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Electricity-producing bacterial communities in microbial fuel cells

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Microbial fuel cells (MFCs) are not yet commercialized but they show great promise as a method of water treatment and as power sources for environmental sensors. The power produced by these systems is currently limited, primarily by high internal (ohmic) resistance. However, improvements in the system architecture will soon result in power generation that is dependent on the capabilities of the microorganisms. The bacterial communities that develop in these systems show great diversity, ranging from primarily δ-Proteobacteria that predominate in sediment MFCs to communities composed of α-, β-, γ- or δ-Proteobacteria, Firmicutes and uncharacterized clones in other types of MFCs. Much remains to be discovered about the physiology of these bacteria capable of exocellular electron transfer, collectively defined as a community of 'exoelectrogens'. Here, we review the microbial communities found in MFCs and the prospects for this emerging bioenergy technology.

Microbial fuel cells make it possible to generate electricity using bacteria

It has been known for almost one hundred years that bacteria could generate electricity [1], but only in the past few years has this capability become more than a laboratory novelty. The reasons for this recent interest in using bacteria to generate electricity are a combination of the need for new sources of energy, discoveries about microbial physiology related to electron transport, and the advancement of fuel-cell technologies. In a microbial fuel cell (MFC), bacteria are separated from a terminal electron acceptor at the cathode so that the only means for respiration is to transfer electrons to the anode (Figure 1). The electrons flow to the cathode as a result of the electrochemical potential between the respiratory enzyme and the electron acceptor at the cathode. Electron transfer from the anode to the cathode must be matched by an equal number of protons moving between these electrodes so that electroneutrality is preserved (Box 1).

From the 1960s until quite recently, it was thought that exogenous mediators needed to be added into the fuel cell to generate reasonable amounts of power. However, Kim and co-workers [2–4] demonstrated that power could be generated by a naturally existing consortium of bacteria in the absence of exogenous mediators. Others then showed that by simply placing an anode into anoxic sediment and a cathode in overlying water, sufficient current could be generated to power subsurface devices [5]. It now seems that electricity can be generated from any biodegradable material, ranging from pure compounds such as acetate, glucose, cysteine, bovine serum albumin and ethanol [6–11] to complex mixtures of organic matter including domestic (human), animal, food-processing and meat-packing wastewaters [10,12–14].

With slight modifications to the MFC architecture and operation, hydrogen can be produced instead of electricity in relatively high yields. If oxygen is removed from the cathode and a small additional voltage is applied to the circuit, hydrogen gas is evolved from the cathode. This process has been referred to as a bio-electrochemically assisted microbial reactor (BEAMR) [15,16] or simply as the bacterial electrolysis of organic matter [17] because the protons and electrons are derived from the organic matter and not water. Water electrolysis is a highly endothermic reaction requiring, in practice, the application of 1.8 V. However, bacterial electrolysis of organic matter is exothermic, providing energy for the bacteria and producing an anode potential of approximately −0.3 V (versus a normal hydrogen electrode). At neutral pH, this is insufficient to generate hydrogen gas from the protons and electrons produced in this system. By adding ~0.25 V (0.11 V in theory), it is possible to produce hydrogen gas at the cathode. Yields of hydrogen production from acetate have reached 2.9 moles of hydrogen per mole of acetate (versus a theoretical maximum of 4 mol/mol) for an energy input equivalent to 0.5 moles of hydrogen. This represents a net energy gain by a factor of 5.8 in terms of the electricity needed for biohydrogen production by the BEAMR process, versus a net loss of energy needed for water electrolysis. The net energy input for biohydrogen is, therefore, sustained by the organic matter degraded by the bacteria.

It is a surprise to many researchers that the most significant block to achieving high power densities in MFCs is the system architecture, not the composition of the bacterial community. Early systems produced very low power densities (<0.1 mW/m^2, normalized by the anode surface area) [4] but power output by MFCs has been consistently increasing over time (Figure 2). In our own laboratory, improvements in system architecture and operation have increased power densities from <1 mW/m^2 to >1500 mW/m^2 using oxygen as the final electron acceptor at the cathode [7,18]. Platinum is used as a cathode catalyst, although cobalt- and iron-based catalysts can both produce similar power densities in these systems [19,20]. Power densities as high as 4.31 W/m^2 have been achieved, although this has required a non-renewable

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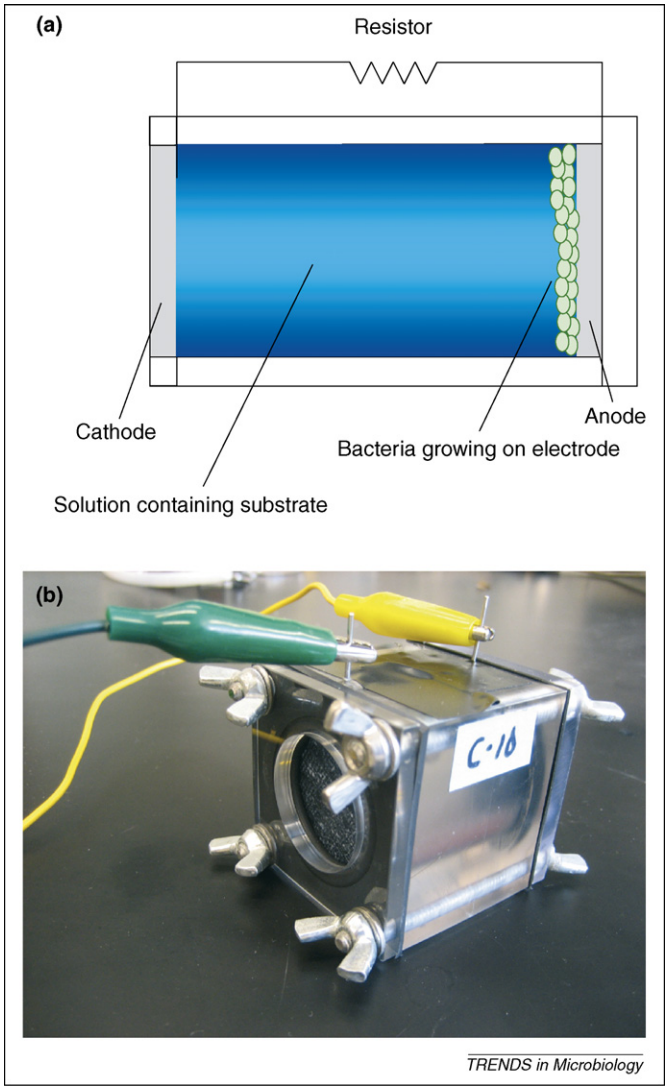

Figure 1. **(a)** A schematic and **(b)** a photograph of a single-chamber microbial fuel cell. The cathode is exposed to air on one side and the solution containing the biodegradable substrate is on the other side. The anode chamber containing the exoelectrogenic bacteria is sealed off from oxygen. The container is made of a solid block of plexiglass bored through to form a single, cylindrical tube and then capped with end plates as shown.

chemical reaction at the cathode involving ferricyanide [21,22]. Power can be increased through system architecture modifications that reduce the internal resistance of the MFC, such as by reducing electrode spacing, providing flow through a porous anode and increasing solution conductivity [7,23,24]. As power densities continue to increase, it is expected that current densities will eventually become limited by the maximum rate of electron transfer that can be sustained by bacteria. Achieving maximum power densities requires a better understanding of the type of bacteria that persist and become predominant in these communities, the mechanisms by which bacteria transfer electrons to the electrode and the ways in which bacteria interact in these systems.

Here, we review the microorganisms that are active in MFCs and the methods of exocellular electron transfer that have been identified to result in electricity generation, and comment on the future prospects of this emerging technology.

Box 1. The efficiency of converting organic matter to current

Coulombic efficiency (CE), or the percentage of electrons recovered from the organic matter versus the theoretical maximum whereby all electrons go to current generation, is an important factor in MFC performance. The complete oxidation of glucose, for example, produces up to 24 moles of electrons per mole of glucose. Some substrate is used for cell synthesis by electricity-generating bacteria but substrate can be lost to other processes as well. When oxygen is used at the cathode, oxygen diffusion through the CEM or cathode (depending on the system) into the anode chamber can be used for aerobic respiration by the same electricity-generating bacteria if they are facultative, or by other bacteria if a mixed culture is used [33]. In two-chamber MFC systems, substrate can also diffuse out of the anode chamber through the CEM into the cathode chamber (J-R. Kim, PhD thesis, Penn State University, 2006). Substrate can also be lost to alternative electron acceptors if they are present in the medium, for example, to nitrate or sulfate or through fermentation and methanogenesis [23,28]. The loss of substrate to other terminal electron acceptors can result in a portion of the bacterial community being sustained by non-electricity-generating processes.

CEs calculated for MFCs vary but, in general, they increase with power density because there is less time for substrate to be lost through competing physical and biological processes. In MFCs using ferricyanide and mixed cultures, CEs were 75% and 78% for acetate [23,27] but decreased to 49% and 65% using glucose, which is a fermentable substrate [21,52]. In recent air-cathode systems, high power densities have also been achieved, resulting in CEs of 60–65% with acetate [7,24,53] but only 19–42% with glucose [8]. For wastewater, which contains fermentable substrates and alternative electron acceptors, CEs range from 0.7–8.1% [28] to 17–96% with ferricyanide [23,52], and 12–28% with oxygen [13,33]. Poising the cathode at a high voltage eliminates the need for ferricyanide or oxygen in laboratory studies because water is electrolyzed to form hydrogen at the cathode. Back diffusion of this hydrogen into the anode might explain the extremely high CE (98.6%) obtained in one study because it was noted that flushing the headspace to remove hydrogen decreased the total electron recovery [54].

Microbial communities are phylogenetically diverse in most MFCs

Community analysis of MFC biofilms shows that there is no single emergent microorganism or 'winner' in the bacterial communities that develop on the anode. This is probably because several different bacteria are capable of electricity production and because of the range of operating conditions, system architectures, electron donors and electron acceptors (at the cathode). In addition, a portion of the community can be sustained by alternative metabolisms such as fermentation, methanogenesis and using terminal electron acceptors that do not result in electricity generation, as evidenced by a low Coulombic efficiency (Box 1). The electrochemically active bacteria in MFCs are thought to be iron-reducing bacteria such as *Shewanella* and *Geobacter* species [4,25] but analysis of the communities reveals a diversity of bacteria much greater than these model iron reducers persisting in the biofilm community [2,6,22,26]. Because high internal resistance has so far limited maximum power densities, comparisons made with different systems using pure or mixed cultures cannot establish which microorganism or microbial community is capable of the highest power density. Indeed, we do not yet know the upper limit of power levels that are achievable using microorganisms. However, we can see trends in the types of bacteria emerging from

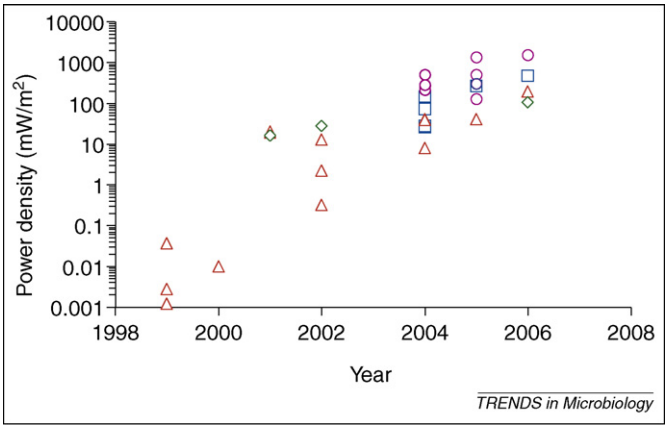

Figure 2. Power production for MFCs shown over time on the basis of published results. In less than a decade, power production by MFCs has increased by several orders of magnitude. Power production continues to be limited by systems that have the cathode immersed in water [aqueous cathodes (red triangles) and sediment MFCs (green diamonds)]. Substantial power production has been possible by using air-cathode designs in which the cathode is exposed to air on one side and water on the other side (blue squares). In general, wastewaters have produced less power than systems using pure chemicals (glucose, acetate and cysteine in the examples shown; purple circles). Not included in this figure are systems that are based on: hydrogen produced by fermentation [49,50] because the substrate is incompletely consumed in fermentation-based reactions or they require light [51]; or systems using ferricyanide at the cathode [21–23,27,28,34,52] because power production by these systems is not sustainable as a result of the need to regenerate chemically the ferricyanide consumed in the reaction.

community analysis of the biofilms with mixed cultures that are associated with the chemical reaction occurring at the cathode.

The high MFC power density of 4.31 W/m^2 was produced using an aerated solution of ferricyanide at the cathode, a graphite electrode system with low internal resistance, and the addition of substrate (glucose) into the reactor without withdrawal of the solution [22]. This lack of replacement of the fluid in the MFC enabled the accumulation of chemicals produced by the cell over many cycles. The analysis of the bacterial community that developed over time using denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S rRNA gene fragments and sequencing of dominant bands showed great phylogenetic diversity, with the identification of sequences derived from bacteria of the taxa Firmicutes, γ-, β- and α-Proteobacteria [22]. Facultative anaerobic bacteria capable of hydrogen production were predominant, such as the Gram-negative *Alcaligenes faecalis* and Gram-positive *Enterococcus gallinarum*, probably as a result of using a fermentable substrate with a mixed culture inoculum. Isolates obtained from this reactor generated electricity and produced large concentrations of highly colored mediators, such as pyocyanin produced by *Pseudomonas aeruginosa*. Therefore, it was deduced that mediator production was the main reason for the high power generation, in concert with the low internal resistance of the system. No attempt was made to limit oxygen diffusion from the cathode chamber into the anode, and the cation exchange membrane (CEM) that was used in this system is permeable to oxygen (J-R. Kim, PhD thesis, Penn State University, 2006) so both oxygen and ferricyanide could have been a factor that affected community development.

In fed-batch systems in which the contents of the anode chamber are replaced after each cycle or in continuous flow systems, different patterns emerge in the development of the microbial community. In a system again using ferricyanide at the cathode, DGGE fingerprinting with sequencing of the major bands revealed that an anaerobic sludge inoculum evolved into a microbial community dominated by the Gram-positive bacterium *Brevibacillus agri*, a member of the Firmicutes [27]. In an upflow reactor in which granules of bacteria remained suspended in the flow (with ferricyanide contained in the cathode chamber), archaea presumed to be methanogens were found to persist in the system, as indicated by fluorescence *in situ* hybridization staining of samples [28]. However, no community analysis was performed on this system.

When oxygen is used for the chemical reaction at the cathode, diverse communities evolve in the system with compositions that vary with the inoculum and substrate. On the basis of the sequences of cloned PCR-derived 16S rDNA fragments with unique restriction fragment length polymorphism (RFLP) patterns, a river sediment evolved into a community dominated by β-Proteobacteria (related to *Leptothrix* spp.) when fed river water, and predominantly α-Proteobacteria (mainly Actinobacteria) emerged when the reactor was fed a glucose–glutamic acid mixture [26]. Sequences from a DGGE-screened 16S rDNA clone library showed that a marine sediment used to inoculate an MFC fed with cysteine resulted in a bacterial community in which 97% of the sequences detected belong to the γ-Proteobacteria but were similar to *Shewanella affinis* KMM 3686 (40% of clones), with *Vibrio* spp. and *Pseudoalteromonas* spp. being the next most frequently detected [6].

In some systems, a large percentage of the clones are uncharacterized. Using wastewater as an inoculum in an MFC with dissolved oxygen at the cathode, the microbial community that developed when fed starch consisted of 36% unidentified clones, 25% β- and 20% α-Proteobacteria, and 19% Cytophaga, Flexibacter and Bacteroides groups on the basis of sequences from RFLP-screened 16S rDNA clones [2]. Using the same community analysis approach, an acetate-fed reactor with the same type of inoculum was found to be similarly diverse, with 24% α-, 7% β-, 21% γ- and 21% δ-Proteobacteria, and 27% others [29]. A γ-Proteobacterium isolated from this reactor (*Aeromonas hydrophila*) was capable of iron reduction and produced current using glucose in an MFC [30].

A clear pattern of dominance by δ-Proteobacteria emerges in sediment MFCs. In these systems, the anode is immersed in anoxic sediment and strict anaerobic conditions are maintained. By contrast, oxygen can diffuse into the anode chamber in other types of MFCs. In a marine sediment MFC, 71% of the sequences obtained in a 16S rDNA clone library from an anode electrode were δ-Proteobacteria, and 70% of these belonged to the family Geobacteraceae [25]. In a similar system, 76% were δ-Proteobacteria, 59% of which were in the family Geobacteraceae with a >95% similarity to *Desulfuromonas acetoxidans* [31]. A comparison of marine, salt marsh and freshwater sediments in five laboratory and field tests revealed the predominance of the δ-Proteobacteria in these samples (54–76% of gene sequences recovered from the anode), with the next most predominant sequences from

γ-Proteobacteria (3 cases, 9–10%), Cytophagales (33%) and Firmicutes (11.6%) [32]. Side-by-side comparisons of the effect of MFC architecture (i.e. sediment, non-sediment, oxygen, ferricyanide and poised-potential MFCs), substrate and inoculum (sediment, river and wastewater bacteria) are needed for a better understanding of the community that evolves in MFC systems.

Wireless and wired communities

The wide diversity of bacteria that evolve in MFC reactors, driven in part by a variety of operating conditions, demonstrates the versatility of bacteria that can either transfer electrons to the electrode or can exist in the reactor as a result of symbiotic relationships with electricity-producing bacteria. A picture is emerging that suggests microorganisms that are not in direct contact with the anode can be integral members of the community, that is, bacteria distant from the anode are capable of electron transfer to the surface. This view of the biofilm as a thick, electrochemically active community arises from several different observations. Live–dead staining of the community and analysis using confocal microscopy shows the presence of 'live' clumps of bacteria, perhaps tens of microns in thickness, on carbon cloth electrodes (Figure 3). Additional evidence of bacteria active at locations distant from the anode is based on calculations of the power that could be produced from a flat electrode surface. With only a monolayer of cells (100% coverage) and typical anaerobic bacterial growth rates, an upper limit of 2.2 W/m^2 of surface area has been suggested [33], a value that has been exceeded in some studies where bacteria produced mediators [21,22].

The ability of bacteria to transfer electrons to a distant surface is also supported by several studies showing the presence of bacterial mediators or electron shuttles (wireless communities), or highly conductive nanowires produced by the bacteria (wired communities). *Pseudomonas aeruginosa* produces phenazines capable of electron transfer between cells and surfaces [22] and tests with pure cultures have shown that the addition of these compounds in MFCs can increase power [34]. However, the effect of these redox-active compounds on the microbial ecology in MFCs systems is unclear because some phenazine derivates also have antibiotic effects on certain fungi and bacteria [22,34,35]. Some time ago, it was shown that *Shewanella oneidensis* MR-1 has cytochromes on its outer membrane [36]. More recent findings conclusively demonstrate that strain MR-1 reduces iron at a distance, which implies that this strain produces mediators [37]. However, specific mediators that can explain observed rates of iron reduction by strain MR-1 have never been conclusively identified.

It has now been shown that both *Shewanella* and *Geobacter* species produce nanowires that are highly conductive. Therefore, cells that are not directly in contact with a surface can achieve electron transfer to that surface [38]. The nanowires produced by *Geobacter sulfurreducens* are long thin strands or filaments, whereas those made by *S. oneidensis* MR-1 seem to be bundles of nanowires containing many individual conductive filaments (like a cable) that together have the appearance of a thick pilus (Figure 4a). Conductive scanning tunneling microscopy (STM) of pili from *S. oneidensis* MR-1 on graphite plates has shown that these filaments are conductive in the z-plane (i.e. between an STM tip and the surface; Figure 4b), although definitive studies on conductivity in the x–y plane remain to be done. When an MFC is inoculated with a pure culture of *S. oneidensis*, the bacteria colonize the electrode and rapidly produce a large number of these nanowires that touch both the surface and other cells (Figure 4c). The chemical composition and properties of these conductive filaments and pili are still being investigated.

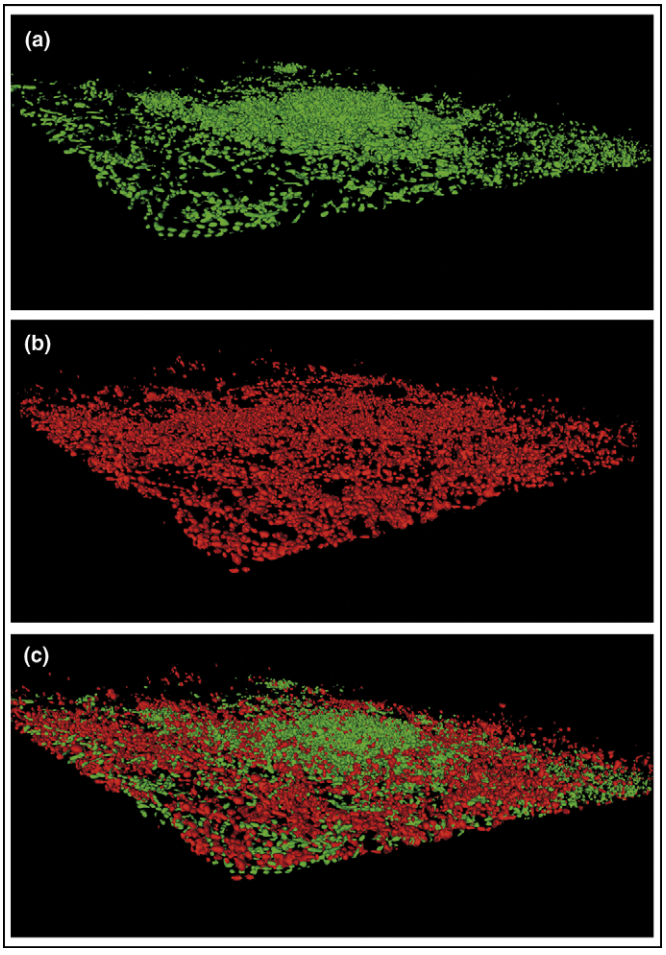

Figure 3. Confocal laser scanning microscopy images (stacked) of a biofilm on a carbon paper electrode obtained from an MFC following several weeks of stable power generation. Cells were stained using a fluorescent stain to show live (green) and dead (red) cells. (Note that such staining is an assay for membrane integrity and, therefore, does not conclusively separate live from dead cells.) The reactor was inoculated with a mixed culture but only electrochemically active bacteria and others that can symbiotically thrive with these bacteria should survive and grow. **(a)** The live-only image shows a mound of live bacteria, even when distant from the surface. **(b)** Dead cells are uniformly distributed because many bacteria in the inoculum might have adhered to the surface but could not use it as an electron acceptor. **(c)** The combined image shows the mounding of live bacteria less clearly than (a). Data were obtained by J. McIntyre. Three-dimensional imaging and figures were prepared by C. Anderson.

The emergence of new bacterial community interactions on the basis of interspecies electron transfer

Electrochemically active bacteria seem to be abundant in a variety of samples used to inoculate MFCs, including wastewaters, sludges, and river and marine sediments. When we inoculate an MFC in our laboratory with domestic wastewater, a repeatable cycle of power production can

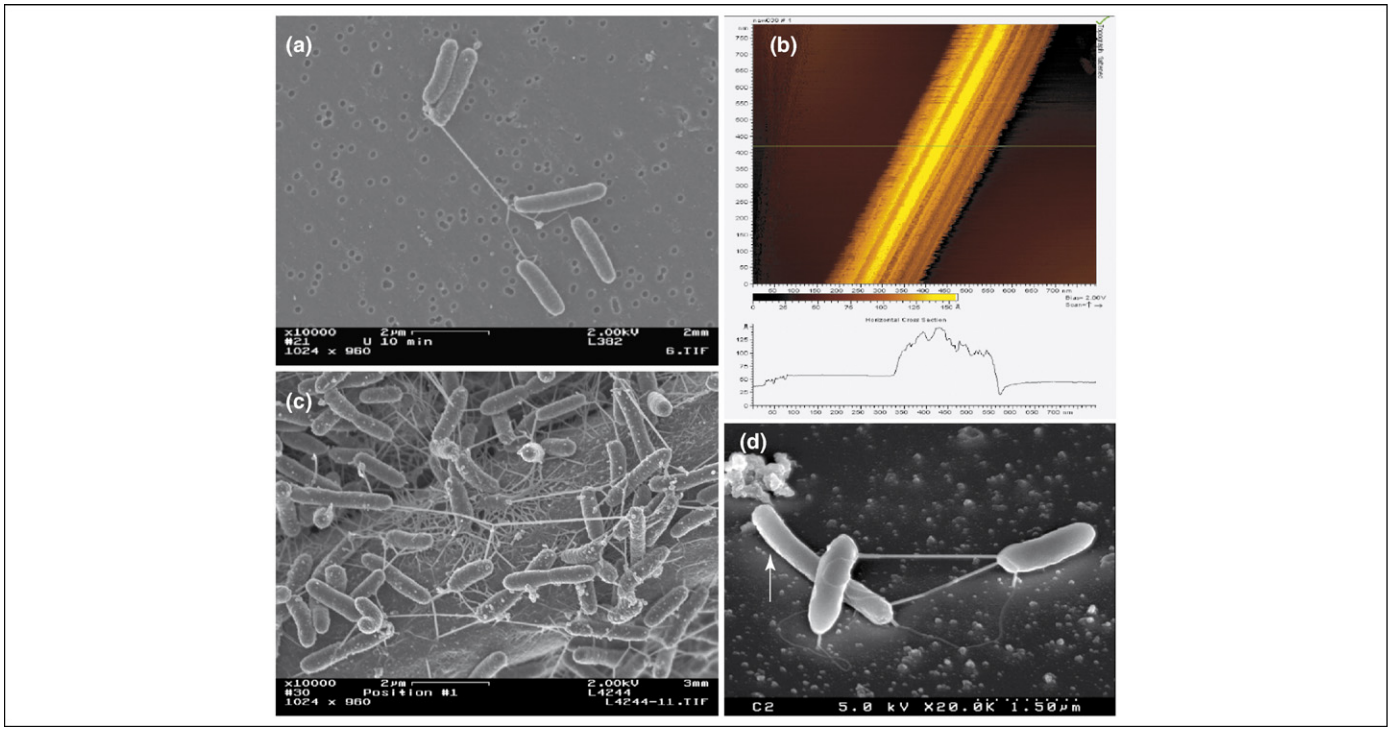

Figure 4. **(a)** A scanning electron micrograph (SEM) of wild-type *Shewanella oneidensis* MR-1 grown under electron-acceptor-limited conditions, showing pilus-type nanowires that connect to other cells. **(b)** STM image of a single pilus-type nanowire from wild-type MR-1 (lateral diameter of 100 nm, topographic height of 5–10 nm) showing ridges and troughs running along the long axis of the structures consistent with a bundle of wires. The corresponding conductivity of the pilus as the tip moves over the indicated surface is shown beneath the STM image. **(c)** The anode from an MFC colonized by *S. oneidensis* MR-1. **(d)** An SEM image of *Pelotomaculum thermopropionicum* and *Methanothermobacter thermautotrophicus* (arrow) in methanogenic co-cultures showing pili connecting the two genera. Subsequent STM imaging has shown that the pili are conductive. Parts (a), (b) and (d) reproduced, with permission, from Ref. [38]; part (c) was provided by Y. Gorby.

occur in as little as three to four days when the reactor is emptied and re-filled with fresh medium on a daily basis [33]. Rapid acclimation might not be so unexpected when using sediments that are already relatively enriched in iron-reducing bacteria. However, we see rapid acclimation of an MFC when using domestic wastewater samples, with no apparent selective pressure for electrochemically active bacteria to be present in this system. It has been found by microautoradiography (MAR) using radiolabeled acetate, inhibition of sulfate reducers and methanogens, and ferric iron as the sole electron acceptor, that iron-reducing bacteria in activated sludge might comprise as much as 3% of the bacteria [39]. Interestingly, the combination of MAR with fluorescence *in situ* hybridization showed that all of the MAR-positive cells hybridized with a bacteria-targeted probe but the Proteobacteria-subclass-targeted probes only identified 20% of these iron reducers as γ-Proteobacteria and 10% as δ-Proteobacteria; 70% did not hybridize with Proteobacteria-targeted probes. This suggests that the diversity of iron-reducing and potentially relevant microbes for electricity production might extend beyond the commonly studied *Shewanella* and *Geobacter* species.

The finding that iron-reducing bacteria are a measurable and, presumably, integral component of the bacterial community in wastewater reactors suggests that bacteria capable of wired (or wireless) electron transfer succeed in the community in unexplained ways. There is now preliminary evidence from a variety of studies for syntrophic relationships on the basis of interspecies electron transfer. Although such evidence is not yet conclusive, there are compelling lines of evidence for cell–cell interactions based on nanowires. For example, consider the symbiotic interactions between fermentative and methanogenic bacteria. It is well established that close proximity is advantageous to both microorganisms to facilitate the exchange of hydrogen, allowing for interspecies hydrogen transfer. However, there now seems to be evidence for direct electron transfer as well. Co-cultures studied by Ishii *et al.* [40] showed the presence of a flagellum-like filament between the propionate fermentor (*Pelotomaculum thermopropionicum*) and a methanogen (*Methanothermobacter thermautotrophicus*; Figure 4d). A closer morphological examination of the filament produced by *P. thermopropionicum* shows that it closely resembles the thick pilus-like appendage produced by *S. oneidensis* MR-1. STM measurements established that the appendage of strain MR-1 is conductive and can be considered to be a nanowire [38] as can the thinner filaments produced by *Geobacter sulfurreducens* [41]. Furthermore, the oxygenic phototrophic cyanobacterium *Synechocystis* PCC6803 also produces conductive nanowires, although the ecological advantage to that bacterium is not known [38]. The examination of micrographs of *Shewanella* biofilms on electrodes shows that some of these wires not only go from the cell to the electrode but also from one cell to another (Figure 4c). Thus, it might be that nanowires have a previously unforeseen role in cell–cell electron transfer and microbial community development.

The observations of the ability of a wide range of bacteria to transfer electrons exocellularly [42] to iron and other metal oxides, to carbon electrodes and possibly to other bacteria suggest that a new term is needed to

classify this functional capability of bacteria. We suggest the term 'exoelectrogens' as a general classification because this name captures the ability of bacteria to generate and transfer electrons outside of the cell. It has recently been suggested that these bacteria be called 'electricigens' [43] but focusing only on electricity generation might be too limited a classification for the functional capabilities of these microorganisms.

Prospects for useful applications of MFCs and related technologies

The MFCs currently in existence are exciting systems for studying microbial communities and improving the understanding of how bacteria transfer electrons to solid substrates. Harnessing that power in an economical manner, however, remains a greater challenge. It is generally considered that the first applications of MFCs will be as power sources for monitoring devices in the environment and for water treatment [44]. Several tests of large systems have already shown sufficient power production by sediment MFCs for long-term, unattended power sources for data-collecting devices [5,31,45]. The replacement of electricity-consuming wastewater-treatment bioreactors with electricity-producing MFC-based systems shows great promise because of the high cost of operating existing systems. Using MFCs as the treatment reactor makes good economic sense because the total infrastructure needed for power generation does not have to pay for itself on the electricity produced alone – only the cost of replacing the existing reactor with an MFC. Accomplishing treatment at a lower overall cost by producing a valuable product (i.e. electricity) saves money and, therefore, makes the process more economical.

Renewable electricity production using MFC-based technologies seems further off in the future but, with advances, MFCs might become practical as a method for producing a mobile fuel from renewable biomass sources. Ethanol is currently viewed as the main prospect for the replacement of fossil fuels for transportation with a renewable, biomass-based resource. Ethanol is currently made from refined sugar, which limits its usefulness and economic potential, although it is hoped that one day cellulosic materials could be used for ethanol production [46,47]. With further development, MFCs or modified MFCs could become practical methods for hydrogen production from the same materials envisioned for ethanol production (sugar and cellulosic wastes) and from other biodegradable materials. Either the electricity produced by MFCs can be used for the electrolysis of water or, more efficiently, hydrogen could be produced directly from biomass sources using the BEAMR process. It was recently shown, for example, that electricity could be produced from corn stover hydrolysates produced by a steam-explosion process that liberates sugars from cellulose and hemi-cellulose components [48]. Any material that produces electricity in the MFC process can be used to produce hydrogen in the BEAMR process, although hydrogen yields cannot yet be reliably predicted and must be measured. Taken together, these biotechnology-based MFC approaches hold great promise as methods for renewable energy production.

Box 2. Accomplishments and outstanding questions

Although there have been dramatic recent improvements in MFC architecture to increase power densities from these systems, advancements in understanding the microbiology of MFC biofilms and the implications of the ecology on system performance are just beginning to emerge. Electrochemically active communities are readily developed from a diversity of environmental sources and maintained with a remarkable range of pure and complex substrates, with stable MFC performance observed for more than five years [3]. It is apparent that the community diversity in MFCs generally extends beyond the model iron-reducing genera, with the exceptions of sediment MFCs that seem to select for Geobacteraceae [25] and a cysteine-fed system that predominantly showed *Shewanella* spp. [6]. Other MFC community characterizations show a broad diversity, including Gram-negative- and Gram-positive-dominated systems and an abundance of unknown 16S rDNA sequences [2,29]. The ecological role of these community members remains unknown because of the lack of direct functional correlation with phylogenetic identity and the possibility of other metabolisms that do not generate electricity. For example, methane production has been observed in MFCs but, apart from a single study that used archaea-targeted probes for fluorescence *in situ* hybridization and reported the detection of probe-positive cells [28], community studies have used bacteria-targeted primers and the importance of methanogens in these biofilms remains largely unexplored. As MFC architecture constraints continue to diminish, it is expected that microbiological constraints will become more pronounced and enable systematic studies of anode-reducing mechanisms and community succession.

Concluding remarks and future perspectives

Building on the extensive literature pertaining to dissimilatory iron-reducing bacteria, studies on exoelectrogens and consortia are beginning to expose the mechanistic and ecological complexities of MFC biofilm communities (Box 2). Different mechanisms of anode reduction have been identified that enable cells to reduce the anode from a distance, thereby imparting electrical conductivity into the biofilm beyond cells directly attached to the anode. Studies of anode biofilm community composition have revealed the persistence of phenotypically uncharacterized bacteria with wide phylogenetic diversity that contribute in unknown ways to the biofilm ecology. Ecological studies show that competition for a limited resource leads to a successive maturation of the community, beginning with early colonizers and invaders that give way to the final dominant organisms. The limited surface area of a non-corrosive carbon electrode in an MFC might represent a unique opportunity to study the effect of limited space for cell respiration on microbial growth and competition. Understanding how the microbial ecology of electricity-producing communities develops and changes over time, in terms of colonization, invasion and succession, therefore opens the door on a new world of exploration and insight into complex microbial communities and the evolution of life. At the same time, it might also open the door to a new method for renewable and sustainable energy production.

Acknowledgements

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