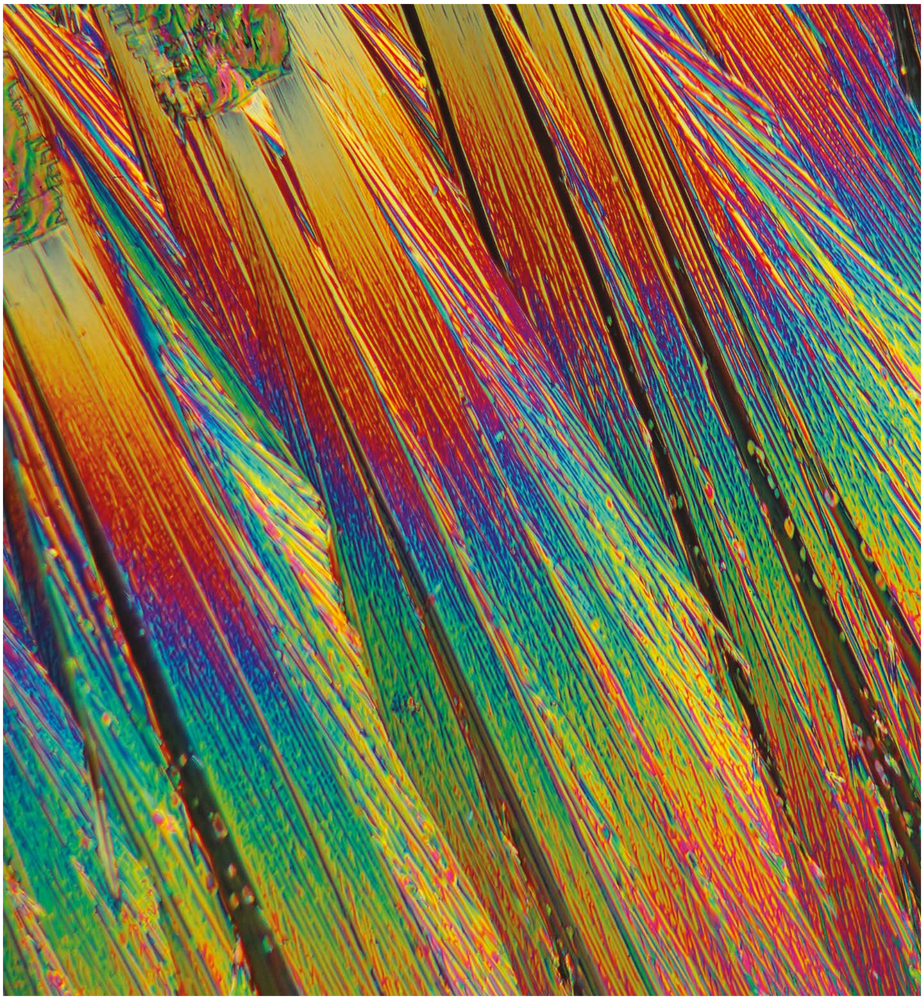

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A sample of lanthanum (III) nitrate under the microscope.

RARE-EARTH METALS: BIOMINING WITH BACTERIA

Amid global demand for rare-earth elements, researchers are adapting bacteria to isolate them without using harsh chemicals. **By Amber Dance**

The fermented drink kombucha seems about as far away as possible from the mining of heavy metals. But Alexa Schmitz, chief executive at the biomining company REEgen in Ithaca, New York, sees parallels between her firm's bacteria-based product and the tangy beverage.

REEgen's bacterial 'soup' dissolves ground-up rocks, waste electronic components and other solids that contain rare-earth elements — metals that have valuable conductive, magnetic and fluorescent properties and that are used in everything from mobile phones to wind turbines. The metals impart strength and hardness to alloys, for example, and are found in superconductors and catalytic converters. But Schmitz notes that the company's product is much less hazardous, both to people and to the environment, than are the chemicals typically used to separate metals from ore. "We're producing a solution that is proving to be about as good as concentrated nitric acid at dissolving solids," says Schmitz. "But it's a little bit like kombucha. You can stick your hand in a vat of it and come out unscathed."

Rare-earth elements include those in the lanthanide series — those with atomic numbers from 58 to 71, usually shown as a pop-out beneath the main periodic table — as well as the group 3 transition metals scandium and yttrium. They are used in products such as magnets, light bulbs and electric cars, and end up in various waste streams, including mining tailings and ash from coal plants.

Despite their name, rare- earth elements (REEs) aren't all that uncommon, but they don't tend to be found in concentrated deposits (unlike, say, a vein of gold). Miners might have to excavate one tonne of rock just to obtain a gram of REEs, says Buz Barstow, a synthetic biologist and Schmitz's former adviser at Cornell University in Ithaca.

They're also difficult to purify. REEs tend to co-occur in natural deposits and are chemically similar. The conventional purification process involves repeatedly separating the metals, in dozens or even hundreds of cycles, using aqueous acids and organic solvents such as kerosene. It's inefficient, costly and damaging to health and the environment. Much of the globe's REE separation currently takes place in China.

Now, Schmitz and a small but growing cadre of researchers are investigating a possible alternative: biomining. Many microorganisms naturally concentrate metals, and some are already used to mine copper and gold. The discovery about a decade ago of microbes that use lanthanides for their metabolism[1,2] allowed researchers to explore the feasibility of adapting the microorganisms or their components to isolate REEs. The US Defense Advanced Research Projects Agency (DARPA) in Arlington, Virginia, has invested around US$43 million in research–industry partnerships to develop biomining for REEs.

There's room for microbes at every step of the biomining process, says Dan Park, an environmental microbiologist and DARPA grant team member at Lawrence Livermore National Laboratory in Livermore, California. For starters, many microbes secrete acids that can solubilize metals from rocks, discarded appliances and other electronic waste. Some make proteins that specifically interact with REEs, giving scientists the opportunity to isolate the elements from other metals, and perhaps even from each other.

But scaling up microbe-based mining and remediation from the bench to an industrial process, in a way that would be practical and economical, involves substantial challenges.

Each DARPA project on REEs, for example, has a goal that is puny by industrial standards: by 2026, the teams must to be able to purify 700 grams of material in a week. "It is really a baby step," says Linda Chrisey, a programme manager for biological technologies at DARPA. "The most important thing is, can we do it?"

Microbe miners

Whatever the starting material, the first step in biomining is to grind it up and isolate the metals from everything else. Acid is often used to solubilize metals, and the acids produced by microbes are environmentally friendly, economical options. Researchers at Idaho National Laboratory in Idaho Falls, for instance, zeroed in on *Gluconobacter oxydans*, an acid-producing bacterium found in garden soil, fruits and flowers, as a potential microbe miner. The organism has no designs on the REEs themselves, says Barstow, who also works with *G. oxydans*. Rather, the acid it produces dissolves phosphates that it then uses in DNA; the liberation of REEs is a collateral benefit that humans can exploit.

In experiments at Idaho National Laboratory, *G. oxydans* secreted a gluconic acid mixture that was better at leaching rare metals from industrial waste than was a comparable concentration of commercial, pure gluconic acid[3]. "We think there are other things being produced besides the gluconic acid," says Vicki Thompson, a chemical engineer at the lab.

Gluconobacter oxydans has a long history in biotechnology applications and a sequenced genome that is accessible to genetic tools. Schmitz, Barstow and their colleagues tapped these tools to optimize leaching of REEs by *G. oxydans*. The researchers began with a gene knockout screen, disrupting 2,733 of the microbe's non-essential genes to identify more than 100 that influence gluconic acid output[4].

Disruption of *G. oxydans* genes involved in the uptake of phosphate resulted in the microbes producing a solution that was more acidic and more effective at leaching REEs[5]. "We convince them they're starving for phosphate," explains Schmitz. Work at REEgen to combine genetically engineered *G. oxydans* with optimization of the firm's processes has boosted leaching by up to five times compared with wild-type microbes, Schmitz says.

Separation anxiety

After leaching, the next step is to isolate REEs from other metals that come out in acid, such as calcium and iron. Here, some surprising biology comes to the rescue. REEs were once thought to have no direct relevance to living organisms. Then, in 2012 and 2013, researchers reported that REEs are used by certain microbes to metabolize methanol[1], and are even vital to the survival of microorganisms living in volcanic mud pots in Italy[2].

Lanthanides, it turned out, provide essential cofactors for microbial enzymes called alcohol dehydrogenases, some of which convert methanol to formaldehyde as part of metabolism. In fact, use of lanthanides as enzyme cofactors is widespread among microbes, even those that don't eat methanol, says Cecilia Martinez-Gomez, a microbial physiologist at the University of California, Berkeley. Researchers are now adapting these microbes, or just their REE-binding molecules, to concentrate the desired elements.

Martinez-Gomez's group, for instance, works with another lanthanide-using organism called *Methylobacterium extorquens*, which is found in a variety of locations, such as plants and the oceans. She and her team identified a set of ten *M. extorquens* genes[6] that produces a small metal-binding molecule that the team named methylolanthanin. The microbes secrete methylolanthanin into their surroundings, where it sticks to nearby lanthanides, which are otherwise insoluble. The complex is then taken up by a microbial transporter and brought into the cell to serve as a cofactor for alcohol dehydrogenase.

M. extorquens also has a system to store lanthanides for later use, saving the metals either in granules or in structures that the researchers called lanthasomes[7]. This, presumably, allows the bacterium to prepare for a lanthanide drought; it can stockpile enough of the metals to last for several microbial generations, says Martinez-Gomez.

> **"The system needs to be incredibly robust, otherwise it won't be economically feasible."**

To improve lanthanide uptake for biomining purposes, she and her team engineered a strain of *M. extorquens* that allowed them to control and scale up methylolanthanin production. This more than tripled the microbes' ability to collect neodymium and other REEs from pulverized magnets, Martinez-Gomez says. Then it's a relatively simple matter of breaking open the cell and precipitating the lanthanides. The process results in REEs that are more than 98.8% pure, says Martinez-Gomez, who co-founded a company, RareTerra in Berkeley, to commercialize accumulation and separation of lanthanides by *M. extorquens*.

The bacterium has also yielded a tool that has become key to the burgeoning field of rare-earth biomining. Discovered in 2018, lanmodulin is a lanthanide-binding molecule[8] that sits between the two outer membranes of the bacterium, alongside the alcohol dehydrogenases that use lanthanides as a cofactor. Co-discoverer Joseph Cotruvo Jr, a biochemist at Pennsylvania State University in University Park, still isn't sure what lanmodulin does there. "We kind of got sidetracked by the interesting properties and technological applications," he says. For example, his group, Martinez-Gomez and others are adapting parts of the protein to create luminescent and fluorescent biosensors. These could highlight where REEs are present or accumulating[9,10], and might even be used to remediate REE contamination of water sources[11].

Lanmodulin has provided researchers with a mechanism for isolating REEs, at least at the benchtop scale. Park, a collaborator of Cotruvo's, immobilized lanmodulin on agarose microbeads to create a column that could capture lanthanides. Starting with coal-mine ash from the northwestern United States, which contained less than 1% lanthanides overall, the team obtained a solution of 88.2% pure lanthanide[12]. "It was so selective that we could take really dilute, poor sources of rare earths, and selectively capture using lanmodulin," says Park.

Getting specific

Lanmodulin and *M. extorquens* are part of a small group of emerging tools for purifying REEs. Researchers have also designed lanthanide-binding peptide tags that can be encoded in a gene of interest. Originally intended to enhance X-ray crystallography and protein assays[13], these are also finding applications in biomining.

Researchers are studying the REE-collecting abilities of the model microbe *Pseudomonas putida*[14] and of *Methylacidiphilum fumariolicum* — the species discovered in volcanic Italian mud pots[15]. And scientists in Germany have discovered that photosynthetic single-celled organisms called cyanobacteria can suck up REEs[16] — although, as with *G. oxydans*, this doesn't seem to be essential for their survival. The cyanobacteria can even absorb heavy metals into their cell walls if they're dead, meaning that it might not be necessary to keep them alive to use them in metal purification, says biotechnologist Thomas Brück at the Technical University of Munich in Germany.

Whatever their source, once REEs are obtained, the most challenging step is to separate them from each other. There are 17 rare-earth metals, which are not necessarily interchangeable for commercial applications. Yet the smallest and largest lanthanide atoms differ in size by less than half an ångström. Their similarities in size and chemistry explain why the current chemical separation process is so laborious. Isolating individual REEs is "the problem that industry wants to solve the most", says Cotruvo.

Here, again, lanmodulin offers possibilities. Cotruvo and his colleagues scanned genome sequences for the most unusual lanmodulins they could find, homing in on a protein from a bacterium called *Hansschlegelia quercus*. It's found on oak buds, where it can live on methanol released by the plant. Lanmodulin from *H. quercus* showed a preference for light lanthanides – those with atomic numbers of 62 or less – rather than for heavy ones with atomic numbers of 63 and up.

Cotruvo's group discovered that *H. quercus* lanmodulin distinguishes between the metals through a selective process. When the molecule encounters a light lanthanide such as neodymium or lanthanum, two lanmodulin monomers stick together to form a dimer, and do so more than 100 times more tightly than they do in the presence of a heavier lanthanide such as dysprosium. The lanmodulin protein probably doesn't dimerize on Park's columns, Cotruvo says, but nonetheless, that preference meant that a column of *H. quercus* lanmodulin could separate a mixture of neodymium and dysprosium into fractions that were more than 98% pure for each element[17].

"That's really a significant breakthrough," says Daniel Nocera, an inorganic chemist at Harvard University in Cambridge, Massachusetts. "It's going down the road to selectivity."

And there are likely to be other tools out there, notes Martinez-Gomez, because microbes seem to have a wide variety of mechanisms for collecting, transporting and using lanthanides. "There are really interesting differences, so this is really a broad and emergent area of study," she says.

To apply these tools to mining and recycling, researchers envision a series of steps. First, they'll remove metals from the ore or waste material, then they'll extract lanthanides from the other metals. At that point, they might enlist *H. quercus* lanmodulin or other tools to separate groups of lanthanides from each other, such as lights from heavies, until they have pure elements.

But biology doesn't have to solve all the separation problems, says Park, because the chemical process remains an option. If microbiologists could go from a low-grade leachate to a solution that is, say, 80–90% REE, they could pass it on to the chemists to finish the job. Even with partial biomining, the whole process might still use less energy and produce less toxic waste than an entirely chemical purification.

Emphasis on 'might': the commercial viability of this biomining approach remains to be seen. "The system needs to be incredibly robust, otherwise it won't be economically feasible," says Marina Kalyuzhnaya, a microbiologist on a DARPA REE project at San Diego State University in California.

The Idaho team has calculated how much it would cost to use *G. oxydans* to recover REEs from hazardous waste originating in petroleum production, and estimated that the process could be economical[18]. The biggest costs in terms of both money and environmental hazards were electricity to power the plant and glucose to feed the microbes, with the sugar alone accounting for 44% of the investment.

CHRISTIAN THOMPSON/ANADOLU AGENCY/GETTY

A worker walks past a stack of waste computers at a recycling yard in Accra, Ghana.

But microbe miners don't necessarily need pure glucose. Alternatives include maize (corn) stover – the stalks, leaves and cobs left over after harvests – or the starchy water that runs off potatoes after they're washed. Switching to either of these inputs, the team calculated, cut costs by 17% or more[19].

Another key question is how long purification columns will last before they have to be replaced. So far in the lab, scientists have run their columns only dozens of times at most, but mining companies could require tens of thousands of runs. "Any time we talk to somebody in industry, that's the first question they'll ask," says Park. "It's still a pretty open question."

"Rare earths are just a test bed for all the other minerals."

Park advises scientists interested in studying this kind of process to talk to people in the mining industry to understand their needs. He's also found "a wealth of expertise" in advice from peers at the Critical Materials Innovation Hub, a collaboration between labs in academia, industry and the US Department of Energy, led by Ames National Laboratory in Iowa. Its goal is to accelerate work on rare-earth and other materials that are key to clean energy. Conferences and journals from the American Chemical Society are also great resources for those interested in REE purification, Park says.

And should lanthanide biomining prove successful, it could be only the beginning. There are other elements found in relatively low-grade ores that manufacturers would love to concentrate, Barstow says. "Rare earths are just a test bed for all the other minerals," he says. "We want to make microbes that are tailored for all the other metals."

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1. Nakagawa, T. *et al. PLoS ONE* **7**, e50480 (2012).
2. Pol, A. *et al. Environ. Microbiol.* **16**, 255–264 (2014).
3. Reed, D. W., Fujita, Y., Daubaras, D. L., Jiao, Y. & Thompson, V. S. *Hydrometallurgy* **166**, 34–40 (2016).
4. Schmitz, A. M. *et al. Nature Commun.* **12**, 6693 (2021).
5. Schmitz, A. M. *et al.* Preprint at bioRxiv https://doi.org/10.1101/2023.02.09.527855 (2023).
6. Zytnick, A. M. *et al.* Preprint at bioRxiv https://doi.org/10.1101/2022.01.19.476857 (2022).
7. Roszczenko-Jasińska, P. *et al. Sci. Rep.* **10**, 12663 (2020).
8. Cotruvo, J. A. Jr, Featherston, E. R., Mattocks, J. A., Ho, J. V. & Laremore, T. N. *J. Am. Chem. Soc.* **140**, 15056–15061 (2018).
9. Featherston, E. R., Issertell, E. J. & Cotruvo, J. A. Jr. *J. Am. Chem. Soc.* **143**, 14287–14299 (2021).
10. Jones, E. *et al.* Preprint at bioRxiv https://doi.org/10.1101/2023.09.26.559553 (2023).
11. Lee, H. D. *et al.* Preprint at bioRxiv https://doi.org/10.1101/2023.01.05.522788 (2023).
12. Dong, Z. *et al. ACS Cent. Sci.* **7**, 1798–1808 (2021).
13. Franz, K. J., Nitz, M. & Imperiali, B. *ChemBioChem* **4**, 265–271 (2003).
14. Wehrmann, M., Billard, P., Martin-Meriadec, A., Zegeye, A. & Klebensberger, J. *mBio* **8**, e00570-17 (2017).
15. Singer, H. *et al. Chem. Commun.* **59**, 9066–9069 (2023).
16. Paper, M. *et al. Front. Bioeng. Biotechnol.* **11**, 1130939 (2023).
17. Mattocks, J. A. *et al. Nature* **618**, 87–93 (2023).
18. Thompson, V. S. *et al. ACS Sustain. Chem. Eng.* **6**, 1602–1609 (2018).
19. Jin, H. *et al. ACS Sustain. Chem. Eng.* **7**, 15311–15319 (2019).