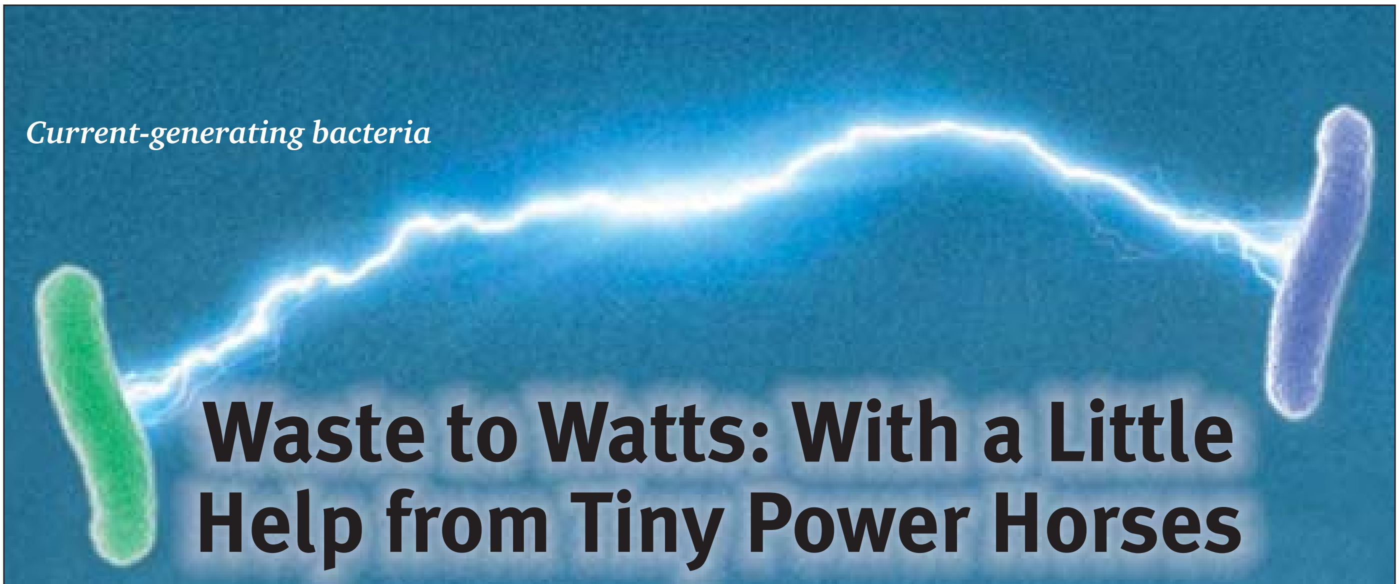


Current-generating bacteria

Waste to Watts: With a Little Help from Tiny Power Horses

Bacteria are jacks of all trades. Some of them are even capable of producing free electrons by digesting organic material. Now, scientists explore whether and how mankind could exploit that peculiar feature.

In 1911 British botanist M. C. Potter was the first to discover that bacteria are able to produce electrical currents. This feature, however, was generally seen as an exceptional oddity. A misconception and a long-lasting mistake. Particularly in the last two decades, it has become an increasingly established fact that bacteria producing free electrons via anaerobic respiration are not just elite members in the universe of microbes. Instead, they are rather widespread, at least in aquatic sediments.

Electric history

One of the few scientists to have been studying such bacteria in detail for decades is Derek Lovley, now working at the University of Massachusetts in Amherst. Some twenty years ago, he discovered a new anaerobic bacterial species in the sediments of the Potomac River and named it *Geobacter*. This strain (initially designated strain GS-15, later known as *Geobacter metallireducens*) turned out to oxidize organic compounds to carbon dioxide using iron oxides as the electron acceptor (*Nature* 1987, vol. 330: 252-54). Two years later, he described that *Geobacter* oxidizes aromatic compounds as benzoate, toluene, phenol or p-cresol with Fe(III) as the only electron acceptor (*Nature* 1989, Vol. 339, p. 297). In the paper, Lovley argued that, given the widespread availability of Fe(III) as potential electron acceptor in subsurface environments, it seems likely that anaerobic microbes "may be capable of removing significant quantities of aromatic compounds from many contaminated aquifers".

Today, we know many microbial genera – i.e. five classes of Proteobacteria, Firmicutes, Acidobacteria phyla – that are able to generate electrons via respiration. They are mostly referred to as exoelectrogenic microorganisms; however, other terms such as electrigens, electrochemically active bacteria, anode respiring bacteria and dissimilatory metal-reducing bacteria are also used. The two most important "models" for the investigation of electron transfer mechanisms today are *Geobacter sulfurreducens* and *Shewanella oneidensis* MR-1.

A microbial fuel cell

Photo: www.microbialfuelcell.org

Role of cytochrome and riboflavin

The core question is easy to guess: how do these bacteria release electrons into the environment? No one knows exactly yet. Though scientists have delved deep into their model genera, the mechanisms of electron transfer to insoluble, extracellular metal oxides still remain nebulous. In general, it's believed that exoelectrogenic microbes use intracellular transmitting molecules for this purpose and/or outer membrane-coupled cytochromes, most presumably c-type cytochromes.

Daniel Bond, Jeffrey Granick and co-workers, for example, discovered that *Shewanella* MR-1 employs self-produced riboflavin (vitamin B2) as primary electron acceptor molecules (*PNAS* 2008, vol. 105: 3968-73). David Richardson from the University of East Anglia in Norwich (UK) and colleagues found out that modules comprising two different cytochrome C-molecules (MtrA and MtrB) can move electrons across a lipid bilayer (*PNAS* 2009, vol. 106: 22169-74). Since these modules exist in a range of bacterial phyla, the authors suggest that they are widely used in electron exchange with the extracellular environment. In addition, other teams were able to confirm that the production of electrons depends on both the abundance of riboflavin and the number of cytochrome genes (*J. Bacteriol.* 2010, vol. 192: 467-74; *Appl. Environ. Microbiol*. 2009, vol. 75: 7674-81).

Similarly vague results have recently come from research on *Geobacter* strains. After the strains were sequenced, genomic as well as transcriptomic analyses revealed high conservation of putative intracellular electron shuttles, such as NADH dehydrogenase and succinate dehydrogenase. However, conservation of cytochrome genes in exoelectrogenic bacteria is poor, leading Lovley's team to the conclusion that C-type cytochromes may not be specific terminal reductases but may rather serve as sinks for electrons somewhere between the inner-membrane electron transport chain and extracellular acceptors (*BMC Genomics* 2010, vol. 11: 40-52). The electrochemical gate between bacteria and the electron acceptor might be OmcZ, an outer surface cytochrome, which is highly upregulated.

However, one explicitly outstanding feature of *Geobacter* is particularly important for electron power: wire-like pili. The bacteria use those pili to transfer electrons onto iron in the surrounding soil (*J. Mol. Microbiol. Biotechnol.* 2009, vol. 16: 146-58). No wonder, therefore, that the genes needed for pili to grow out belong to the most highly upregulated genes of *Geobacter*.

Powerful microbes

It is also no wonder that the ability to produce electrons has made the bacteria an interesting object of bioenergy research. In 1999, Korean scientists revealed the first direct proof of electrical current generation by *Shewanella* in a special battery-like chamber. Since then, several teams have been trying to develop little microbial fuel cells (MFCs) that operate in a similar manner to conventional fuel cells. While in the latter, electrons are produced by oxidizing hydrogen gas or methanol at the anode with platinum or palladium as catalysts, MFCs run without any expensive catalysts – just on the basis of bacteria, plus organic material as food for the greedy microbes and anaerobic conditions provided.

At a metallic anode, which is separated from the cathode by a selective membrane, electrons are generated via anaerobic oxidation processes releasing protons that pass the membrane. Those electrons flow through an external circuit to the cathode where they fuse with oxygen and protons to produce water. Since the bacteria are not choosy, many sources of organic material can be used as an energy supply, ranging from simple molecules like carbohydrates or proteins to complex mixtures, such as are present in waste water and sludge. Optimal nutrition provided, the microbes divide constantly and propagate on the surface of the anode. This way, the whole system is self-sustaining and, potentially, should last indefinitely – the dream of any researcher looking for renewable energy resources.

Sludge bacteria most efficient

In the early days of MFC research, scientists used pure cultures of certain soil bacteria. In the meantime, however, it has turned out that diverse bacterial communities, like those living in the slick sludge or waste water, are more robust and often perform more efficiently in power production than cultivated strains. "For our MFC studies we always take bacteria from the local wastewater treatment plants because they work just excellent," says Uwe Schröder, professor for Sustainable Chemistry & Energy Research at the Technical University in Braunschweig (Germany). "With mixed cultures we routinely get a current density of one milliampere per square centimetre [anode area, the ed.].” In Schröder’s waste water MFCs, microbes develop biofilms on the anode that reveal a strong similarity to *Geobacter sulfurreducens* biofilms, indicating a dominating role of this bacterium or related genera in electrogenic biofilms (*Biosens. Bioelectron*. 2008, vol. 24: 1006-11).

Hardware solutions

On the other hand, special strains also emerged as powerful energy producers. The isolate *Rhodopseudomonas palustris* DX-1, for example, produces about 60% more power (2,720 mW per m^2) than the original inoculum from which *DX-1* was collected (*Environ. Sci. Technol*. 2007, vol. 41: 3341-46). And an astounding 7.6 A/m^2 was produced by the *G. sulfurreducens* strain KN 400, which Lovley isolated from a wild-type strain biofilm that itself was sevenfold less effective (*Biosens. Bioelectron*. 2009, vol. 24: 3498-503). How? In an email to *Lab Times*, Lovley wrote, “One of the key features is that it appears to produce more of the electrically conductive pili, known as microbial nanowires that appear to be important in conducting electrons through the biofilm on the anode of microbial fuel cells.” The TIME magazine rated this strain as one of the “50 Best Innovations of 2009”.

Varying dimensions

For readers who are wondering why various dimensions – watts, ampere – are used to describe MFC power capacities: it depends on the scientist’s professional background. Environmental technologists tend to measure power in watts per cubic metres, reflecting their interest in bioreactors; electrochemists like

Possibly soon run by *Shewanella* and *Geobacter* alone

Photo: Fotolia/view7

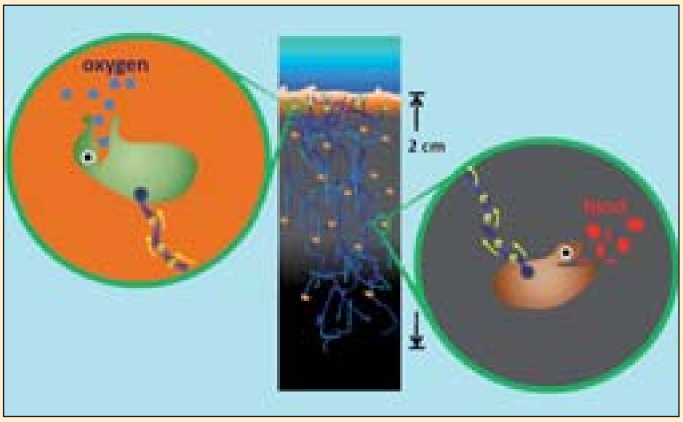
Conducting Mud

Time and again nature reveals amazing stories. Even the smallest creatures are capable of astonishing skills. Who would have thought that deep under the water surface, billions of bacteria are networking together true to the motto “You breathe for me and I eat for you”? Moreover, that for this purpose, those tiny organisms hook up to form giant, natural batteries that generate electrical current for producing food? Surprising, isn’t it? Sounds more like part of a science fiction story. However, it’s true.

Lars Peter Nielsen at Aarhus University in Denmark, together with colleagues from Japan, discovered that bacteria from the marine sediment team up with colleagues living in the overlying water. Okay, so their communication distance is only two centimetres. But we’re talking about bacteria - mean length two micrometres. In a human’s world that distance would correspond to about 20 kilometres. Incredible!

The bacteria evolved some sort of labour division. Those living in the upper part of the sediment, having direct contact to water and oxygen, produce energy by consuming oxygen in the form of yet-to-be identified, energy-rich molecules. This energy is being transported to bacteria that live two centimetres deeper under anaerobic conditions, enabling them to reduce hydrogen sulphide and organic carbons. Electrons produced by this process are then transported back to the sediment surface.

The scientists suppose that bacteria build up networks via nanowires to accomplish this task. When the scientists incubated sulphidic marine sediment in the presence of sea water, hydrogen sulphide disappeared from the surface. Some weeks later, the suboxic zone – 10-20 mm deep – was devoid of both oxygen and sulphide. When they depleted oxygen from the overlying water, hydrogen sulphide con-



Labour division among marine bacteria.
(Graphic: Lars Peter Nielsen, Aarhus University, Denmark)

centration increased within an hour. When they re-introduced oxygen, the system changed again to the opposite. These reactions, occurring over a distance of 10-20 millimetres, were so quick that they could not be explained by diffusion. The environment in the control experiment –

Schröder use maximal power points in ampere per square metre [A/m²] to describe their devices.

Certainly, the MFC capacity not only depends on the microbes but also on its technical parameters, such as resistance of the chamber and the electrodes, as well as size. In a recently published review, Bruce Logan from Penn State University, even argued that taken together, it's not so much the inoculated isolate or mixed culture that restricts the power density of an MFC but rather its specific architecture, electrode spacing and solution conductivity. Therefore, he designed a new form of anode, a graphic fibre reminiscent of a bottle brush. This structure provides much more surface area and, subsequently, power production was increased two to fourfold when compared to conventional flat anodes. "We are not yet at the upper limits of maximum power densities for microorganisms in MFCs on either a volumetric or projected-anode area basis," he wrote (*Nature Rev. Microbiol*. 2009, vol. 7: 375-81).

Importance of pili and biofilms

Besides all reflections on power densities, one feature of MFCs is particularly amazing: its great efficiency. Whereas conventional fuel cells efficiency lies between 35 and 65 percent, bacteria in an MFC transfer almost all respiratory electrons to the anode. They can boast 80 to 95 percent efficiency. However, electron transfer strictly depends on healthy and active bacteria. For example, *Shewanella* mutants, which are unable to form biofilms due to a defect in cytochrome type MtrC, generate less power. Thus, biofilm growth seems to be important for high en-

without oxygen in the water – remained sulphidic from the surface to the bottom. So, the scientists concluded audaciously, "[...] that the remote coupling between oxygen reduction and sulphide depletion is due to the presence of natural electric currents." (*Nature,* Vol. 463, p. 1071). That's nothing other than a huge "biogeobattery".

"This study adds a new dimension to the understanding of biogeochemical interactions, in that natural electric currents allow oxidation and reduction processes to be spatially separated yet instantly and intimately coupled," the scientists wrote in *Nature*. Right they are!

Already in 2005, the Danes had hints on the processes deep under the water surface. They demonstrated that bacteria of the genus Beggiatoa (white sulphur bacteria) could deplete hydrogen sulphide from suboxic sediments, with the help of nitrate, and transport the obtained molecular sulphur to the sediment surface for aerobic oxidation. At that time, however, the scientists did not speculate about how that transport could be accomplished (*Appl. Environ. Microbiology*, 2005, Vol. 71, p. 7575-77).

Until now, Lars Peter Nielsen and his colleagues don't have any direct evidence that nanowires really exist. They have never actually seen them. But since they are the most plausible devices, by which bacteria could transport electrons over long distances in a short time, one can be sure that the scientists will continue their search.

Karin Hollricher

ergy production. In a similar manner, *Geobacter* mutants, which are neither able to grow out pili nor form biofilms, are also not as efficient as their wild type fellows.

Knowing that, the question immediately arises: how can electrons be transferred from bacteria that are not in direct contact with the anode? This has not yet been determined but it's clear that electrons from bacteria on the outside or in the centre of the biofilm do pass electrons to the electrode at the same rate as bacteria with closer contact to the anode.

Self-sustaining energy resources

Now practically speaking, how and where could MFCs be used? Since bacteria can grow from digesting many organic molecules – acetate, glucose, starch, cellulose, wheat straw, phenol as well as complex mixtures of molecules – they can be used for the treatment of waste water as well as for self-sustaining energy sources.

The first MFC application was reported by Leonard Tender from the Center of Bio/Molecular Science and Engineering at the Naval Research Department in Washington. In their experiments, benthic MFCs working with naturally occurring bacteria in marine sediments generated power for meteorological buoys that measured climate parameters and transferred the data by real-time telemetry. The authors of the paper (*J. Power Sourc*. 2008, vol. 179: 571-75) believe that such MFCs fixed to aquatic sediments could work indefinitely because they are independent from any exogenous energy supply.

Photo: Wikimedia Commons, MDs

Bacteria could power weather buoys like this one

Tests in waste water plants have already started. Since traditional waste water treatment uses aerobic and facultative bacteria, the water has to be enriched with oxygen – an energy-consuming process. "I think that waste water treatment could be completely self-sustaining if one would change to anaerobic cleaning conditions and use the power generated by anaerobic bacteria for running the plant," comments Schröder.

Microbial desalination

Another option is powering water desalination plants by MFCs. Bruce Logan, together with scientists from the Tsinghua University in Beijing, have recently yielded a proof of concept. They created a small three-chambered-MFC, with the middle chamber being for water desalination. When bacteria produced a current by digesting acetate on the anode in the first chamber, ionic species in the middle chamber were transferred to the two outside electrode chambers, thereby desalinating the water in the middle chamber. Though not yet optimised for this process, the microbial desalination cell (MDC) produced a maximum of 2 W/m^2, whilst simultaneously removing about 90% of the salt in a single desalination cycle. "Water desalination can be accomplished without electrical energy input or high water pressure, by using a source of organic matter as the fuel to desalinate water," Logan *et al.* conclude (*Environ. Sci. Technol*. 2009, vol. 43: 7148-7152.)

Most recently, the electrogenic bacteria community made a U-turn when recognising in their experiments that electrons can also flow in the opposite direction, meaning that microorganisms can use electrons derived from the electrode for the reduction of carbon dioxide to organic products.

Brewery waste water projects

Korneel Rabaey from the University of Queensland (Australia), for example, is now even exploring the possibilities of using the electrical current generated by microorganisms in this way to fabricate chemicals. A couple of years ago, he set up an MFC pilot plant in an Australian brewery for cleaning their waste water. Despite plans to scale up that system he stopped the project. "It has been a great learning exercise. We found out, which aspects are the key impediments to scale up and how to address these. I hope to write down the results in the coming months," he wrote in an email to *Lab Times*. Instead, Rabaey now uses the microbes' power to produce chemicals such as caustic soda and hydrogen peroxide. "These chemicals provide a higher economic and environmental benefit than the generation of electrical power," he wrote. That's why he is now investigating microorganisms that can use electrical currents to drive the bioproduction of valuable chemicals such as butanol, ethanol and bioplastics. This process is called "microbial electrosynthesis", the technology "bioelectrochemical systems" and the energy is derived from waste water or solar power. "The opportunities for bioelectrochemical systems are vast and, potentially, the production rates are much higher than any alternative," Rabaey states.

New discoveries to be expected

Well, after all of that, it's clear that the constantly growing MFC science scene hasn't yet left the discovery phase. It is safe to assume, there might well be some astonishing abilities of exoelectrogenic bacteria yet to be uncovered – not to mention any hitherto inconceivable applications.

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