
Credit: Getty Images / eleonora galli

Closing the recycling circle

Capturing the value back from plastic waste has been the holy grail of recyclers. Biotechnology is taking us closer to a solution.

Laura DeFrancesco

In April, the French industrial biotech company Carbios published work that signals a new era in the world of plastic recycling. In a public–private partnership with the Toulouse Biotechnology Institute, company researchers reported in Nature the recycling of plastic waste into fully functional plastic bottles. Most recycled plastic bottle waste — roughly 35 million tons per year — is turned into pellets or material of lesser value, or “downcycled” in industry parlance. Applying some clever genetic engineering tricks to leaf compost cutinase (LCC), the research team was able to enzymatically reduce plastic waste made from poly(ethylene terephthalate) (PET) into its two component molecules — ethylene glycol and terephthalic acid (benzene-1,4-dicarboxylic acid) — and reform them into a bottle that matched bottles made from virgin PET, completing the cycle.

What preceded this accomplishment was decades of work by the research community scouring landfills for microbes that could live on plastic, likely because they have enzymatic pathways capable of metabolizing these xenobiotics. Reports of such bacteria date at least as far back as the 1990s. This is the easy part. Going from there to an activity that works efficiently and on a large scale is not.

Whether Carbios’s approach for PET will work for the tons of other, harder-to-degrade olefins and polyesters, accumulating in landfills and oceans as plastic waste, remains to be seen (Fig. 1). In the meantime, the hunt is on to find and hone enzymes, organisms or even consortia of organisms that can take a bite out of the plastic waste problem.

Getting after PET

PET, a semicrystalline polyester made up of repeating units of ethylene glycol and dimethyl terephthalate is an important industrial polymer, used in packaging and textiles. Although it currently is the most recycled of the big five polymers (polyethylene, polypropylene, PET, polyvinyl chloride and polystyrene), industrial processes for recycling PET degrade the

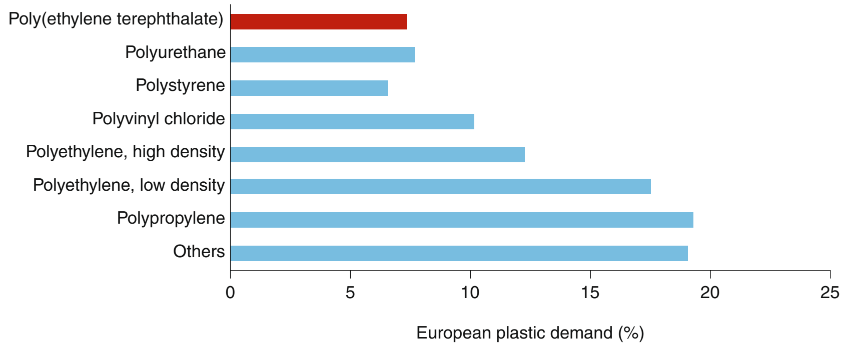

Fig. 1 | The demand for plastic resins in Europe during 2017 as a percentage of total plastic demand. Source: PlasticsEurope.

monomers, leading to the loss of potential value of the byproducts. So researchers have been looking at enzymes as a less destructive, more ecofriendly way to recycle PET, to both spare the environment and recapture the plastic's value. Whereas activities against ester bonds exist in nature, in order to work on something like a bottle, enzymes would have to thermostable, as they must function at temperatures at or above the glass transition of the polymer. This is the point at which semi-crystalline, largely impenetrable structures become partially amorphous, allowing enzymes access to the polymers.

The first real breakthrough in the modern era from enzymatic approaches to recycling PET was reported in 2016, when Japanese scientists discovered a bacterium that could both degrade and assimilate PET. Through analyzing hundreds of samples containing consortia of microorganisms living on plastic bottles in a recycling bin, they isolated a novel bacterial species they named *Ideonella sakaiensis* 201-F6 that can degrade plastic film made of PET. After attaching itself to the plastic, this bug secretes a PET-degrading enzyme called PETase; takes up the main byproduct, mono-2-hydroxyethyl terephthalate (MHET); and degrades it further into usable metabolites by means of a second enzyme, MHET hydrolase (Fig. 2).

Although this report garnered a lot of attention from the press, the dirty little secret was that the enzymes performed poorly, in comparison to previously described enzymes from bacteria and fungi capable of degrading the ester bonds holding the PET polymer together. It took six weeks for these bacteria to fully degrade a PET film, which itself is not the major form of PET found in landfills. Nonetheless, the finding was noteworthy for its novelty: the bacteria and both activities were unique, the PETase showing only modest homology to other known PET-degrading hydrolases and the MHET hydrolase being perhaps totally unique. Also remarkable is that the bacteria appear to have evolved to use PET as an energy source, even though the material entered into wide use as recently as the 1970s.

The Japanese group and others have drilled down to learn more about the *I. sakaiensis* enzymes. Several groups have solved the crystal structures of the two enzymes. A group of collaborators from National Renewables Energy Laboratory in Golden, Colorado (NREL) and the University of Portsmouth in the United Kingdom have improved upon the activity of the PETase by engineering the catalytic site to resemble that of previously studied, more active cutinases. By modifying just two amino acids, they improved the enzyme's ability to degrade crystalline PET, suggesting a path to creating industrially useful tools. This was a heady time for the team, used to working in relative obscurity, garnering them a spotlight in the popular press.

According to John McGeehan, director of the Centre for Enzyme Innovation at the University of Portsmouth, it was in part due to the timing of their publication, which coincided with the airing of the BBC series Blue Planet. By several accounts, this program was a turning point, at least in Europe, capturing the attention of the public to the problem of plastic waste. It had immediate benefits to McGeehan, as GlaxoSmithKline, located a few miles up the road from his institute, reached out to offer their fermenters for producing the enzyme. This connection in turn led to an important industrial insight that the researchers might have missed. "The first thing they said was 'What is the product of this reaction, ethylene glycol and terephthalic acid? Do you know the market price of this?'" he recalls. He was told this would "markedly impact the viability of this system."

Reports of other promising enzymes were to follow. Wolfgang Zimmermann and coworkers at Leipzig University described naturally occurring hydrolases from a thermophilic actinomycete, *Thermobifida fusca* KW3 (TfCut), and an LCC isolated from a compost metagenome. They improved on the activity at high temperatures of TfCut by replacing several amino acids in the substrate binding pocket to make it more closely resemble a cutinase. Also notable is the 2018 publication by Richard Gross at Rensselaer Polytechnic Institute describing a LCC that outperforms the *I. sakaiensis* enzyme at high temperature, although its activity was still too low. However, they discovered that low activity at high temperature was due enzyme aggregation, a problem they solved by expressing the protein in *Pichia pastoris*, which glycosylated the enzyme at its natural glycosylation sites.

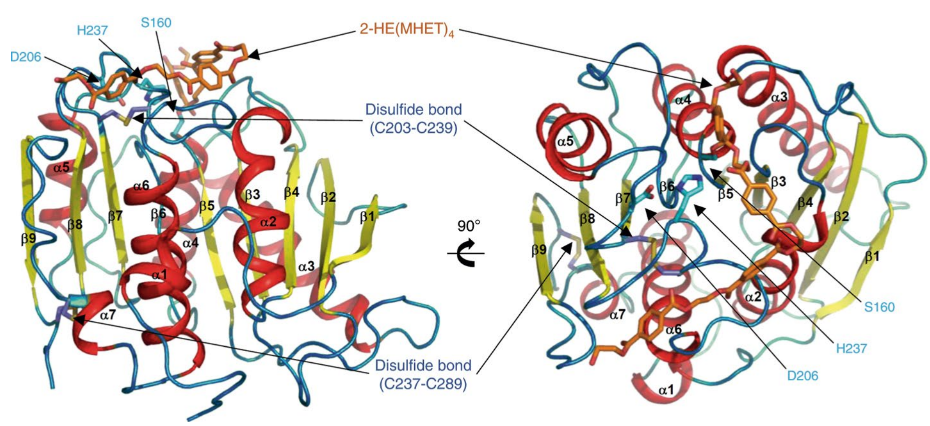

Fig. 2 | Structure of PETase from *I. sakaiensis*. The three-amino-acid catalytic site is in cyan and the substrate 2-H(MHET)4 molecule in orange at the active site. Reprinted with permission from S. Joo et al. *Nat. Commun.* **9**, 382 (2018).

Box 1 | Funding ramps up

Whether it was the airing of the series Blue Planet or the horrifying images of sea life engorged with plastic, funding agencies around the globe are finally putting money where their collective mouths have been. Numerous pronouncements about the plastic problem have been made, especially since the Chinese stopped accepting it (as if that were a solution), but it appears that, until recently, not much was behind the pronouncements. The funding opportunities (see table) are notable in that all the program release dates are from 2019 or later.

In the United States, for example, the final act of former Secretary of Energy in the Trump administration Rick Perry as he departed in late 2019 was establishing the Plastics Innovative Initiative, the first major overarching initiative at DOE addressing the problem of plastic waste. Under this rubric, Nichole Fitzgerald, a technology manager at DOE's Bioenergy Technologies Office, has helped establish a consortium of three universities and three national labs, Bio-Optimized Technologies to keep Thermoplastics out of Landfills and the Environment (https://www.bottle.org), whose mission includes "efficient, scalable technologies to deconstruct and up cycle plastic." The group brought together stakeholders from all segments in the supply chain to brainstorm the problem of plastic waste. Companies "have heartburn over the issue of packaging something as transient as food with something that will be in a landfill for hundreds of years," says Fitzgerald.

Funding initiatives for recycling plastic waste

Name of initiative	Funding or sponsoring agency	Amount of funding available	Date announced	Goals
Plastics Innovation Challenge	DOE	$50 million	November 2019	Umbrella agency
Bottle.org	DOE	$2 million	December 2019	Keep plastics out of landfills
BOTTLE Funding Opportunity	DOE	$25 million	March 2020	Create new recyclable plastics and a circular economy for recycling plastic waste
REUSE	ARPA-E	Not specified	August 2019	Create feedstock from plastic waste
REMADE	DOE	$12 million (not all for plastic waste)	November 2019	Reduce embodied energy and decrease emissions
SBIR	DOE Office of Energy Efficiency and Renewable Energy	$1 million for phase 1	December 2019	Develop novel utilization strategies for ocean plastic waste
Horizon 2020	European Union	€100 million ($109 million)	2020	Improve recycling possibilities
Enabling Research in Smart Sustainable Plastic Packaging	UKRI, UK National Environmental Research Council	£8 million ($10 million)	February 2020	Research recycling technologies and other topics relating to plastic packaging
Emerging Frontiers in Research and Innovation	NSF	$30 million	November 2019	Engineer the elimination of end-of-life plastics

ARPA-E, Advanced Research Projects Agency–Energy; NSF, US National Science Foundation; REUSE, Recycle Underutilized Solids to Energy; REMADE, Reducing Embodied Energy and Decreasing Emissions; SBIR, Small Business Innovation Research; UKRI, UK Research & Innovation; DOE, US Department of Energy.

Getting cute with PET

Carbios and its collaborators had a different goal in mind. According to the company's CSO, Alain Marty, they wanted not only to improve the ability of an enzyme to depolymerize PET, but also to recover the two monomers (ethylene glycol and terephthalic acid) and use them to recreate plastic bottles made from PET. Marty says when Carbios CEO Jean-Claude Lumaret tasked him with recycling PET, his initial reaction was that it would be impossible to do economically. And his results for the first few years seemed to prove him right, as they were getting but a few percent conversion after weeks of degradation. But the discovery of the enzyme from *I. sakaiensis* gave them some hope. With collaborators Isabel André and Sophie Duquesne, researchers at the Toulouse Biotechnology Institute, they evolved a cutinase (chosen as a good starting point as its native activity bested several other hydrolases that they considered) that eventually led to a modified LCC that was thermostable at PET's glass-transition temperature (75–78 °C). They did this by using computer-aided targeted mutagenesis to design mutants with changes at 11 sites in the substrate binding groove, did site-specific saturation mutagenesis, tested 209 thus-generated variants on commercially available amorphous PET, and isolated several with activity that was 75% higher. They further enhanced the stability at high temperature of one such variant by introducing one stabilizing disulfide bridge. With these modifications, they could achieve, in only ten hours, a 90% depolymerization of amorphous PET, something others in the field consider remarkable. "Kudos to them. It's really quite impressive," says Jennifer Le Roy, chief technical officer of a California-based startup, BioCellection, a recycling and green materials company.

Although pretreatment was still required to make the polymers accessible — the researchers used a combination of extrusion and micronization, which Marty says is "industrially relevant" — the usual mechano-thermal methods employed for recycling PET leave the monomers degraded to the point that they can only be used to create lower value products, like carpet liners. But using recovered and purified terephthalate monomers, the Carbios team was able to reconstitute a bottle with the mechanical properties of virgin PET, closing the proverbial loop.

Several partnerships with large commercial players will both enable Carbios's team to scale up enzyme production (Novozymes) and assist with the creation of an industrial-scale platform (Pepsico, L'Oréal and Suntory, among others). Carbios sees their process becoming industrialized by 2024, with actual recycled bottles hitting the market in 2025.

Carbios has another iron in the fire: a self-degrading plastic. In collaboration with the group at Toulouse, they created an extremely thermostable enzyme, another feat that Marty originally thought impossible. This enables the enzyme to withstand the extrusion temperature of polylactic acid (170 degrees Celsius), so they can embed the enzyme during manufacture. A joint venture with Limagrain and BpiFrance called Carbiolice is taking this project forward.

Not the end of the story

Although Carbios received accolades for their paper in *Nature*, which was featured on the cover of the journal, a daunting road lies ahead. For one thing, PET is one of the best recycled of the major sources of plastic waste, with 50% of it recycled presently. And technology exists to convert PET into its two monomers and create food-grade plastic containers. However, the challenge is to do it at a price that can compete with virgin PET, according to Le Roy. That's why so few of these technologies have been adopted, she says: "It's not because the technology doesn't exist; it's because they can't compete on price." Le Roy would know. Her company started out working on enzymatic platforms to depolymerize polyethylene film — a more recalcitrant polluter — but pivoted to an ecofriendly chemical method when they realized their process would never be price competitive.

Furthermore, the Carbios method still requires an energy-intensive pretreatment step and, at least as described in their paper, uses petrochemical-based ethylene glycol monomers in the rebuilding phase. "When companies claim that they can fully degrade biodegrade PET bottle, it's not completely true. They have to do some chemical or mechanical pretreatment. It's not fully biological," says Zimmerman. He calls such a process "semi-green."

And that's just PET; there are the other big four plastics. "It would be really exciting to see the same enthusiasm to enzymatically recycling PET to more challenging plastics," says Le Roy.

Gregg Beckham, who is leader of the group at NREL and is also heading up a new US Department of Energy (DOE) program, the Bottle.org consortium (Box 1), is working to fill that gap. He and his collaborators are on the hunt for dozens to hundreds more enzymes, to "offer a bigger buffet," he says. They are using a combination of standard bioinformatics screening techniques to fish out of the billions of extant microbial sequences those that resemble the 25 known activities, followed by machine-learning hidden Markov models to determine those most likely to be thermophilic. "We only fish out the thermophilic ones," he says.

Another challenge is mixed plastic, which includes materials that blend plastics as well as streams of mixed plastic consumer materials that all get tossed together. To get at this problem, researchers are developing methods for biological funneling to separate out usable monomers from a mixture. "You can take mixed plastics and bust them apart into little [bits] and use the selectivity of biology to convert the mixture of stuff to a single molecule that is interesting and hopefully of value. I think that's where biotech has a really cool role to play," says Beckham.

It may be premature to rule chemistry out of the picture, as it might take a combination of chemistry and biology to solve the mixed waste issue. At NREL, for example, they are using a combination of biological funneling with chemical catalysis to convert the waste from lignin (another aromatic polymer) produced when the polymer is used to make biofuels into several intermediates, including adipic acid, a precursor to nylon.

Another interesting possibility is creating consortia of microbes, rather than focusing on individual enzymes. Zimmerman's group at Leipzig had focused on enzymes, but recently started taking a closer look at organisms and the possibility of expanding them into consortia. "That's what happens in nature. It's probably not just one bug doing that but it may have synergistic activities," he says. A new European Union project under the auspices of European Research Area's CoBioTech program called MIPLACE will be working on this possibility.

Ultimately, industrializing the process of enzyme-based recycling of plastic waste means making it price competitive with thermomechanical processes. At least for now, mechanical size reduction as well as decrystallization may still be required to create industrially relevant technologies. Carbios calculated that the price of the enzyme will represent 4% of the price of the virgin PET, but that's only one part of the picture. Beckman sums it up: "Whether all that is scalable and economical is the $64,000 question." ❐

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