

BATTERIES NOT INCLUDED

Among their many talents, bacteria are the world's best electrochemists, creating a life-powering flow of electrons in a startling range of conditions. In the first of two features, **Nick Lane** asks what limits, if any, constrain this ability. In the second, **Charlotte Schubert** meets the people trying to put this microbial ingenuity to practical use.

What can't bacteria do?

In 1977 Engelbert Broda, a physical chemist, then at the University of Vienna in Austria, made a startling prediction¹. Nitrate and ammonia can and will react together in the way that oxygen and organic carbon molecules do. And so, in theory, it would be possible for a creature to make a living by breathing nitrate and eating ammonia, just as we do by breathing oxygen and eating proteins, carbohydrates or fats. The creatures Broda had in mind for this bizarre diet were not some race of aliens floating in a soup of ammonia under red, nitrate-laden skies, but earthly bacteria of a sort never before imagined.

The discovery of bacteria capable of this 'anaerobic ammonia oxidation' or anammox reaction, in the late 1990s², was a high-water mark for the rising tide of what might be called microbial triumphalism. The anammox reaction, it transpires, is a dazzlingly hard trick to pull off — it produces hydrazine (rocket fuel) as an intermediate, which the bacteria have to tuck away in internal sacs made of lipids.

If bacteria have found a way to take advantage of nitrates' propensity to oxidize ammonia, despite having to cope with a toxic by-product, is there anything they cannot do? Subsequent discoveries — of bacteria that 'breathe' metal oxides, or feed on bleach — have strengthened the case for bacterial omnipotence. Last month, one of the last gaps in the bacterial skill set was closed when some of them (in concert with helpful members of another class of single-celled organism, the archaea) were discovered using nitrates to get energy from methane³.

And yet there are still some energy-producing pathways down which no bacteria have been seen to stroll. No known bacteria seem to live by oxidizing manganese; none derive their energy from molecular nitrogen; and no photosynthetic bacterium is known that uses ammonia, phosphine (phosphorus hydride), or even methane, although the laws of thermodynamics show that all those reactions could provide energy. Are such organ-

isms really absent, or have we just not found them yet? And if they are absent, does this reflect a fundamental limitation on life's ingenuity? Or does it reveal set truths about the Earth's environmental chemistry?

"I can imagine that evolution's random walk has failed to locate some reactions," argues Franklin Harold, an emeritus professor of biochemistry and molecular biology at Colorado State University in Fort Collins. "If no bacteria live by molybdenum reduction, no profound principle is indicated." But there is some reason to believe that the missing tricks could explain why bacteria specialize so narrowly, forming rich metabolic networks and ecosystems only through collaboration. Indeed, bacteria's inabilities may be fundamental to the question of why there are ecosystems at all, rather than one all-conquering bug.

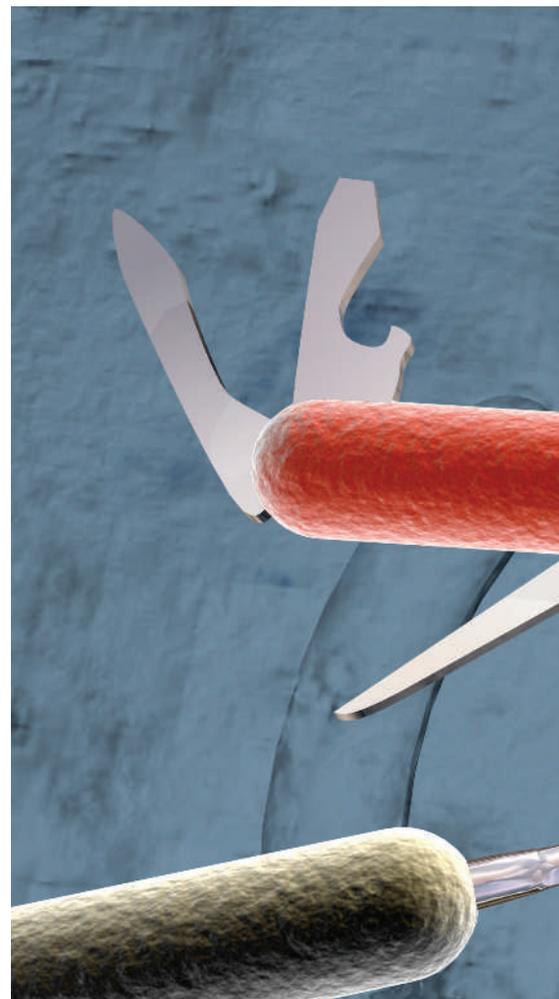
Working in pairs

"It's a dangerous game to put up your hand and say 'bacteria can't do this or that', as we probably only know about 1 to 5% of bacteria on the planet" says microbiologist Ken Nealson, at the University of Southern California, Los Angeles. "But I'd wager that there are constraints on what's possible, beyond whether a reaction yields energy; some of these are still an incredible, wonderful mystery."

The common factor in all the forms of respiration known is the involvement of what chemists call a redox pair — an electron donor and an electron receptor. The process by which electrons are stripped from the donor is called oxidation, because oxygen is the most familiar stripper of electrons (and also a very powerful one). The electrons are transferred to an acceptor, which is said to be reduced; hence the term 'redox'. Non-chemical ways of generating energy are conceivable (see 'The first electrochemist') but don't seem to be used.

Life's dependence on redox reactions led the great geneticist Hermann Muller to describe it as "a fancy form of rust". But it is rust with a

purpose. As the electrons go from willing donor to willing acceptor, they go down a thermodynamic gradient; this means they lose energy. In respiring cells, the energy from this flow of electrons is used to build up a gradient of protons across membranes. When the protons flow down this artificially created gradient back across the membrane, ATP is created



— a molecule that stores energy in a form that can be given up wherever it is needed to drive the cell's molecular machinery. (Humans may prefer to use these electrons for other purposes; see page 277.)

In the case of aerobic respiration, used by most animals and many bacteria, the electron acceptor is oxygen, but it can be anything that has a hunger for electrons: carbon dioxide, nitrate, perchlorate (used in bleaches) or plain protons will do. All that matters is that the recipient's redox potential — its tendency to acquire electrons — is higher than that of the donor. The greater the difference in potentials, the more energy is released (see graphic, overleaf).

In photosynthesis, the energy of the Sun is used to tear electrons from unwilling donors, driving redox reactions thermodynamically uphill. The absorption of energy by a chlorophyll molecule turns it into a fierce oxidant. Like a dentist pulling teeth it then wrenches electrons from a bystander that would normally be loath to surrender them. The electrons are ultimately transferred to carbon dioxide (the electron acceptor) to form sugars.

Whether the electrons come from willing donors, as in respiration, or unwilling ones, as in photosynthesis, the bacteria need a way to get rid of the reduced molecules where the electrons accumulate once the reactions have

run their course. In anaerobic systems, the reduced molecule in question will often be hydrogen. "Unless the hydrogen is removed, the whole ecosystem gums up; that's why hydrogen consumers such as methanogens [archaea that produce methane] are so important," says Nealson. Other organisms in the ecosystem benefit: "By removing hydrogen, shifting the equilibrium, they make other processes thermodynamically possible, such as using protons as electron acceptors, which generates more hydrogen."

Current thinking

A build up of end products is not the only obstacle. The need for specially lined rocket-fuel tanks that the anammox reaction forces on bacteria highlights the fact that intermediates can also be a problem. So do recent findings about bacteria that oxidize perchlorate, notes Stuart Ferguson, a biochemist of the University of Oxford, UK.

So far, six bacterial species are known to use perchlorate as an electron acceptor⁴. At first, the key component was thought to be an enzyme shared by these species that normally passes electrons to nitrate or nitrite, and which serendipitously happens to be able to deal with perchlorate too (the receptor molecules are not dissimilar in size and shape). But more



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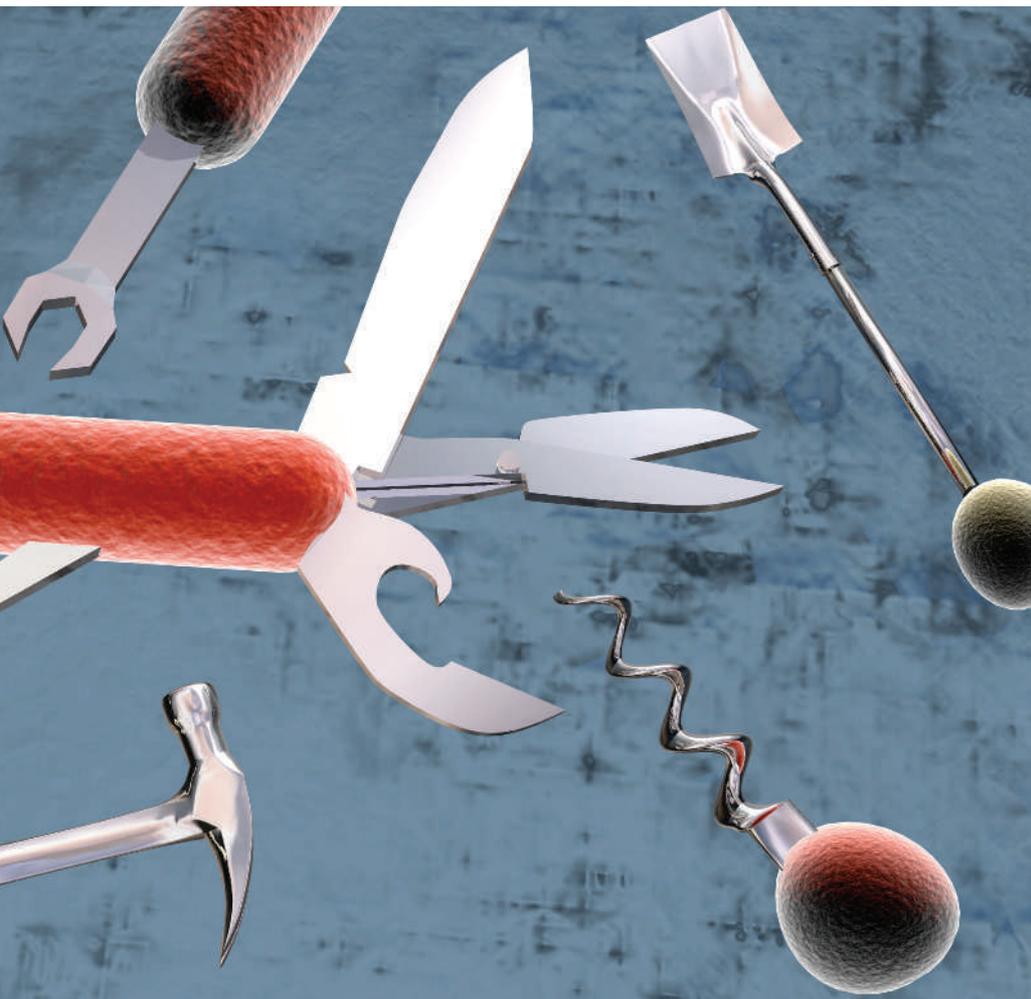
recent work has shown that the crucial common denominator is in fact an enzyme that can detoxify the most toxic intermediate, chlorite⁵. Bacteria with the enzyme chlorite dismutase can cope; those lacking it could not. Being able to cope with toxicity, not the ability to carry out the appropriate redox chemistry, is the bottleneck.

Is this a general truth? "Toxicity can be a big challenge for microbes," says geochemist Donald Canfield of the University of Southern Denmark. Canfield thinks that there may be molecules that are too toxic for bacteria to deal with. "I don't know of any microbes that can oxidize phosphine, for example. Perhaps it's just too toxic — it ignites spontaneously in air." This is despite there being environments such as swamps and rice paddies where phosphine is plentiful. "From a thermodynamic point of view it would make good sense," argues Canfield. In oxygen-free settings, phosphine's spontaneously combustible nature might be less of a problem. If there are phosphine-eaters, the most likely place for them to turn up would be in anaerobic sediments such as those of Lake Taihu in China — a site of ongoing research into the phosphate cycle.

Another factor that might stump bacteria is kinetics — the speed at which a reaction takes place naturally. For example, a potential source of energy in many marine sediments is the reaction of iron oxides such as rust with hydrogen sulphide that can bubble up from below. "But this reaction happens so fast naturally that, apparently, no bacteria can tap into it for energy," says Canfield. "They would have to come up with an enzyme that could actually slow it down."

On the other hand, some thermodynamically favourable reactions are hard to get started, and go slowly. Enzymes are good at speeding up such reactions through catalysis, but the degradation of certain compounds needs a huge pulse of energy to get going. For such compounds, with a very high activation energy, the reaction is too slow to be profitable for microbes despite their enzymatic cleverness.

"A good rule of thumb is to look for what



accumulates”, says biochemist Rolf Thauer, at the Max Planck Institute for Terrestrial Microbiology in Marburg, Germany. Lignin is a good example. When lignin, a key component of hard wood, first evolved around 375 million years ago, nothing could metabolize it; the great reserves of coal in the Carboniferous period attest to that failure. Even today, it remains a challenge, and one that is met for the most part by aerobic fungi, says Thauer. “The molecule is too large for most bacteria to handle, and its activation energy is too high.”

Conversely, a lack of build up can be evidence of microbial activity. Geochemists knew that methane was ‘missing’ from ocean sediments long before the anaerobic bacteria and archaea (another group of microbes) that oxidize it were discovered. It should have been accumulating, but wasn’t, so something had to be oxidizing it. “Microbiologists said ‘that’s not possible,’” recalls Thauer, “but clearly it was going somewhere.” Benzene is a similar case. Its disappearance from anaerobic environments means something must be consuming it. But the organisms and metabolic pathways involved have yet to be elucidated.

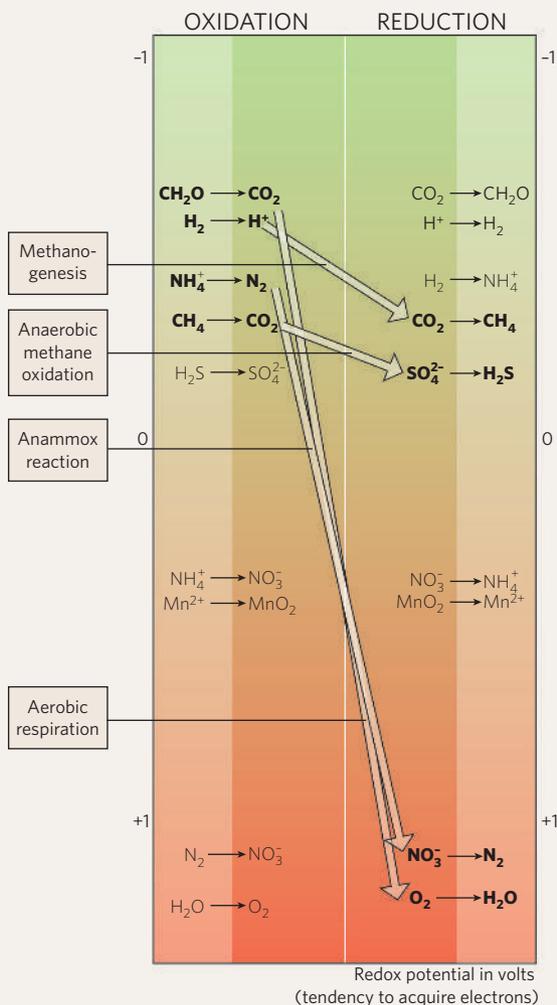
Accumulating evidence

Perhaps the most interesting accumulation is of the molecule currently filling 80% of your lungs. Atmospheric nitrogen is mostly made by bacteria that reduce nitrates. Reducing the nitrogen further, to make ammonia, could in principle be a way of getting energy out of various organic fuels; but this doesn’t seem practical. “Bacteria do reduce nitrogen,” says Friedrich Widdel, at the Max Planck Institute for Marine Microbiology in Bremen, Germany. “But none can glean energy from this process: the activation energy needed to break the triple bond of nitrogen is too high.”

Widdel points out that the opposite process, using nitrogen as an electron donor and thus forming nitrate, is also possible. He thinks that ecological constraints may be blocking

MIX AND MATCH REACTIONS

Bacteria and archaea can tap into the energy made available when electrons released from an oxidation reaction are used in an electron-absorbing reduction that is lower down the energy scale. (The length of the thick arrows indicates the amount of energy released.)



the use of this reaction by bacteria. “Perhaps the problem is the high energy ‘hills’ on the way to nitrate,” he speculates. “But photosynthetic organisms should have unlimited energy: they ‘ought’ to be able to use nitrogen as an electron donor.”

Robert Blankenship, a biochemist at Arizona State University in Tempe, agrees that

there are many potential forms of photosynthesis that bacteria don’t seem to use, and also suggests an ecological explanation — scarce resources. “Because sulphur compounds are used as electron donors for photosynthesis, you might predict that phosphorus and nitrogen compounds would be used too, but to the best of our knowledge they’re not,” he says. “I suspect they’re too important for other purposes — they’re in short supply, and limit growth because they’re needed for proteins and nucleotides.”

Creatures of habit

If there were no other electron donor available, this constraint might not be so strong. But there almost always is. Cyanobacteria, algae and plants benefit from a type of photosynthesis that uses water as its electron source; this process can take place anywhere where there is sunshine, water and carbon dioxide (the electron acceptor).

What’s more, with this kind of oxygen-generating photosynthesis, the reduced compounds that might otherwise be used as electron donors in photosynthesis are mopped up: the compounds react chemically with oxygen in the surroundings, or properly equipped bacteria burn them up in respiration. Few reduced compounds survive long where light is plentiful, because of the oxygenating effects of photosynthesis. This may well be why ammonia-based photosynthesis, another possible form of metabolism that Broda discussed in the 1977 paper¹, has never been seen.

These constraints may hamper the iron-oxidizing photosynthetic bacteria first discovered by Widdel and his colleagues in the early 1990s⁶. The purple bacteria use soluble ferrous iron as an electron donor for photosynthesis, precipitating insoluble ferric iron as rust. But they seem to be restricted to sediments rather than open waters. Part of the problem is that ferrous iron is rarely found high in the water column, where it is quickly oxidized; so iron-oxidizing bacteria thrive only in

THE FIRST ELECTROCHEMIST

Bacteria may be the best electrochemists going, but their obsession with redox reactions is a curiosity in itself. Why not use heat or mechanical forces for energy? Plenty of bacteria live in hydrothermal conditions — life on Earth may even have originated there. Yet none has been found that can tap the heat gradient of vents directly

— perhaps by using a long thin cellular structure to stretch across the gradient from cold to hot.

Or why not use water currents? Bacteria often move around by rotating rigid corkscrew-like flagellae, using tiny molecular motors powered by redox chemistry. There is no reason in principle not to fix the

cell to a base, use water currents to rotate the flagellum in reverse and capture the mechanical force.

The dependence of all microbes on electrochemistry tells us something about early life on Earth. At the very least, it shows that the last ancestor of all microbes must have been an electrochemist. Either it was so

successful that it supplanted all life using other forms of energy in its first flush of youth, or else the origin of life itself involved redox chemistry. “We may never know which until we see how life operates on other planets,” says microbiologist Ken Nealson, at the University of Southern California, Los Angeles.

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the sediments of shallow waters where mud low in oxygen is exposed to sunlight.

The things bacteria don't do mostly seem to be things the environment doesn't easily let them do. But although, as a group, bacteria can make use of more or less every reaction within thermodynamic and ecological limits, any given bacterium can probably only manage a few of them. Given the apparent ease with which bacteria and archaea swap genes, this might seem surprising. Why don't photosynthetic sulphur bacteria, for example, ever reduce sulphate in the dark? Why are different bacteria needed to oxidize ammonia to nitrate and then reduce the nitrate back to nitrogen. In short, why is there not one super-bacterium that does everything?

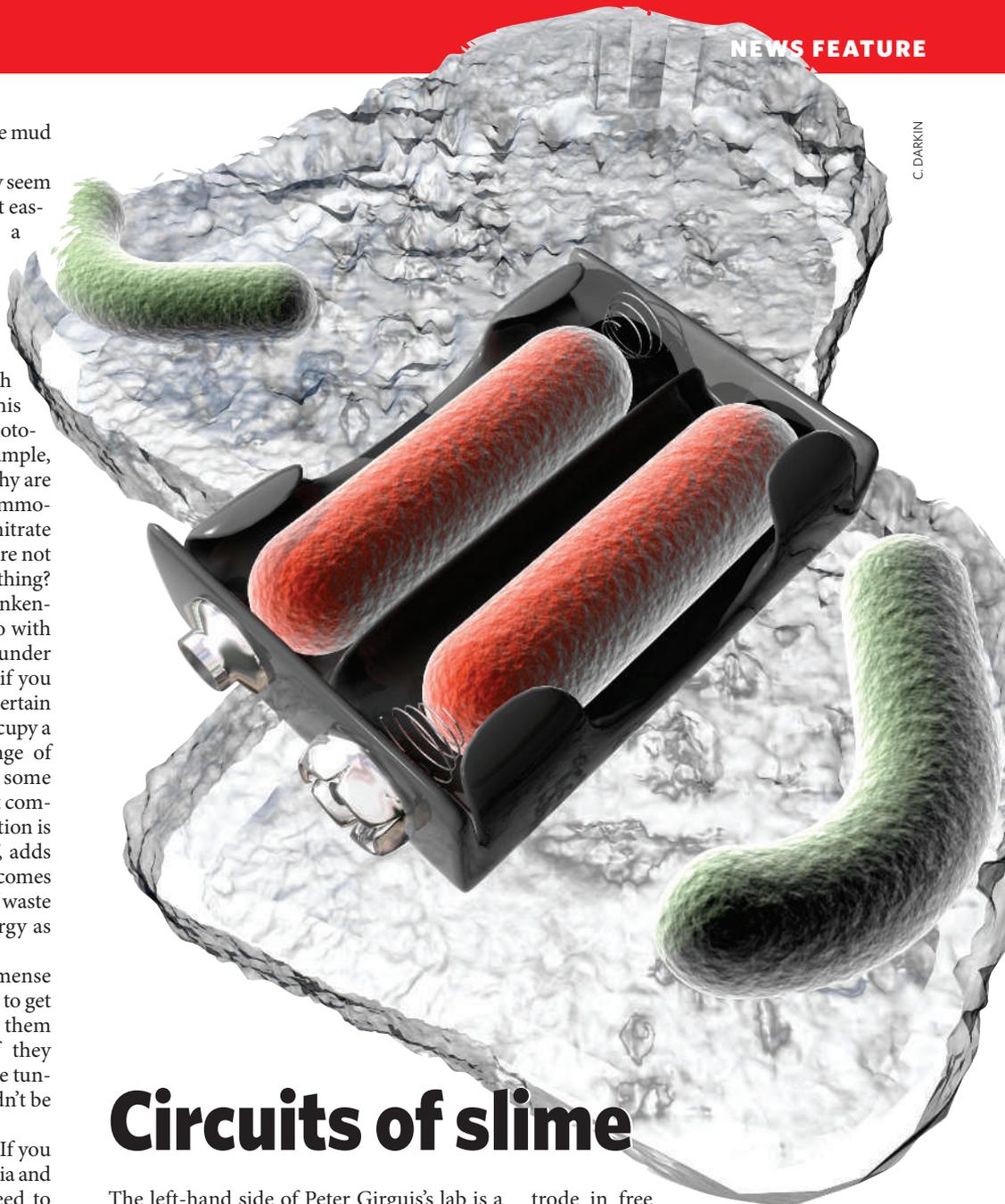
"That's a vexed question!" says Blankenship. "I'd say it was something to do with redox reactions running one way under some conditions, and another way if you change the conditions. So to make a certain reaction work, a bacterium has to occupy a particular niche." If a specific range of redox potentials are needed for some processes to work, but others are not compatible with that, then "the best solution is to cooperate with your neighbour", adds Blankenship. The ecosystem thus becomes a way of dealing with the build up of waste products, and getting as much energy as possible out of the environment.

If Blankenship is right, the immense subtlety with which bacteria are able to get one thing done may be what stops them from getting everything done. If they weren't constrained by their own fine tuning of redox conditions, there wouldn't be any diverse microbial systems.

And there's an obvious corollary. If you really want to understand how bacteria and archaea make their livings, you need to study communities, not individuals, whether they be tightly coupled partnerships such as those between archaea and bacteria that oxidize methane or the looser, more complex communities found wherever the redox potential of a sediment varies with depth. And that approach requires, among other things, a new culture of microbiology. As Neelson puts it: "When I was a student, you were thrown out for working with mixed cultures, but today the interest is in how bacterial consortia operate. If you fish out two bacteria in a cling, they're doing something together, but we've hardly begun to look!"

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Circuits of slime

The left-hand side of Peter Girguis's lab is a mucky place. Dried mud flecks benches, beakers and scales, and buckets of slime clutter the floor. Fresh ooze is flown in regularly from California. A skein of pipes fed by a 2,000-gallon tank of sea water in the basement keeps everything moist. On the floor, wires gush out of dismembered plastic cocoons, and electrodes poke out of various tanks.

The messy workplace is a design shop for electrical generators. This summer, microbiologist Girguis and his colleagues at Harvard University in Cambridge, Massachusetts, will shove graphite electrodes, like those used in this lab, into the sea floor of Monterey Bay in California. The current that flows between the electrodes will power a suite of scientific instruments, including a wave and tide meter, and a fluorometer that measures levels of chlorophyll in sea water.

Girguis's work depends on the fact that when bacteria respire they pull electrons off organic debris. Catch those electrons on an electrode that is hooked up to a second elec-

trode in free water, say, or in air, or in another layer of sediment where the microbes use a different sort of electrochemistry (see page 274) — and the electrons will flow from the first to the second electrode. That gives you a current with which you can power things.

The currents produced by these microbial fuel cells are small, but their potential, in the non-voltage sense, may be surprisingly large. After all, microbes can produce electrons from sediment, sewage, food scraps or pig slop. Girguis, a self-confessed gearhead, takes some delight in a simple demonstration of their current-generating abilities using just a bucket, some hardware-store supplies and a bag of cow manure.

People have been trying to harness the power of microbes in this way since the early 1900s, although with little practical success. Engineering advances and molecular biology's new tools, some of which have a home in the clean half of Girguis's lab, have allowed him and like-minded