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Plastic trash recovered from an island in the South Pacific Ocean shows decay and bite marks from marine life.

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THE PLASTIC EATERS

Bacterial enzymes can digest some plastic waste. Scientists want to harness them for recycling By Warren Cornwall

uhammad Reza Cordova is searching for treasure amid the water bottles, plastic bags, and plastic foam cups that choke the beaches, reefs, and mangrove forests around Jakarta, Indonesia. In the microbe-rich slime coating some of that trash, he hopes to find organisms to help solve the vexing problem of what to do with the plastic flooding the planet.

Cordova, a marine biologist, collects samples of the slime and brings them back

to his lab at Indonesia's Research Center for Oceanography, where he plans to culture the microbes and feed them only plastic to see what thrives. "We are hoping that we find the most effective microbes that can eat or degrade the plastic," he says.

Researchers across the globe are on the same quest. They are looking for plastic-munching microbes in searing hot springs in Yellowstone National Park, remote island beaches in the Pacific Ocean, and a plastic recycling factory in Japan, among other places. Some scientists have already found bacteria that wield enzymes able to break down a common plastic used to make water bottles and clothing.

The scientists think the mi-

crobes' enzymes-proteins that speed chemical reactions-might help recycle some kinds of plastic, much of which gets buried in landfills, burned, or washed into rivers and oceans. Although industrial chemicals can break down plastics, using enzymes is potentially a greener approach, requiring less energy, that can also target specific plastics mixed with trash. "Nature is the most amazing recycler because it wastes nothing," says John McGeehan, a structural biologist at the United Kingdom's University of Portsmouth who leads an enzymehunting project that Cordova is part of.

A company in France is already building a demonstration factory that will use enzymes to turn plastic trash into raw material for new bottles. Scaling up further means overcoming major challenges, however. Finding enzymes is just a first step. Moving from the laboratory to a recycling factory requires overcoming technical and economic hurdles in an industry with razor-thin profits, and where new plastic can be cheaper than recycling. On top of that, the microbes largely fail to dent some of the most widespread plastics.

"Think about the sheer scale on which



After 48 hours with an enzyme that breaks down the plastic polyethylene terephthalate, it loses nearly 98% of its mass (right).

we manufacture plastics and the low value of these plastics," says Susannah Scott, a chemical engineer at the University of California, Santa Barbara, who is developing metal-based catalysts, synthesized in the laboratory, to recycle plastic. "It's a tall order to ask biology to do that well."

PLASTIC IS in many ways a recycler's nightmare. Built to last, plastic encompasses dozens of different molecules, made of long chains of carbon atoms. Those molecules all resist breaking apart, and each has distinct chemical properties that must

be tackled differently. Often a single itema potato chip bag, for example-is a maddening fusion of plastics, confounding the goal of easily extracting pure materials to develop a new product.

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grow 70% by 2050, to almost 600 million tons per year.

recycling bins meets a variety at University of fates. Though some is reused, much is incinerated, buried in landfills, or dumped in the environment. Until recently, more than half the plastic collected in the United States was shipped overseas; of the shipped material, as much as one-quarter was too contaminated to be recycled, according to an estimate in a 2020 Science Advances study. In 2018, China stopped accepting most imported plastic waste, and U.S. recyclers reported sending unwanted bales of plastic to landfills. "We can't keep buying more and more plastic, putting it into the blue bin, and feeling

like that's OK," says Kara Lavender Law, an oceanographer at the Sea Education Association in Woods Hole, Massachusetts, who has worked to measure global plastic pollution.

Enzymes are an appealing solution. Unlike many industrial chemicals, enzymes work at relatively low temperatures and are choosy about which molecule they interact with—enabling an enzyme to target a single plastic in a stew of polymers. Scientists began hunting for such enzymes in earnest in 2016, after Japanese researchers analyzing mud near a plastic recycling factory found a bacterium with an unusual

<u>of</u>

Maryland College Park on

November 30,

2022

Coming full circle

Scientists are engineering enzymes to recycle plastic. These modified versions of natural proteins work at relatively low temperatures, target specific plastics in a mixture, and produce pure monomers that can then form new plastic.



Breaking bonds

PET is held together by bonds between carbon and oxygen, which require less energy to break in a chemical reaction than those formed by links between two carbon atoms. Those bonds, found in many common plastics such as polyethylene and polypropylene, are harder to break.



appetite for plastic (Science, 11 March 2016, p. 1196). The organism produced two enzymes that together enabled it to feed on polyethylene terephthalate (PET) by breaking it into its building blocks, terephthalic acid and ethylene glycol. PET is found in single-use drink bottles and fibers in polyester clothing, and it makes up about one-fifth of worldwide plastic production.

Fabric is challenging to recycle today because it is often mixed with other materials. Although PET bottles are simpler, just 29% of PET bottles in the United States were recycled in 2018.

Before reading about the finding, McGeehan had been studying how organisms use enzymes to break down tough plant fibers. Now, he turned his eye to plastic, setting out to find other enzymes that could target polymers. He went on to recruit enzyme-hunting scientists in some of the world's plastic pollution hot spots, thinking all the plastic trash might have led to the evolution of microbes that attack it. Indonesia is one place he's looking, ranking second after China in one study examining sources of ocean-polluting plastic (Science, 13 February 2015, p. 768).

In Indonesia, in addition to collecting bacteria from plastic litter, Cordova plans to delve into the muck at the roots of mangrove trees. Microbes that originally fed on tough mangrove leaves would have had decades to evolve the ability to break down the plastic bags that cling to the roots. He will also suspend small tags of various plastics in Jakarta Bay to see whether any microscopic creatures start to feed on them.

Any promising microbial candidates that Cordova finds will be shipped to McGeehan's lab. His team crystallizes promising enzymes, then uses x-ray crystallography to peer into their structures, deciphering how they bind to polymers and help break their chemical links. The work has already yielded insights about which enzyme shapes hold the most promise. PETbreaking enzymes, for example, have a valley in their surface into which the plastic molecule nestles. There, a distinctive trio of amino acids attacks the molecular bond joining units of the polymer.

Using that information, McGeehan and others are scouring databases of bacterial genomes for DNA sequences that code for similar molecules, signaling potential plastic-cracking enzymes. Researchers in his lab then use computers to model how the proteins might be artificially improved. The goal is to modify the genes that encode the natural enzymes to make them into powerful plastic-busting tools. Already the team has altered the enzyme uncovered by the Japanese researchers to make it more efficient. "We're not looking for superenzymes from nature. That's pretty unlikely," McGeehan says. "We're just looking for enzymes that tickle plastic."

His group isn't the only one on the hunt. A consortium of European and Chinese labs is also working to find and cultivate bacteria whose enzymes break down plastic-and other enzymes that can turn the breakdown products into valuable chemicals. A group of researchers from Germany, France, and Ireland that included members of the consortium recvcled PET by using a modified version of an enzyme found in a compost pile that takes apart the waxy layer on leaves. A strain of lab-evolved bacteria then used the raw materials to build two new kinds of plastic.

GRAPHIC: V. ALTOUNIAN / SCIENCE

IF THOSE QUESTS succeed, some plastics might be recycled by washing them in enzymes, much as enzyme-based detergents break down food stains in dirty clothes, says Gregg Beckham, a chemical engineer at the U.S. Department of Energy's National Renewable Energy Laboratory (NREL). Beckham heads a \$32 million DOE initiative to develop new plastic recycling methods and collaborates with McGeehan on the enzyme search. In late May, NREL scientists gathered to test a scheme in which enzymes would help soldiers turn their plastic trash into building blocks for battlefield essentials.

The vision is "a box ... where they could put in plastic and other types of waste, like paper. And out the back of it would come he says. "We'll blow that out of the water."

The French company that developed the enzyme, Carbios, plans to build the world's first enzyme-fueled plastic recycling factory. The endeavor will start with a small demonstration plant in the city of Clermont-Ferrand to test the enzyme's ability to convert plastic trash into raw material for new PET plastic. The company says it will open a full-scale factory in 2024 with a goal of producing the ingredients for 40,000 tons of recycled plastic each year.

SUCH ENZYMES might not break the recycling logjam. They work best on plastic made from carbon atoms joined by an oxygen atom. Such polymers, called polyesters,

Panoply of plastics

The millions of tons (Mt) of plastics produced each year include a vast array of materials tuned for different functions. Recycling processes vary depending on a plastic's identity, so a mix often can't be recycled together. But enzymes may be able to selectively break down a single plastic in a mix.

Polyethylene (116 Mt) •Sandwich bags •Trays and containers •Food packaging film	Polypropylene (68 Mt) •Food packaging •Snack wrappers •Microwavable containers •Automotive parts	Polyvinyl chloride (38 Mt) • Window frames • Cable insulation • Garden hoses • Inflatable pools
Other (75 Mt) • Touch screens • Optical fibers • Hub caps • Surgical devices	Polyethylene terephthalate (33 M •Water bottles •Soft drink bottles) Polystyrene (25 Mt) •Dairy and meat
	Polyurethane (27 Mt) •Building insulation •Pillows and •Kitchen sponges mattresses	•Disposable cutlery

*Total mass does not include that of polyester fibers in clothing.

something like food or gun oil," Beckham says of the project, which is sponsored by the Defense Advanced Research Projects Agency (DARPA).

As with many DARPA projects, the research is a long way from practical deployment. Scientists at the NREL lab in Golden, Colorado, cut sheets of PET plastic into small squares. The squares were submerged in a brew of warm water, salt, and a version of the leaf-digesting enzyme found in compost, altered by French researchers to bind more tightly with the plastic and withstand higher temperatures.

When the Colorado researchers returned 24 hours later, 84% of the plastic had dissolved—suggesting the enzyme had broken the plastic down into smaller molecules. Beckham's target is to break down 95% of 5 kilograms of plastic in 1 month, are also found in plant fibers, which bacteria have had millions of years to evolve to feed on.

By contrast, plastics with bonds linking carbon atoms directly are tougher. Representing more than half of plastics made, they include the polyethylene of ubiquitous grocery bags and the polypropylene that forms a dizzying array of products, as diverse as syrup bottles and car dashboards.

In recent years, scientists have reported organisms that can feed on such plastics, including larvae of wax moths. But Beckham and others doubt the chemical talents of those organisms will translate into practical recycling of polyethylene or polypropylene.

Enzymes can be finicky, failing at the high temperatures needed to coax chemical reactions in many plastics other than PET. Enzymes also tend to work more slowly than industrial chemicals, Scott says, making them inefficient. "You're always coming back to the techno-economic analysis," says George Huber, a chemical engineer at the University of Wisconsin (UW), Madison, who heads a DOE-funded research project on recycling plastic into high-value products. "The economics dominate everything."

That's true for all enzymatic recycling methods. The raw materials—natural gas and oil—that go into most plastic are relatively cheap. Even if a recycled material were cheap enough to compete with a new one, it would have to be integrated into a vast manufacturing infrastructure and meet the exacting demands of companies buying plastics, says William Banholzer, a chemical engineer who was chief technology officer until 2014 at Dow, one of the world's largest plastic manufacturers. "The truth is, recycling still is too expensive and gives crappier products," says Banholzer, now at UW.

Amid a growing public outcry over plastic pollution, major chemical companies are investing in new forms of plastic recycling. But those approaches rely largely on industrial chemicals. In January, two major petrochemical companies, Eastman Chemical Company and SABIC, a subsidiary of the oil and gas giant Saudi Aramco, each announced plans to build factories using chemical treatments to turn plastic trash into materials to help make new plastics.

SABIC's chief technology and sustainability officer, Bob Maughon, says enzymeboosted reactions move too slowly for plastic recycling. At the moment, "I think enzymatic is not realistic," he says.

But Alain Marty, a biologist and chief technology officer for Carbios, says he is confident his company's enzyme-based approach can chew through PET fast enough to find a place in the market. Although the first factory probably won't compete with the price of unrecycled raw materials, companies will pay a premium for recycled plastic that can be sold to environmentally conscious consumers, he says. "It is another product, and there is a great demand."

Despite Beckham's excitement about enzymes, he says he is agnostic about whether they will win out over other new ways to recycle. The DOE project he oversees is also investigating using heat, light, and electricity to break down plastics. Whatever the method, he just wants to see an increase in the fraction of plastic that goes into his recycling bin and really gets recycled. "My hope," Beckham says, "is that our recycle bins become much, much bigger."



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