Silicon chips use bacteria to sense danger

The role that bacteria will play in aerospace programs continues to expand. NASA-supported researchers have been working to interface bacteria to silicon chips and have now created a device that can sense almost anything.

Just as a canary in a mine can provide an early warning of deadly gas before miners can smell it, a microbe too will often sense environmental dangers before humans can sense them. It is easy to see a canary's reaction: It dies. It is more difficult to tell what a microbe is sensing. How can such an organism be coaxed to communicate?

One way is to interface it with a silicon chip.

Messages from microbes

University of Tennessee microbiologist Gary Sayler and his colleagues have developed a device that uses chips to collect signals from specially altered bacteria. The researchers have already used these devices, known as BBICs, or bioluminescent bioreporter integrated circuits, to track pollution on Earth. Now, with the support of NASA's Office of Biological and Physical Research, they are designing a version for spacecraft.

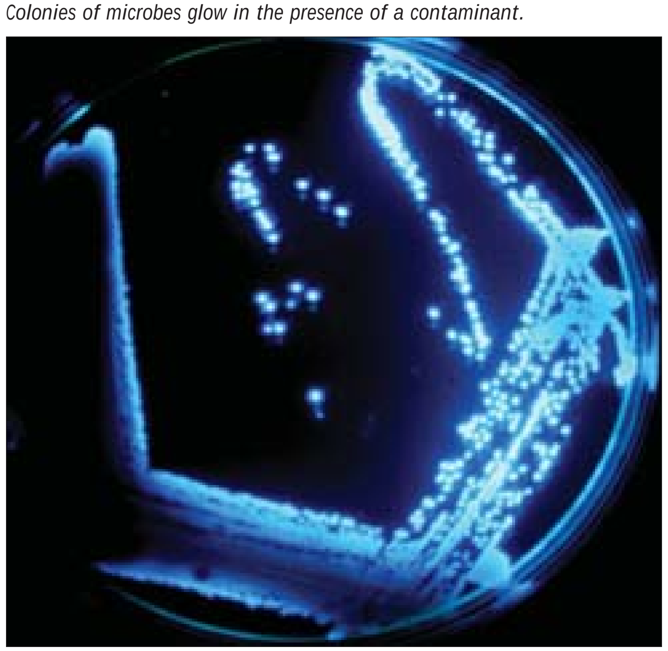
Sayler's group includes Tennessee researchers Steve Ripp, Syed Islam, and Ben Blalock, as well as collaborators at JPL and NASA Kennedy. The scientists bioengineer microbes that glow blue-green in the presence of contaminants. Then they join those bacteria to microluminometers, which are chips designed to measure the emitted light.

BBICs offer a low-cost, low-energy way to detect pollutants, explains Sayler. They are tiny—each BBIC is about 2×2 mm. The entire device, including its power source, will probably be about the size of a matchbox. Moreover, the devices monitor their surroundings continuously.

Bioreporters are intact, living microbial cells that have been genetically engineered to produce a measurable signal in response to a specific chemical or physical agent in their environment. They contain two essential genetic elements: a promoter gene and a reporter gene.

The promoter gene is turned on (transcribed) when the contaminant, or target agent, is present in the cell's environment. In a normal cell, the promoter gene is linked to other genes that are likewise transcribed and then translated into proteins that help the cell in either combating or adapting to the contaminant.

Colonies of microbes glow in the presence of a contaminant.



In the case of a bioreporter, these genes, or portions of the genes, have been removed and replaced with a reporter gene. Consequently, turning on the promoter gene now causes the reporter gene to be turned on. Activation of the reporter gene leads to production of reporter proteins that ultimately generate some type of detectable signal. Therefore, the presence of a signal indicates that the bioreporter has sensed a particular target agent in its environment.

NASA is interested in sensing contaminants, because spaceships are tightly sealed. Unseen fumes from scientific experiments or toxins produced by molds and other biofilms can accumulate and pose a hazard to astronauts. BBICs can be crafted to sense almost anything: ammonia, cadmium, chromate, cobalt, copper, proteins, lead, mercury, PCBs, ultrasound, ultraviolet radiation, and zinc, among other substances.

The system is surprisingly rugged. Microbes thrive in a wide range of environments. It is possible to design BBICs that can survive in extreme or highly contaminated surroundings. "They can actually do their job sitting in things such as jet fuel-water mixtures," says Sayler.

Smaller, faster, cheaper

Sayler's group used BBICs at a groundwater research facility at Columbus AFB, Miss. The facility was contaminated with a simulated jet fuel mixture consisting of naphthalene, toluene, ethyl benzene, and p-xylene. Numerous multilevel sampling wells installed upstream and downstream of the contaminant source allowed for monitoring of these substances. Typically, water would be pumped up from designated wells and sent to an off-site laboratory for contaminant analysis using gas chromatography/mass spectrometry (GC/MS) techniques.

GC/MS analysis is extremely sensitive and accurate, and is by far the best method available for detecting chemical contaminants in environmental sources. However, it also requires expensive and bulky instrumentation, a trained technician, the use of hazardous chemicals, and a significant allotment of time.

As an alternative, the researchers proposed using bioreporters as sensors for the groundwater contaminants. Two were used, Pseudomonas fluorescens 5RL, a bioreporter for naphthalene, and Pseudomonas putida TVA8, a bioreporter for toluene.

Analysis occurred on-site, where bioreporters were simply combined with groundwater samples and allowed to incubate for a set time. Resulting bioluminescence was measured using a field portable photomultiplier unit interfaced to a laptop computer. Duplicate samples were sent to an off-site lab for GC/MS determination of toluene and naphthalene concentrations.

Bioluminescent bioreporters consistently predicted contaminant concentrations within 50% of the GC/MS analytic measurements. Although not highly quantitative in this case, these bioreporters did provide a rapid, general assessment of contaminant presence within the groundwater aquifer, and established an overall snapshot of plume dynamics within a few hours of initial sampling, at a cost approximately one-tenth that of GC/MS analysis.

Care and feeding

Although the microbes can protect themselves from toxins, they still have a variety of needs—food, for example. Sayler says that keeping them alive is a significant portion of the work.

One problem is that microbes must be immobilized so that they remain right next to the chip. The challenge, says Sayler, is trying to figure out how this can be done in a way that enables the microbes to survive as long as possible.

The researchers are testing various substances that will keep the microbes in place. Something with good optical transparency is critical, of course, so that if the microbes light up, the chip can perceive that change. The immobilant has to be porous, so that any contamination can flow in and reach the microbes. It must contain nutrients for the microbes to feed on. And it has to allow the microbe enough, but not too much, room.

"We are basically trying to feed the immobilized organisms in the matrix without them growing. We really do not want them to grow very much, if at all. If they grow, it changes the total amount of cells in the system, and it confounds the issue of how much light corresponds to how much contaminant."

There must be a few thousand microbes per chip, says Sayler, in order to generate enough light. That number would be only about enough to cover the tip of a pin.

Sayler hopes to develop gels in which the microbes can be kept functional for several months. The sensors would probably be attached to the spaceship walls, continuously monitoring the craft's atmosphere. They would monitor themselves as well, to make sure that the microbes are still viable. "We can electrically induce cells to make light, so we can pulse the system every once in a while to see if the organisms are still physiologically active.

"After, say, six months, the chip would send a signal that says, 'oops, time to replace your bug sensor.' An astronaut would go and get a freeze-dried package of seed microbes, add a little moisture, and stick it in the sensor," says Sayler. "Nothing more need be done until the next time the signal goes off, six months later. It is a low-maintenance system.

Integrated circuit microluminometer measures only 2×2 mm.

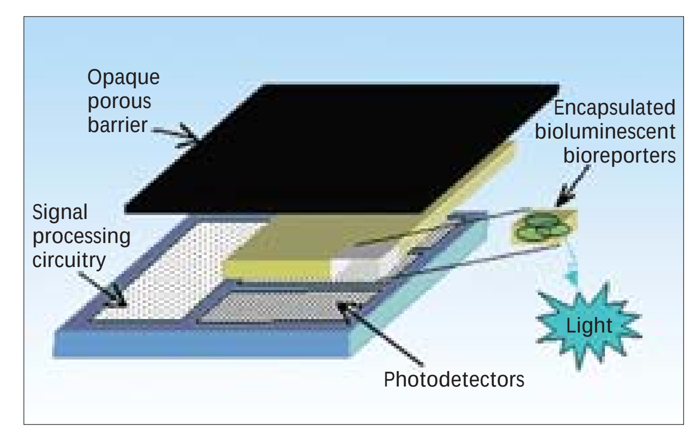
Cancer detection and other uses

These BBICs are useful here on Earth, too. They can detect formaldehyde emitted by pressed wood furniture or hard-to-detect molds often implicated in sick building syndrome. "If this device works as planned, it could turn out to be a very inexpensive kind of monitoring system," Sayler points out. "You could go to your corner drugstore, buy one of these, take it home, and stick it up on your wall. It could tell you whether your carpets are degassing, or whether you've got problems like black mold."

Advanced BBICs could serve as bioterrorism monitors for homeland security, detect DNA radiation damage in astronauts, or act as a diagnostic tool for doctors. A promising approach for advanced identification of cancerous or precancerous cells is the detection of tumor-specific secreted proteins in body fluids. This unique approach allows for the detection of cancer cells based on signature protein secretions rather than on identifying a distinguishable tumor mass or other major physiological change.

The researchers believe it would be possible to develop a sentry of reporter

The basic architecture of a BBIC consists of an integrated circuit with a photodetector, an encapsulation matrix containing the bioluminescent bioreporters, and a porous barrier on top.



cells that will emit bioluminescent signals in response to signature tumor protein molecules. They expect that this approach will allow cancer cells to be accurately recognized before tumor development initiates. The result would be that appropriate preventive medical treatment could be promptly and effectively administered.

The researchers have also developed BBIC prototypes that detect toluene and naphthalene in part-per-billion concentrations. Although their previous work has typically concentrated on environmental chemical monitoring, they are currently advancing BBIC technology into medical applications. They are now developing a eukaryotic bioluminescent bioreporter that will respond specifically to glucose.

Ultimately, they envision an implantable BBIC that can be incorporated into diabetic therapies for continuous monitoring of blood glucose levels. Inclusion of an on-chip radio frequency transmitter will allow the BBIC to communicate remotely with an insulin delivery system. The researchers believe this technology is fully adaptable to tumor-specific protein detection and monitoring as well.

Much more research needs to be done before these ideas become reality. Making BBICs work on spacecraft is a good place to start.

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Microbe turns space waste into electricity

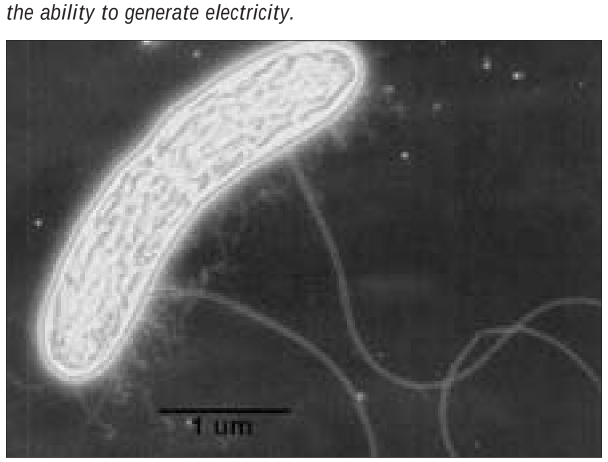
The prospect of sending astronauts to Mars requires a thoughtful look at one unavoidable aspect of space travel: What is to be done with the waste products that accumulate during a long trip?

One answer would be to jettison the material into space, just as ships once disposed of their garbage on the open ocean. However, there is another requirement in space—namely, the need to recycle every drop of moisture.

In much the same way that a land-based sewage disposal plant will process waste products to a point where the fluid can be sent into a river without spreading disease, so too will a space-based processing system do the same for the astronauts. However, because there are no rivers in space, the moisture processed would be recycled as drinking water. Moreover, NASA scientists are certain that the water produced from such a system would be safer to drink than the water that comes from one's tap at home.

Not content with merely providing safe drinking water from waste products, NASA-supported researchers are also working to develop a fuel cell that can extract electricity from human waste. According to one estimate, on a two-year trip to Mars, a crew of six will generate more than six tons of unwanted solid organic material—much of it human waste.

Geobacter metallireducens, shown here in a photomicrograph, has the ability to generate electricity.



Help from a new-found germ

Right now, astronaut waste gets shipped back to Earth. However, for long-term exploration, it should be recycled, because it holds resources the astronauts will need. It will provide pure drinking water. It will provide fertilizer. It is also a rich source of electrons—and with the help of a recently discovered microbe, it will provide electricity.

Like many bacteria, Geobacter (a member of the Geobacteraceae family) feeds on, and can decompose, organic material. Geobacter microbes were first discovered in the muck of the Potomac River downstream from Washington, D.C., in 1987. They like to live in places where there is no oxygen and plenty of iron. They also have the unexpected ability to move electrons into metal. That attribute means that under the right conditions, Geobacter microbes can process waste and generate electricity.

The "right conditions" might be found in a new type of power source called a membrane microbial fuel cell. This device is currently under development by a NASA-funded research team at Northwestern University.

Bruce Rittmann, a professor at the university, leads the team. Other researchers have demonstrated that these microbes will generate electricity; however, Rittmann expects that his team will develop a more refined microbial fuel cell. He says the project is in the early stages of development and hopes to have a prototype in about a year.

All fuel cells generate electricity by producing and controlling a flow of electrons. Conventional cells, including those used onboard the space shuttle and in some prototype automobiles, obtain the electrons for this flow by pulling them off hydrogen atoms. In order to do that, these fuel cells must be given a constant supply of hydrogen.

By contrast, microbial fuel cells obtain their electrons from organic waste. The bacteria at the heart of the device feed on the waste. As part of their digestive process, they pull electrons from the waste material. Geobacter microbes, as well as a few other types, can be coaxed to deliver these electrons directly to a fuel cell electrode, which conducts the electrons into a circuit—a wire, for example. As they flow through the circuit, the electrons generate electricity.

Researchers are already experimenting with microbial fuel cells for use on Earth. For example, one prototype is being used at Pennsylvania State University to generate electricity as it purifies domestic wastewater.

Other projects include the Geobacter Project at the University of Massachusetts (U. Mass.) at Amherst. Researchers were the first to discover the Geobacter microbe in the Potomac River. Similar microbes were discovered in an oil field in Oklahoma. At U. Mass., researchers have set up a laboratory apparatus that draws electricity from river muck.

Proposed design

To make such fuel cells practical for space travel, says Rittmann, you have to have "a very efficient, very compact configuration." The device cannot take up much room. To meet this requirement, Rittmann is considering a fuel cell composed of tightly packed fibers, each one of which will be a fuel cell all by itself.

Each fiber would consist of three layers, like three straws, one inside another. Each layer corresponds to one of the layers of a fuel cell: the anode (outer), the electrolyte-membrane (middle), and the cathode (inner). A slurry of liquefied waste would be pumped past the outer layers, where Geobacter microbes (or other similar bacteria) can grab electrons and move them to the anode, into the circuit, and then to the cathode.

Rittmann says that while he and his team have developed a design for the fibers, they have not yet fabricated them. He hopes to develop a prototype of the fiber and, within a year's time, to have a small-scale pilot study under way.

Speeding up the process

Before any such concept can be put into practice, Rittmann and his team must first decipher the exact mechanism by which the bacterium transfers electrons to the electrode. In laboratory tests so far, the transfer rate is too slow. "We need to know how we can make that faster," says Rittmann, "and so generate more power."

He has a couple of ideas about what the holdup might be. "The electron actually has to move from the outer surface of the microbe to the electrode, and it could be that it is limited by physical contact." Even though the bacteria live attached to the surface of the anode, only a tiny bit of each microbe actually touches the metal, and the smallness of the contact area may be what is hindering electron movement.

Another factor is the voltage on the electrode. This voltage has to be high enough to coax the microbes into giving up their electrons. "Microbes move electrons around in order to gain energy. In fact, they only move the electrons when they do gain energy," Rittmann explains. What is the best voltage? "That is one of the questions we are trying to answer.

"Let us say, for example, that the total voltage difference between the fuel and the anode is 2 V. Then the microorganisms, as they give up their electrons, might take 0.5 V to sustain themselves, leaving 1.5 V for doing work in the circuit. These are just made-up numbers," says Rittmann, "but they illustrate what we are trying to learn."

Rittmann believes that in the long run, the Geobacter microbe may not be as important as it now seems—other bacteria may be found that will supplant it. Noting how recently Geobacter was discovered, he says work on incorporating it into a fuel cell is still in the beginning stages. Yet, if the project succeeds, such devices may soon be used not only in space, but also in our own homes—astronauts are not the only ones who produce organic waste.

"You have to treat the wastes anyway," Rittmann points out. "So why not make the process an energy gainer, instead of an energy loser? By producing electricity, microbial fuel cells would make the process of purifying waste streams much more economical."

Moreover, he continues, "they change our focus. Microbial fuel cells transform something we think of as undesirable into a resource."

TIGR deciphers the genome

To achieve a greater understanding of how these metal-reducing microbes operate, scientists at The Institute for Genomic Research (TIGR) and collaborators have deciphered and analyzed the genome of Geobacter sulfurreducens. They found that the bacterium—a member of a family of organisms that can remove dissolved uranium from groundwater—possesses extraordinary capabilities to transport electrons and "reduce" metal ions as part of its energy-generating metabolism.

Reduction is a chemical process during which electrons are added to metal ions. As a result, the metals become less soluble in water and precipitate into solids, which are more easily removed. Small charges of electricity are also created through the reduction process.

Geobacter's capability for reduction is enhanced by its genes, more than 100 of which appear to encode for various forms of c-type cytochromes. Those are proteins that facilitate electron transfers and metal reduction during the organism's energy metabolism. The presence of those c-type cytochrome genes—the highest number and the greatest variety found so far in a bacterial species—is thought to give G. sulfurreducens a significant capability and flexibility to reduce metals or create electricity.

Barbara Methé, the TIGR researcher who led the genome project, says that research based on genome data has shown that this microbe can sense and move toward metallic substances, and in some cases can survive in environments with oxygen.

TIGR's collaborator on the project was Derek Lovley, a professor of microbiology at U. Mass. in Amherst. Lovley discovered the Geobacter family of bacteria and has led projects to assess their biology and their potential for bioremediation. G. metallireducens, isolated from Potomac River sediments in 1987, was the first Geobacter species to be discovered. G. sulfurreducens was isolated later in Oklahoma from a soil sample that was contaminated by hydrocarbons.

The U. Mass. team had previously reported that Geobacter sulfurreducens and other Geobacter species have the novel ability to harvest electricity from mud and other forms of inorganic waste matter. Scientists say this capability shows promise for such uses as powering electronic devices at the bottom of the ocean and at other remote locations, for converting sewage and renewable biomass into electricity, and for developing improved microbial fuel cells.

One discovery about the G. sulfurreducens surprised TIGR researchers: A high percentage of its genes are devoted to sensing environmental conditions and then regulating its metabolism in response to changes in those conditions. The scientists say this characteristic sheds light on why Geobacter species are so successful at rapidly colonizing changing environments. The finding also suggests that the species are ideal candidates for the development of novel biosensors.

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