

Running on Microbes

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USC College's Ken Neelson leads a multidisciplinary team of biologists, chemists, earth scientists and engineers developing a microbial fuel cell capable of powering small devices that might include tiny surveillance planes and environmental sensors. Here Neelson examines a prototype of the fuel cell in his lab. Photo credit: Phil Channing

What's cleaner than coal, as renewable as solar energy and as ingenious as any of the cutting-edge alternative energy sources now being proposed for cars? The answer is microbe power, and if a USC team's efforts to harness its electrical punch succeed, it may one day find uses in applications both big and small.

Imagine a sewage treatment plant that uses its own waste to power itself, incidentally producing less sludge destined for landfills. Or perhaps an insect-like flying machine that can refuel itself by grazing off the land. In the ocean, hundreds or thousands of fish-like units might form an

environmental sensor network that monitors pollution or blooms of poisonous algae.

Geobiologist Kenneth Nealson leads a USC College-based effort to develop bacteria-powered fuel cells that could act as remote, portable power supplies for a multitude of purposes, ranging from remote sensors to tiny insect-like surveillance drones for use in combat zones.

In 2006, the U.S. Air Force Office of Scientific Research awarded Nealson and his team a \$4.5 million Multidisciplinary University Research Initiative (MURI) grant to take the microbial fuel cell from great idea to usable power source. This has allowed the USC consortium to launch a major effort into understanding just how these microbial machines work.

Of course, the bacteria at the heart of the USC microbial fuel cell aren't just any bugs. It's *Shewanella oneidensis* MR-1, a microbe whose extraordinary abilities have kept Nealson enrapt for 17 years and counting. First discovered by Nealson, *S. oneidensis* MR-1 is a kind of microbial superhero. In addition to generating electricity, MR-1 and its relatives can "breathe" metal, clean up toxic residue in water and even keep brass, iron, copper and aluminum corrosion free.

Original work with this unique microbe, funded by the Department of Energy, revealed many of its interesting and potentially useful properties, and led to the initiation of the microbial fuel cell investigations.

One of the most exciting things about the project is that the microbes can use such a wide variety of fuels — ordinary milk would work, but so would honey or a dead fish — to make the current flow. MR-1 have been successfully fed 75 different types of carbon-containing compounds.

In a rare occurrence, the project team is made up almost entirely of USC scientists. “Almost anywhere else,” Nealson said, “I would have to go thousands of miles to find all the different kinds of expertise we need for this. Here, it’s all within 200 meters.”

Engineering with Bacteria

Orianna Bretschger, out of college for five years with a growing resume in software and data analysis, wasn’t planning on going back to school. But after stints working on missile guidance software and facial recognition systems, the Arizona native figured out what she really wanted to do — be part of the effort to develop an alternative source of power.

“I thought, ‘What are the major problems we’re facing as a society?’ Energy and running out of fuel resources was one that really interested me. I wanted to find ways to improve the alternative energy sources, and I got into fuel cells,” she said.

That led her to USC, where chemists at the Loker Hydrocarbon Research Institute in the College had developed a promising new type of fuel cell that runs on liquid methanol, not hydrogen. She discovered scientists at the USC Viterbi School of Engineering working on other innovative green technologies. Impressed, she applied for and was accepted into the Viterbi School’s Ph.D. program in materials science.

For the past three years she’s worked with electrochemist Florian Mansfeld, whose previous collaboration with Nealson led to the discovery that MR-1 could protect metals from corrosion caused by other bacteria.

In another joint project, Mansfeld’s lab built a simple battery with two different kinds of metal in a liquid medium, electrons flowing through a

wire from one metal to the other — the setup used in elementary physics classes. Without MR-1, the battery runs for a few days, and then runs down. But when researchers added MR-1 to this setup, creating a bacterial battery, the power steadily increased during the 90-day experiment. Much like what happens chemically in a regular battery, bacteria in fuel cells can strip electrons from organic material and produce an electric current.

Bretschger, 28, jumped at the chance to get involved in their next project: the microbial fuel cell. The physics-trained engineering student has spent most of the last year at her bench in Nealson's bacteria-laden lab. She builds the prototype microbial fuel cells and has done much of the hands-on work to optimize them.

Kicking Up Power

The first prototypes worked, but produced electricity very weakly, generating only a few microamps of current.

“For the applications we’re talking about, we needed to increase that as much as a thousand-fold,” Nealson said.

Thanks to the team's use of a combination of approaches, they have already made progress in kicking up energy production. The group now has systems that work in the milliamp range — about enough electricity to power a digital watch or a refrigerator light bulb.

Chemist G.K. Surya Prakash and his graduate student Federico Viva played a critical role in improving the microbial fuel cell's efficiency. Prakash, the Olah Nobel Laureate Chair in Hydrocarbon Chemistry in USC College and a member of the MURI team, is the co-inventor of the highly efficient liquid-methanol fuel cell developed at the Loker Institute. This chemical fuel cell has found its way into laptop computers

and a commercial, portable power generator.

Bretschger brought the microbial fuel cell prototype to Prakash's team, which added a better membrane and assembly that houses the membrane and electrodes (the anode and the cathode) in the fuel cell. With the new parts, the fuel cell produced about 100 times more power. "We're also experimenting with a number of newer designs for the microbial fuel cells, which we expect will increase the power and efficiency even more," Prakash said.

Right now, Nealson said, understanding just how these bacteria interact with the fuel cell anode to produce useful electric energy is the major challenge. Once this is understood, he expects that upping the electrical output of the fuel cells should be a straightforward bioengineering problem.

Power Genes and Live Wires

Nealson, along with Steven Finkel and Byung-Hong Kim, lead the search for biological and genetic solutions to the challenge.

In 2002, Nealson identified genes thought to be responsible for electrical production in MR-1. His team is following up by comparing the power output of the original MR-1 bug with strains they've genetically altered in an attempt to home in on the genes most important to power output. Nealson hopes that by understanding the biological mechanisms involved in the microbe's electrical current production, he will be able to genetically engineer an MR-1 strain that will produce hundreds to thousands times the amount of energy of its forebears.

In another tack, the team has seen some rise in power output from changing the bacterial growth conditions in the fuel cell device. In their earliest studies, the MR-1 were grown in a liquid medium. But when the

bacteria were allowed to grow onto the solid anode surface for four days, they formed a pinkish, slimy coating on the fuel cell's electrode and generated more electricity. The slime is known by scientists as a biofilm — a complex, organized and highly interactive bacterial community.

A 2006 paper by Yuri Gorby of the J. Craig Venter Institute in San Diego and co-authored by Nealson suggested a reason for the increase in power. The Proceedings of the National Academy of Sciences report revealed a network of living nanowires linking the bacteria in a kind of electrical grid. Nealson speculates that the network of nanowires, actually bacterial filaments called pili, offers a more efficient pathway for electrons traveling to the anode and thus a stronger current.

Listening to Bacteria

A microbiologist fluent in the language of genetics, Steven Finkel, assistant professor of biological sciences in the College, is focused on two strategies to boost the bug's power. First, he's studying ways to increase the survival time and growth for the MR-1 microbes living in the fuel cell. Finkel also is looking at ways to increase the electron output for each cell. And he is doing so in a new way.

“We think we know some of the genes involved in the process of electron transport,” Finkel said. “Through genetic engineering we can turn them ‘on’ or ‘off.’ ” But a better, faster approach, he thinks, is to use directed evolution.

“That way we're not limited by what we know, or don't know, in coming up with a solution,” he said. “We are just selecting for those cells that have the qualities we want — they may grow more robustly, survive better or produce more electricity.”

A member of the USC Center for Excellence in Genomic Science,

Finkel has long studied the molecular mechanisms underlying genetic mutation and evolution in *Escherichia coli*. He has used the directed evolution technique extensively to understand how, in stressful conditions, bacterial cells can switch on a system that promotes mutations — or more positively, genetic diversity.

In bacteria, one cell with an advantageous mutation can quickly repopulate an entire culture, allowing a population to survive even in harsh conditions, such as when there are few nutrients available.

One promising direction, Finkel said, involves growing the MR-1 bacteria on a proton-rich carbon source that allows the cells to produce more electrons. But excess protons mean, inevitably, higher acidity, which would prove fatal to most cells.

“So we need to find the ones that can tolerate high acid levels,” Finkel said. By growing the bacteria in an acidic environment, Finkel can select for the MR-1 that — through randomly generated genetic mutations — can outcompete ordinary cells.

“Populations are so large that in a fraction of an ounce, you can have bacteria with every possible mutation represented in the population — including cells with advantageous mutations,” Finkel said. And because bacteria reproduce so quickly, Finkel can study genetic changes over many generations in a single day.

As long as you choose the right selective tool, he said, “you can get solutions you’d never get any other way. We need to listen to the bugs.”

But the scientists won’t be listening only to MR-1.

Another element of the team’s study will be to see if adding other bacteria to the mix can enhance the fuel cell performance — by

breaking down waste, by using materials MR-1 can't use, or by changing acidity or other parameters.

Byung-Hong Kim, a leader in microbial fuel cells who provided the original prototype for the USC team's device, began studying mixed microbial communities in fuel cells while director of the Microbial Ecology Fuel Cell group at the Korean Institute of Science and Technology in Seoul. Now a visiting scientist at USC and a co-investigator on the MURI grant, Kim was the first to show that MR-1, sent to him by Nealson, could produce an electrical current.

"Once we have an optimal cell, the engineers will start looking at how to make this a thousand times bigger or a thousand times smaller," Finkel said.

In fact, Bretschger recently began working with the team in aerospace and mechanical engineer Paul Ronney's lab, helping to set up microbial fuel cells in their lab. Ronney, an astronaut and world authority on micro-scale power generation, will use techniques developed for his research on combustion with conventional fuels to understand the dynamics of the microbes living in the fuel cell.

Given more food, bacteria multiply. More bacteria eat more food, potentially producing more electricity in a feedback loop not unlike that created in a fire: more fuel creates more heat which sets fire to more fuel creating a larger, hotter fire. Ronney and fellow mechanical engineer Hai Wong, both of the Viterbi School of Engineering and co-investigators on the MURI project, will use data collected from the prototypes to build a mathematical model that will predict an optimal design for the microbial fuel cell.

"It is the modeling component that makes this multidisciplinary team uniquely suited to solving this question," Nealson said.

Bretschger noted that working with the interdisciplinary team, which also includes geochemist Andreas Luttge of Rice University, provides an unparalleled perspective onto a scientific problem. “We have the big picture of what’s going on, as well as all of the details — the microbiology, genetics, electrochemistry, microscopy — all of it,” she said.

Source: By Eva Emerson / Eric Mankin, USC College

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