. Production of electricity during wastewater treatment using a single chamber microbial fuel cell. *Environ. Sci. Technol.* **38**, 2281–2285 (2004).
48. Rismani-Yazdi, H. *et al.* Electricity generation from cellulose by rumen microorganisms in microbial fuel cells. *Biotechnol. Bioeng.* **97**, 1398–1407 (2007).
49. Tender, L. M. *et al.* Harnessing microbially generated power on the seafloor. *Nature Biotech.* **20**, 821–825 (2002).
50. Liu, H., Grot, S. & Logan, B. E. Electrochemically assisted microbial production of hydrogen from acetate. *Environ. Sci. Technol.* **39**, 4317–4320 (2005).
A study showing that the formation of H_2 at the cathode of a BES allows the production of H_2 from acetate without a requirement for light.
51. Rozendal, R. A., Hamelers, H. V. M., Euverink, G. J. W., Metz, S. J. & Buisman, C. J. N. Principle and perspectives of hydrogen production through biocatalyzed electrolysis. *Int. J. Hydrogen Energy* **31**, 1632–1640 (2006).
A second study to independently develop the same system as that described in reference 50.
52. Gil, G. C. *et al.* Operational parameters affecting the performance of a mediator-less microbial fuel cell. *Biosens. Bioelectron.* **18**, 327–334 (2003).
53. Rozendal, R. A., Hamelers, H. V. M. & Buisman, C. J. N. Effects of membrane cation transport on pH and microbial fuel cell performance. *Environ. Sci. Technol.* **40**, 5206–5211 (2006).
54. Rabaey, K., Bützer, S., Brown, S., Keller, J. & Rozendal, R. A. High current generation coupled to caustic production using a lamellar bioelectrochemical system. *Environ. Sci. Technol.* **44**, 4315–4321 (2010).
55. Rozendal, R. A., Leone, E., Keller, J. & Rabaey, K. Efficient hydrogen peroxide generation from organic matter in a bioelectrochemical system. *Electrochem. Commun.* **11**, 1752–1755 (2009).
56. Zhu, X. P. & Ni, J. R. Simultaneous processes of electricity generation and *p*-nitrophenol degradation in a microbial fuel cell. *Electrochem. Commun.* **11**, 274–277 (2009).
57. Gregory, K. B., Bond, D. R. & Lovley, D. R. Graphite electrodes as electron donors for anaerobic respiration. *Environ. Microbiol.* **6**, 596–604 (2004).
A demonstration that *Geobacter* spp. can use electrodes as electron donors for nitrate and fumarate reduction. Pure-culture growth was not evaluated in this study.
58. Wrighton, K. C. *et al.* Bacterial community structure corresponds to performance during cathodic nitrate reduction. *ISME J.* 3 Jun 2010 (doi:10.1038/ismej.2010.66).
The first manuscript to describe an in-depth investigation of microbial populations that use electricity as an energy source; despite the challenging conditions on the cathode, highly diverse populations can thrive.
59. Cournet, A., Bergel, M., Roques, C., Bergel, A. & Délia, M.-L. Electrochemical reduction of oxygen catalyzed by *Pseudomonas aeruginosa*. *Electrochim. Acta* **55**, 4902–4908 (2010).
60. Freguia, S., Tsujimura, S. & Kano, K. Electrontransfer pathways in microbial oxygen biocathodes. *Electrochim. Acta* **55**, 813–818 (2010).
61. Thrash, J. C. & Coates, J. D. Review: direct and indirect electrical stimulation of microbial metabolism. *Environ. Sci. Technol.* **42**, 3921–3931 (2008).
62. Hongo, M. & Iwahara, M. Application of electro-energizing method to L-glutamic acid fermentation. *Agric. Biol. Chem.* **43**, 2075–2081 (1979).
63. Hongo, M. & Iwahara, M. Determination of electro-energizing conditions for L-glutamic acid fermentation. *Agric. Biol. Chem.* **43**, 2083–2086 (1979).
64. Kim, T. S. & Kim, B. H. Electron flow shift in *Clostridium acetobutylicum* by electrochemically introduced reducing equivalent. *Biotechnol. Lett.* **10**, 123–128 (1988).
65. Saint-Amans, S., Girbal, L., Andrade, J., Ahrens, K. & Soucaille, P. Regulation of carbon and electron flow in *Clostridium butyricum* VPI 3266 grown on glucose-glycerol mixtures. *J. Bacteriol.* **183**, 1748–1754 (2001).
66. Thrash, J. C. *et al.* Electrochemical stimulation of microbial perchlorate reduction. *Environ. Sci. Technol.* **41**, 1740–1746 (2007).
67. Butler, C. S., Clauwaert, P., Green, S. J., Verstraete, W. & Nerenberg, R. Bioelectrochemical perchlorate reduction in a microbial fuel cell. *Environ. Sci. Technol.* **44**, 4685–4691 (2010).
68. Clauwaert, P. *et al.* Biological denitrification driven by microbial fuel cells. *Environ. Sci. Technol.* **41**, 3354–3360 (2007).
69. Virdis, B., Rabaey, K., Yuan, Z. & Keller, J. Microbial fuel cells for simultaneous carbon and nitrogen removal. *Water Res.* **42**, 3013–3024 (2008).
70. Aulenta, F. *et al.* Electron transfer from a solid-state electrode assisted by methyl viologen sustains efficient microbial reductive dechlorination of TCE. *Environ. Sci. Technol.* **41**, 2554–2559 (2007).
71. Aulenta, F. *et al.* Characterization of an electro-active biocathode capable of dechlorinating trichloroethene and *cis*-dichloroethene to ethene. *Biosens. Bioelectron.* **25**, 1796–1802 (2010).
72. Strycharz, S. M. *et al.* Graphite electrode as a sole electron donor for reductive dechlorination of tetrachlorethene by *Geobacter lovleyi*. *Appl. Environ. Microbiol.* **74**, 5943–5947 (2008).
73. Gregory, K. B. & Lovley, D. R. Remediation and recovery of uranium from contaminated subsurface environments with electrodes. *Environ. Sci. Technol.* **39**, 8943–8947 (2005).
74. Clauwaert, P. *et al.* Combining biocatalyzed electrolysis with anaerobic digestion. *Water Sci. Technol.* **57**, 575–579 (2008).
75. Sakakibara, Y. & Kuroda, M. Electric prompting and control of denitrification. *Biotechnol. Bioeng.* **42**, 535–537 (1993).
76. Angenent, L. T., Karim, K., Al-Dahhan, M. H. & Domiguez-Espinosa, R. Production of bioenergy and biochemicals from industrial and agricultural wastewater. *Trends Biotechnol.* **22**, 477–485 (2004).
77. Lithgow, A. M., Romero, L., Sanchez, I. C., Souto, F. A. & Vega, C. A. Interception of the electron-transport chain in bacteria with hydrophilic redox mediators.1. Selective improvement of the performance of biofuel cells with 2,6-disulfonated thionine as mediator. *J. Chem. Res.* **5**, 178–179 (1986).
78. 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).
79. Ghosh, B. K. & Zeikus, J. G. Electroenergization for control of H_2 transformation in acetone butanol fermentations. *Abstr. Pap. Am. Chem. Soc.* **194**, 79 (1987).
80. Stombaugh, N. A., Sundquist, J. E., Burris, R. H. & Orme-Johnson, W. H. Oxidation–reduction properties of several low potential iron-sulfur proteins and of methyl viologen. *Biochemistry* **15**, 2633–2641 (1976).
81. Park, D. H., Laivenieks, M., Guettler, M. V., Jain, M. K. & Zeikus, J. G. Microbial utilization of electrically reduced neutral red as the sole electron donor for growth and metabolite production. *Appl. Environ. Microbiol.* **65**, 2912–2917 (1999).
In this pivotal study, microorganisms are shown to grow on electrical current assisted by a mediator, and both fumarate reduction and methanogenesis are achieved.
82. Park, D. H. & Zeikus, J. G. Utilization of electrically reduced neutral red by *Actinobacillus succinogenes*: physiological function of neutral red in membrane-driven fumarate reduction and energy conservation. *J. Bacteriol.* **181**, 2403–2410 (1999).
83. Steinbusch, K. J. J., Hamelers, H. V. M., Schaap, J. D., Kampman, C. & Buisman, C. J. N. Bioelectrochemical ethanol production through mediated acetate reduction by mixed cultures. *Environ. Sci. Technol.* **44**, 513–517 (2010).
This article describes the production of alcohols by a microbial population using electrical current and the corresponding fatty acids.
84. Peguin, S., Goma, G., Delorme, P. & Soucaille, P. Metabolic flexibility of *Clostridium acetobutylicum* in response to methyl viologen addition. *Appl. Microbiol. Biotechnol.* **42**, 611–616 (1994).
85. Ter Heijne, A., Hamelers, H. V. M., deWilde, V., Rozendal, R. A. & Buisman, C. J. N. A bipolar membrane combined with ferric iron reduction as an efficient cathode system in microbial fuel cells. *Environ. Sci. Technol.* **40**, 5200–5205 (2006).
86. Aulenta, F., Reale, P., Catervi, A., Panero, S. & Majone, M. Kinetics of trichloroethene dechlorination and methane formation by a mixed anaerobic culture in a bio-electrochemical system. *Electrochim. Acta* **53**, 5300–5305 (2008).
87. Strycharz, S. M. *et al.* Reductive dechlorination of 2-chlorophenol by *Anaeromyxobacter dehalogenans* with an electrode serving as the electron donor. *Environ. Microbiol. Rep.* **2**, 289–294 (2010).
88. Virdis, B., Rabaey, K., Yuan, Z. G., Rozendal, R. A. & Keller, J. Electron fluxes in a microbial fuel cell performing carbon and nitrogen removal. *Environ. Sci. Technol.* **43**, 5144–5149 (2009).
89. Cheng, S. A., Xing, D. F., Call, D. F. & Logan, B. E. Direct biological conversion of electrical current into methane by electromethanogenesis. *Environ. Sci. Technol.* **43**, 3953–3958 (2009).
90. Flickinger, M. C., Schottel, J. L., Bond, D. R., Aksan, A. & Scriven, L. E. Painting and printing living bacteria: engineering nanoporous biocatalytic coatings to preserve microbial viability and intensify reactivity. *Biotechnol. Prog.* **23**, 2–17 (2007).
91. Carothers, J. M., Goler, J. A. & Keasling, J. D. Chemical synthesis using synthetic biology. *Curr. Opin. Biotechnol.* **20**, 498–503 (2009).
92. Reda, T., Plugge, C. M., Abram, N. J. & Hirst, J. Reversible interconversion of carbon dioxide and formate by an electroactive enzyme. *Proc. Natl Acad. Sci. USA* **105**, 10654–10658 (2008).
Using a single enzyme and electrical current, this study finds that formate can be produced from CO_2.
93. Peters, V., Janssen, P. H. & Conrad, R. Transient production of formate during chemolithotrophic growth of anaerobic microorganisms on hydrogen. *Curr. Microbiol.* **38**, 285–289 (1999).
94. Gottschalk, G. & Braun, M. Revival of the name *Clostridium aceticum*. *Int. J. Syst. Bacteriol.* **31**, 476–476 (1981).
95. Rozendal, R. A., Jeremiasse, A. W., Hamelers, H. V. M. & Buisman, C. J. N. Hydrogen production with a microbial biocathode. *Environ. Sci. Technol.* **42**, 629–634 (2008).
96. Villano, M. *et al.* Bioelectrochemical reduction of CO_2 to CH_4 via direct and indirect extracellular electron transfer by a hydrogenophilic methanogenic culture. *Bioresour. Technol.* **101**, 3085–3090 (2010).
97. Ishizaki, A. & Tanaka, K. Production of poly-β-hydroxybutyric acid from carbon-dioxide by *Alcaligenes-eutrophus* ATCC 17697. *J. Ferment. Bioeng.* **71**, 254–257 (1991).
98. Steinbusch, K. J. J., Hamelers, H. V. M. & Buisman, C. J. N. Alcohol production through volatile fatty acids reduction with hydrogen as electron donor by mixed cultures. *Water Res.* **42**, 4059–4066 (2008).
99. Thauer, R. K., Jungermann, K. & Decker, K. Energy-conservation in chemotropic anaerobic bacteria. *Bacteriol. Rev.* **41**, 100–180 (1977).
100. Cao, X. X. *et al.* A completely anoxic microbial fuel cell using a photo-biocathode for cathodic carbon dioxide reduction. *Energy Environ. Sci.* **2**, 498–501 (2009).
101. Peguin, S., Delorme, P., Goma, G. & Soucaille, P. Enhanced alcohol yields in batch cultures of *Clostridium acetobutylicum* using a 3-electrode potentiometric system with methyl viologen as electron carrier. *Biotechnol. Lett.* **16**, 269–274 (1994).
102. Shin. *et al.* Evaluation of an electrochemical bioreactor system in the biotransformation of 6-bromo-2-tetralone to 6-bromo-2-tetralol. *Appl. Microbiol. Biotechnol.* **57**, 506–510 (2001).
103. Emde, R. & Schink, B. Enhanced propionate formation by *Propionibacterium freudenreichii* subsp. *freudenreichii* in a 3-electrode amperometric culture system. *Appl. Environ. Microbiol.* **56**, 2771–2776 (1990).
104. Rabaey, K. *et al.* Microbial ecology meets electrochemistry: electricity-driven and driving communities. *ISME J.* **1**, 9–18 (2007).
105. Freguia, S. et al. Microbial fuel cells operating on mixed fatty acids. *Bioresour. Technol.* **101**, 1233–1238 (2010).
106. Ginley, D., Green, M. A. & Collins, R. Solar energy conversion toward 1 terawatt. *MRS Bull.* **33**, 355–372 (2008).
107. De Schamphelaire, L. & Verstraete, W. Revival of the biological sunlight-to-biogas energy conversion system. *Biotechnol. Bioeng.* **103**, 296–304 (2009).
108. Cheng, S. & Logan, B. E. Ammonia treatment of carbon cloth anodes to enhance power generation of microbial fuel cells. *Electrochem. Commun.* **9**, 492–496 (2007).
109. Freguia, S., Rabaey, K., Yuan, Z. & Keller, J. Non-catalyzed cathodic oxygen reduction at graphite granules in microbial fuel cells. *Electrochim. Acta* **53**, 598–603 (2007).

110. Logan, B., Cheng, S., Watson, V. & Estadt, G. Graphite fiber brush anodes for increased power production in air-cathode microbial fuel cells. *Environ. Sci. Technol.* **41**, 3341–3346 (2007).
111. Pant, D., Van Bogaert, G., De Smet, M., Diels, L. & Vanbroekhoven, K. Use of novel permeable membrane and air cathodes in acetate microbial fuel cells. *Electrochim. Acta* 1 Dec 2009 (doi:10.1016/j.electacta.2009.11.086).
112. Logan, B. E. Scaling up microbial fuel cells and other bioelectrochemical systems. *App. Microbiol. Biotechnol.* **85**, 1665–1671 (2010).
113. Freguia, S., Rabaey, K., Yuan, Z. G. & Keller, J. Sequential anode-cathode configuration improves cathodic oxygen reduction and effluent quality of microbial fuel cells. *Water Res.* **42**, 1387–1396 (2008).
114. Torres, C. I., Lee, H. S. & Rittmann, B. E. Carbonate species as OH^- carriers for decreasing the pH gradient between cathode and anode in biological fuel cells. *Environ. Sci. Technol.* **42**, 8773–8777 (2008).
115. Sprott, G. D. & Patel, G. B. Ammonia toxicity in pure cultures of methanogenic bacteria. *Syst. Appl. Microbiol.* **7**, 358–363 (1986).
116. Foley, J. M., Rozendal, R. A., Hertle, C. K., Lant, P. A. & Rabaey, K. Life cycle assessment of high-rate anaerobic treatment, microbial fuel cells, and microbial electrolysis cells. *Environ. Sci. Technol.* **44**, 3629–3637 (2010).
117. Heijnen, J. J. in *Bioprocess technology: fermentation, biocatalysis and bioseparation* (eds Flickinger, M. C. & Drew, S. W.) 267–291 (Wiley & Sons, New York, 1999).
A seminal book chapter on the use of thermodynamics for microbial growth calculations concerning yield and energy balance.
118. Gunther, H. & Simon, H. Artificial electron carriers for preparative biocatalytic redox reactions forming reversibly carbon hydrogen bonds with enzymes present in strict or facultative anaerobes. *Biocatal. Biotransformation* **12**, 1–26 (1995).

Acknowledgements
K.R. is supported by the Australian Research Council (grants DP0879245 and DP0985000), the Queensland, Australia government (Queensland Sustainable Energy Innovation Fund (QSEIF)) and The University of Queensland (UQ) Foundation (UQ Foundation Research Excellence Award 2009). R.R. is supported by the Queensland government (QSEIF) and UQ (UQ Early Career Researcher 2009; UQ Postdoctoral Research Fellowship 2010).

Competing interests statement
The authors declare no competing financial interests.

FURTHER INFORMATION
UQ Advanced Water Management Centre's homepage:
http://awmc.uq.edu.au

ALL LINKS ARE ACTIVE IN THE ONLINE PDF