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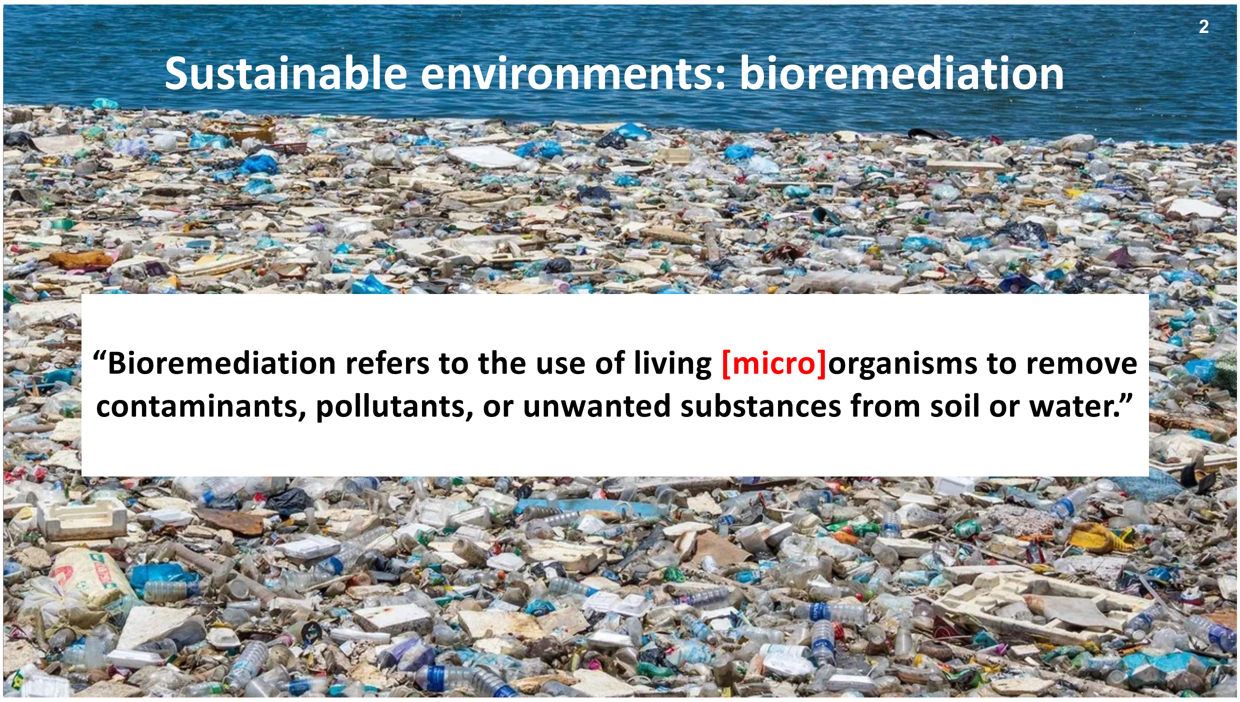
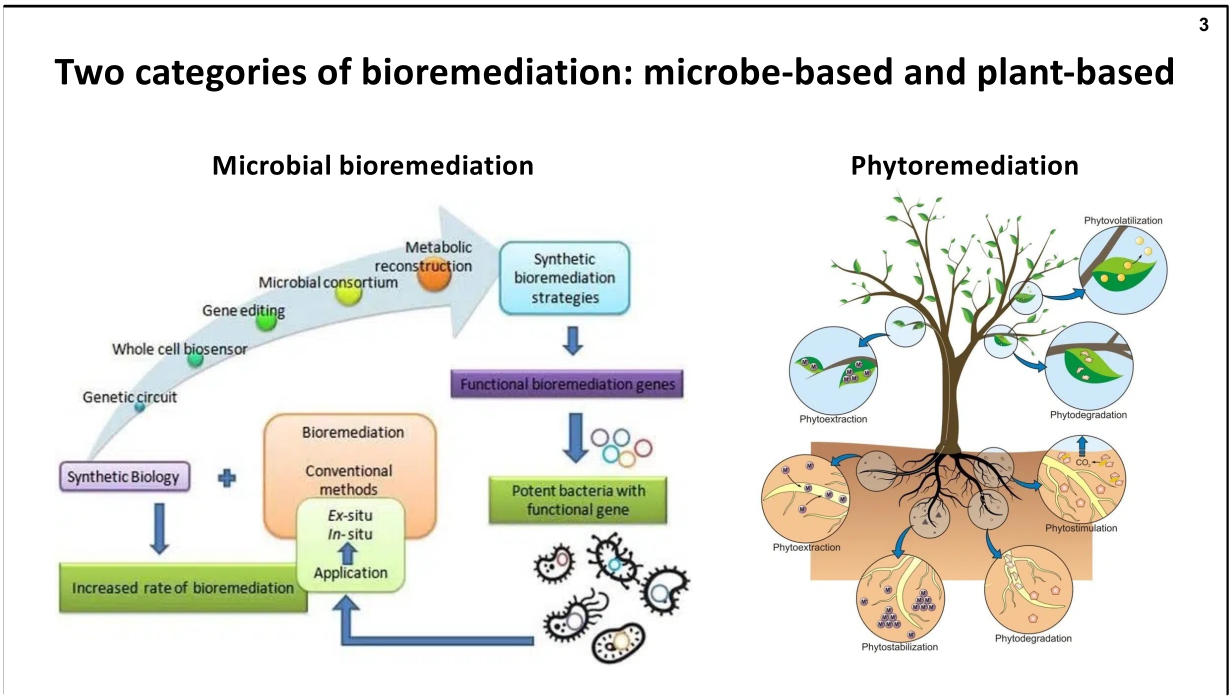
Sustainable environments: bioremediation



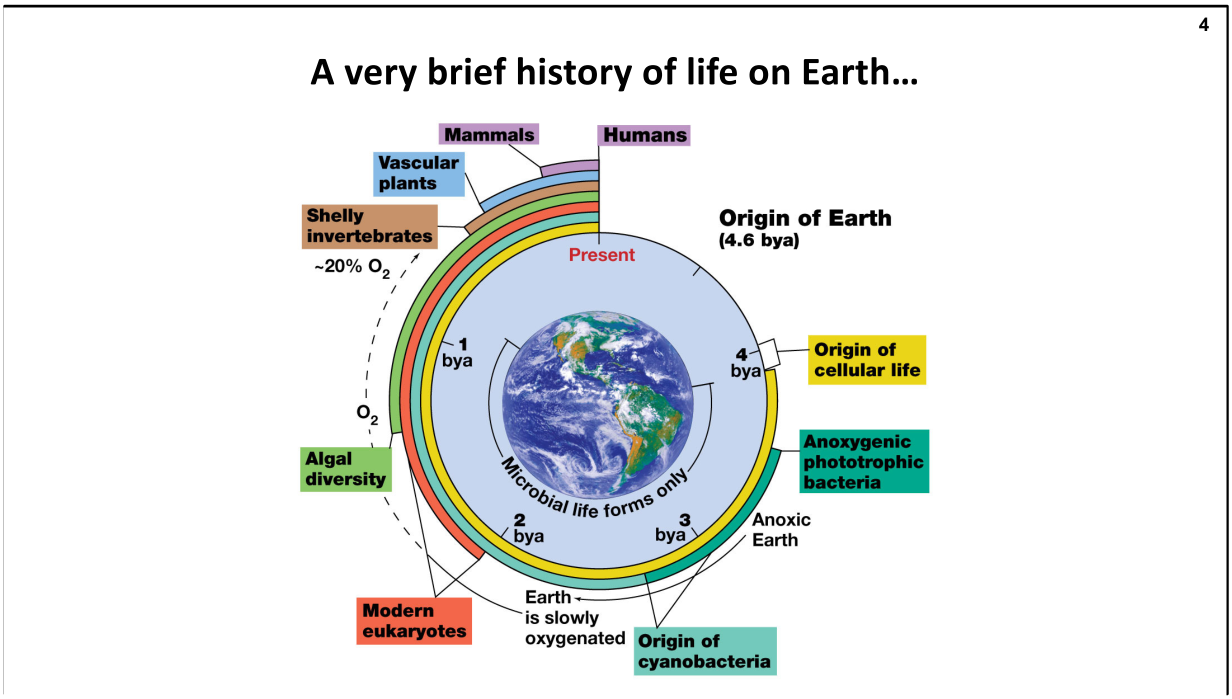
Source: Lidder P, Sonnino A (2012) Biotechnologies for the management of genetic resources for food and agriculture. *Advances in Genetics* 78: 1-167 PMID: 22980921.



Source: https://sigmaearth.com/principles-of-bioremediation/
Source: https://microbenotes.com/bioremediation/

Principles of Bioremediation
Bioremediation can be broadly categorized into two categories: Microbial bioremediation (based on microorganisms) and Phytoremediation (based on plants).

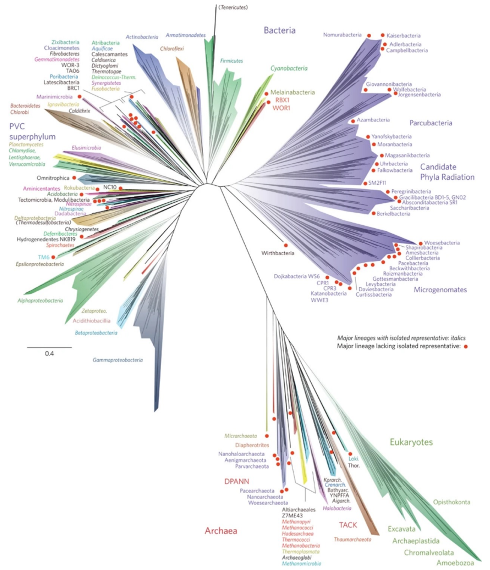
Microbial Bioremediation
1.Natural Attenuation:- It is the in-situ process of bioremediation. It can be applied when proper nutrients, moisture, microbes, and temperature are available naturally. This helps reduce the mass of pollutants significantly in soil or groundwater without any human intervention. This process needs to be monitored until the remediation process occurs entirely or up to an acceptable level.
2.Biostimulation:- It is one of the method remediation processes used in an in-situ environment by stimulating the growth of the native microbes by adding nutrients in the contaminated site (soil or water). This method effectively works for the removal of metals or hydrocarbons.
3.Bioaugmentation:- Another type of in-situ remediation process is when native, non-native, or genetically modified microbes are added to the contaminated site to speed up biodegradation and make the site cleaner. This technique is majorly implemented for municipal wastewater treatment and soil pollutants with chlorinated and aromatic hydrocarbon. Sometimes introducing non-native microbes will compete with native microbes and eliminate them, changing the soil microbiology so that site needs proper management.



Source: *Brock Biology of Microorganisms [15th edition for Kindle]*, Chapter 1: The Microbial World (pp. 37-69), published by Pearson Education Inc., San Francisco © 2019.

Figure 1.5. A summary of life on Earth through time and origin of the cellular domains. At the time of its origin, Earth was sterile. Cellular life was present on Earth by about 3.8 billion years ago (bya). Cyanobacteria began the slow oxygenation of Earth about 3 billion years ago, but current levels of oxygen in the atmosphere were not achieved until about 500-800 million years ago. Eukaryotes arose about 2 billion years ago. Eukaryotes are nucleated cells (by definition) and include both microbial organisms (the overwhelming majority) and multicellular organisms (a tiny minority).

Earth may be home to as many as one trillion (10^{12}) species, and almost all of them are microorganisms



Source: Hug LA et al. (2016) A new view of the tree of life. 1: 16048 PMID: 27572647.

The tree includes 92 named Bacterial phyla, 26 Archaeal phyla, and all 5 of the Eukaryotic supergroups. Major lineages are assigned arbitrary colors and named, with well-characterized lineage names, in italics. Lineages lacking an isolated representative are highlighted with non-italicized names and red dots. Eukaryotic supergroups are noted but not otherwise delineated due to the low resolution of these lineages. The CPR (Candidate Phyla Radiation) phyla are assigned a single color as they are composed entirely of organisms without isolated representatives, and are still in the process of definition at lower taxonomic levels.

Source: Bakalar N (2016) Earth may be home to a trillion species of microbes. *New York Times* Section D, page 4 March 23, 2016.

According to a new estimate, there are about one trillion species of microbes on Earth, and 99.999 percent of them have yet to be discovered. As recently as 1998, the number of microbial species was thought to be a few million at most, little more than the number of insect species. But estimates have been growing ever since. Now Kenneth J. Locey and Jay T. Lennon, biologists at Indiana University, have used two techniques to conclude that the number of microbial species is larger than any previous researchers have imagined (Locey KJ et al. 2016 Scaling laws predict global microbial diversity *Proc Natl Acad Sci USA* 113:5970-5975 PMID: 27140646). The first method extrapolates from the available data for microbes, based not on individual organisms but on samples of DNA. "So if we say a million, we mean a million pieces of DNA that we think belong to different organisms and among them represent different species," Dr. Locey said. The second approach was to use a well-known model of biodiversity as a basis for making predictions. "If you know the number of individuals, you can predict the most abundant species," Dr. Locey said. "So we used those two inputs — the number of individuals over all and the number of individuals belonging to the most abundant species — to estimate the total number of species." The two methods provided numbers that matched: Earth contains 10^11 to 10^12 species of microbes. "We think our approach was rigorous in that we used a theoretical prediction and a statistical estimate," he said. "We ended up with intersecting predictions based on different methods." Finding the number of species has broader implications, Dr. Locey said. "How many species could have actually evolved in four billion years? What are the upper constraints of evolution on Earth? How many species have evolved, how many could have evolved? "As far as I know, no one has approached those questions. We're very far away from discovering what's really out there."

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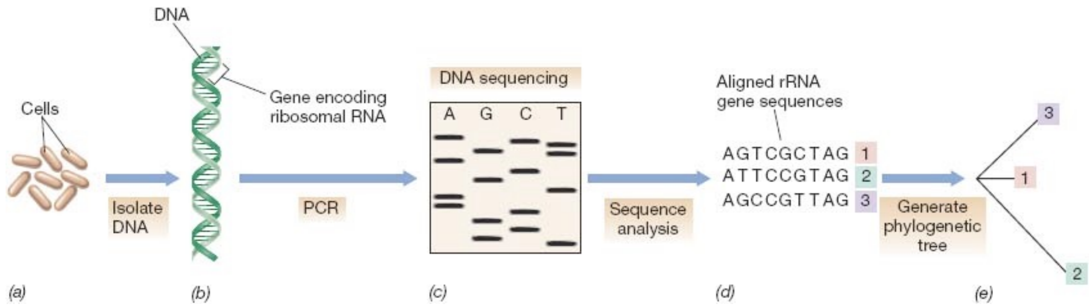
Modern phylogenetic trees are based on rRNA (ribosomal RNA) sequences (Karl Woese, 1977)



>Objective >Rigorous >Unambiguous >Quantitative

Source: Madigan MT, Martinko JM, Stahl DA, Clark DP (2012) *Brock Biology of Microorganisms [13th edition]*. Pearson Education Inc., San Francisco.

Figure 2.16. Ribosomal RNA (rRNA) gene sequencing and phylogeny. (a) Cells are broken open. (b) The gene encoding rRNA is isolated, and many identical copies are made by the technique called the polymerase chain reaction. (c, d) The gene is sequenced, and the sequence obtained is aligned with other rRNA gene sequences. A computer algorithm makes pairwise comparisons and generates a phylogenetic tree (e) that depicts the differences in rRNA sequence between the organisms analyzed. In the example shown, the sequence differences are as follows: organism 1 versus organism 2, three differences; 1 versus 3, two differences; 2 versus 3, four differences. Thus organisms 1 and 3 are closer relatives than are 2 and 3 or 1 and 2.

Carl Richard Woese (born July 15, 1928; died December 30, 2012) was an American microbiologist and biophysicist. Woese is famous for defining the Archaea (a new domain or kingdom of life) in 1977 by phylogenetic taxonomy of 16S ribosomal RNA, a technique pioneered by Woese that revolutionized the discipline of microbiology. He was also the originator of the RNA world hypothesis in 1977, although not by that name.

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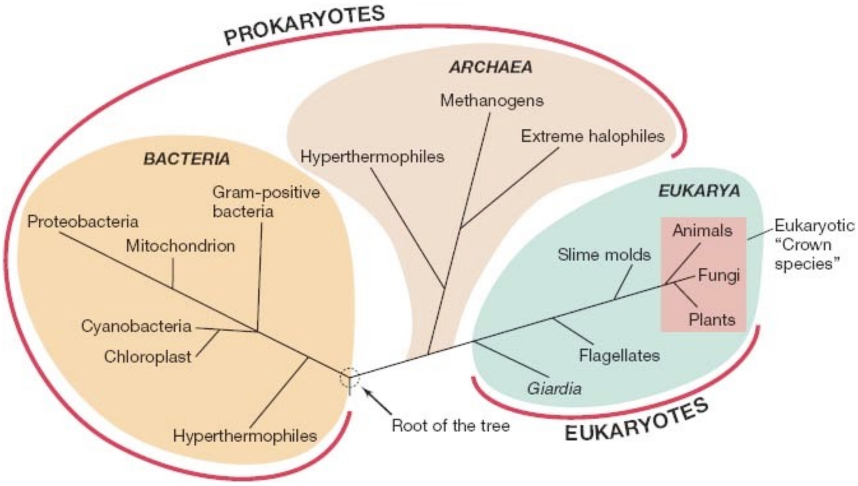
Phylogenetic "tree of life" based on rRNA: microbes account for almost all of the diversity of life on Earth

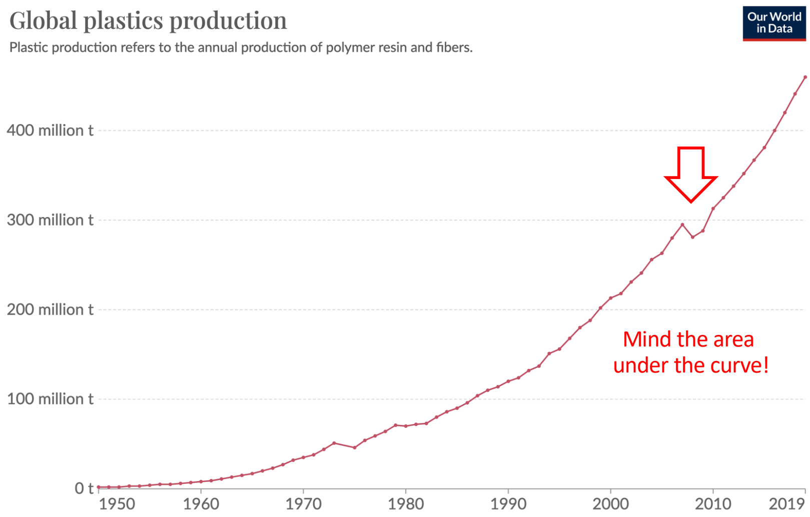


Three domains of life: Bacteria, Archaea, Eukarya

Source: Madigan MT, Martinko JM, Stahl DA, Clark DP (2012) *Brock Biology of Microorganisms [13th edition]*. Pearson Education Inc., San Francisco.

Figure 2.17. The phylogenetic tree of life as defined by comparative ribosomal RNA gene sequencing. The tree shows the three major domains of organisms (*Bacteria*, *Archaea*, *Eukarya*) and a few representative groups in each domain. All *Bacteria* and *Archaea* and most *Eukarya* are microscopic organisms. Only plants, animals, and fungi contain macro-organisms. Phylogenetic tree of each domain can be found in Figures 2.19, 2.28, and 2.32. LUCA, last universal common ancestor.

Global plastic production since 1950: up, up, and away…



Source: https://ourworldindata.org/grapher/global-plastics-production
Source: https://ourworldindata.org/plastic-pollution

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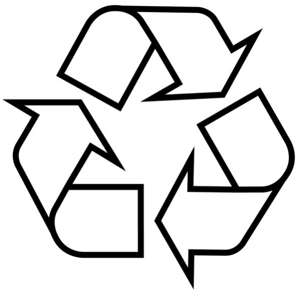
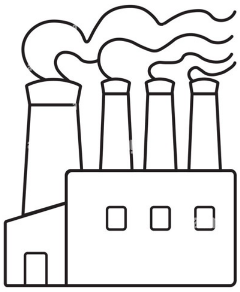
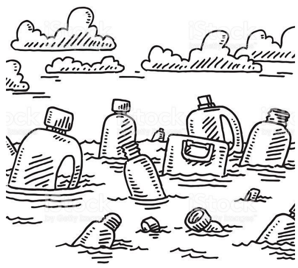
What happened to all that plastic? Where did it go?



9%
recycled



12%
incinerated



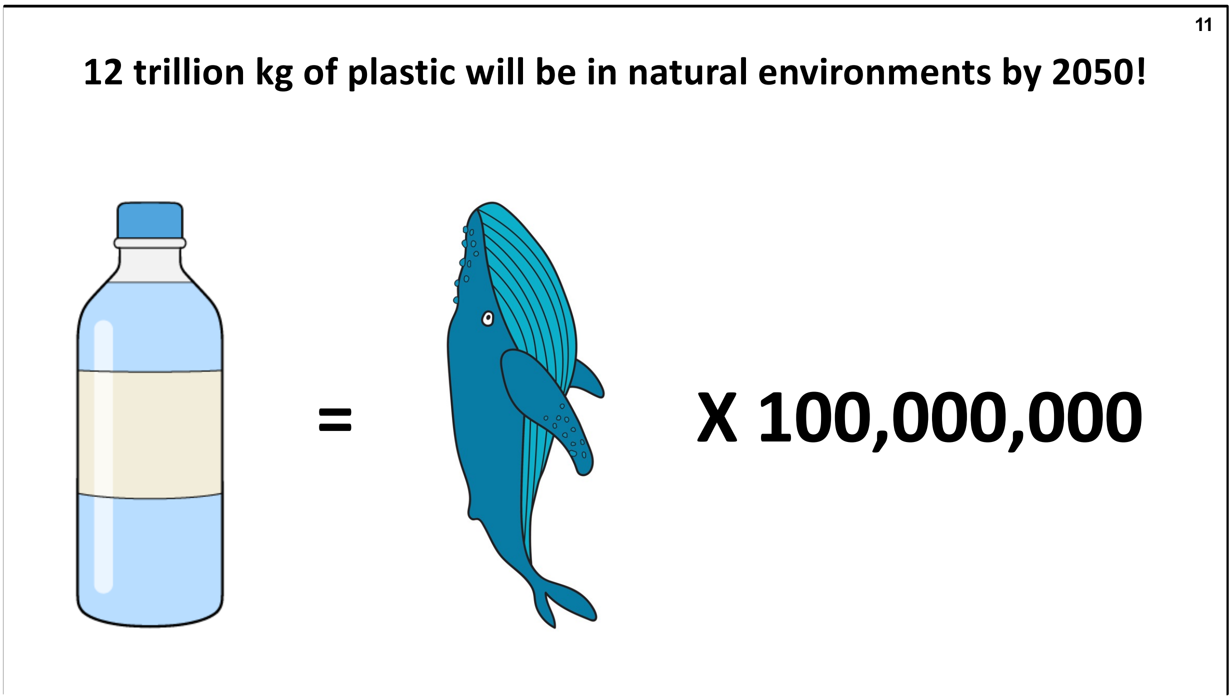
79%
environmental

Source: https://www.greenpeace.org/usa/the-ocean-plastic-crisis/
Source: Geyer R, Jambeck JR, Law KR (2017) Production, use, and fate of all plastics ever made. *Science Advances* 3(7): e1700782 PMID: 28776036.

Only about 9% of plastic has been recycled, 12% has been incinerated (thereby polluting the air with toxic gases), and the remaining 79% remains in the environment. If current production and waste management trends continue, by 2050 there will be 12 billion tons of plastic in natural environments. That’s the weight of 100 million blue whales, equal to 5,000 times the actual mass of the blue whale population left on Earth.

Source: https://www.asbmb.org/asbmb-today/science/091023/the-living-things-that-feast-on-plastic
Source: https://www.condorferries.co.uk/plastic-in-the-ocean-statistics
Source: https://www.iucn.org/resources/issues-brief/marine-plastic-pollution

Ever since plastics manufacturing began in earnest in the 1950s, production has soared. Estimates suggest that we make close to 460 million tons of plastic annually, equivalent to the weight of roughly 2.3 million blue whales.



Source: https://www.greenpeace.org/usa/the-ocean-plastic-crisis/
Source: Geyer R, Jambeck JR, Law KR (2017) Production, use, and fate of all plastics ever made. *Science Advances* 3(7): e1700782 PMID: 28776036.

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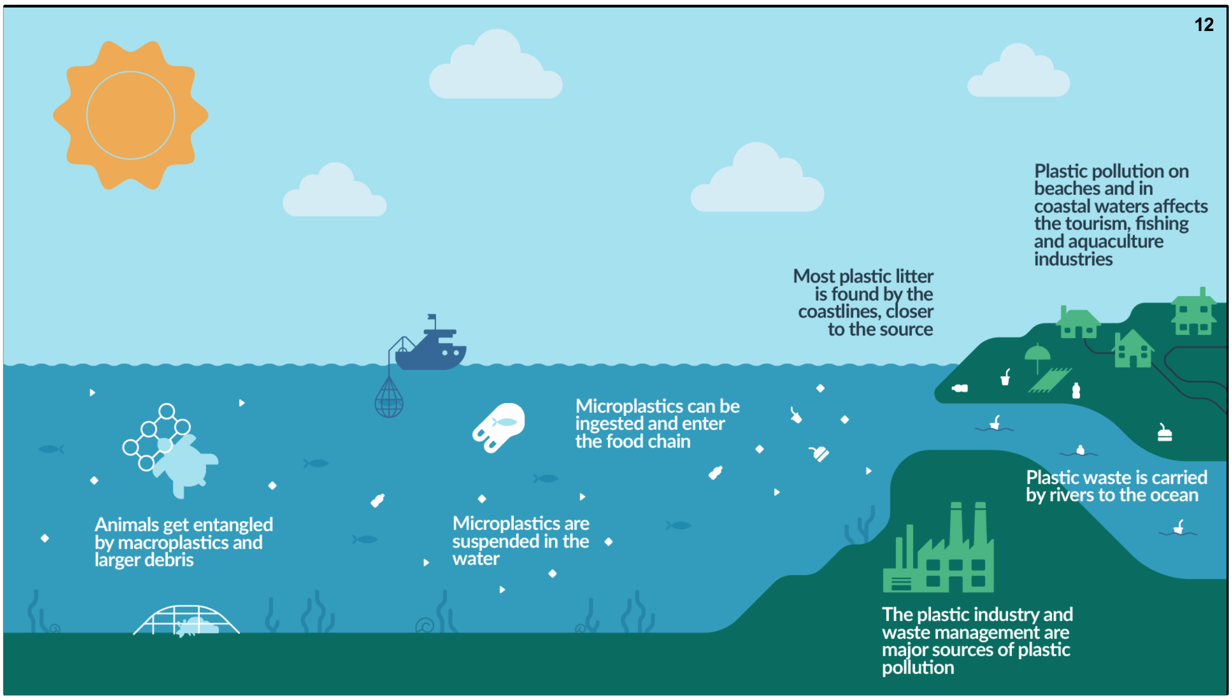


Source: https://marine.copernicus.eu/explainers/phenomena-threats/plastic-pollution

Where plastic goes when it “remains in the environment”

Albatross

Sea Turtle

Accumulation of plastic in the oceans is driven by 5 regional "gyres"

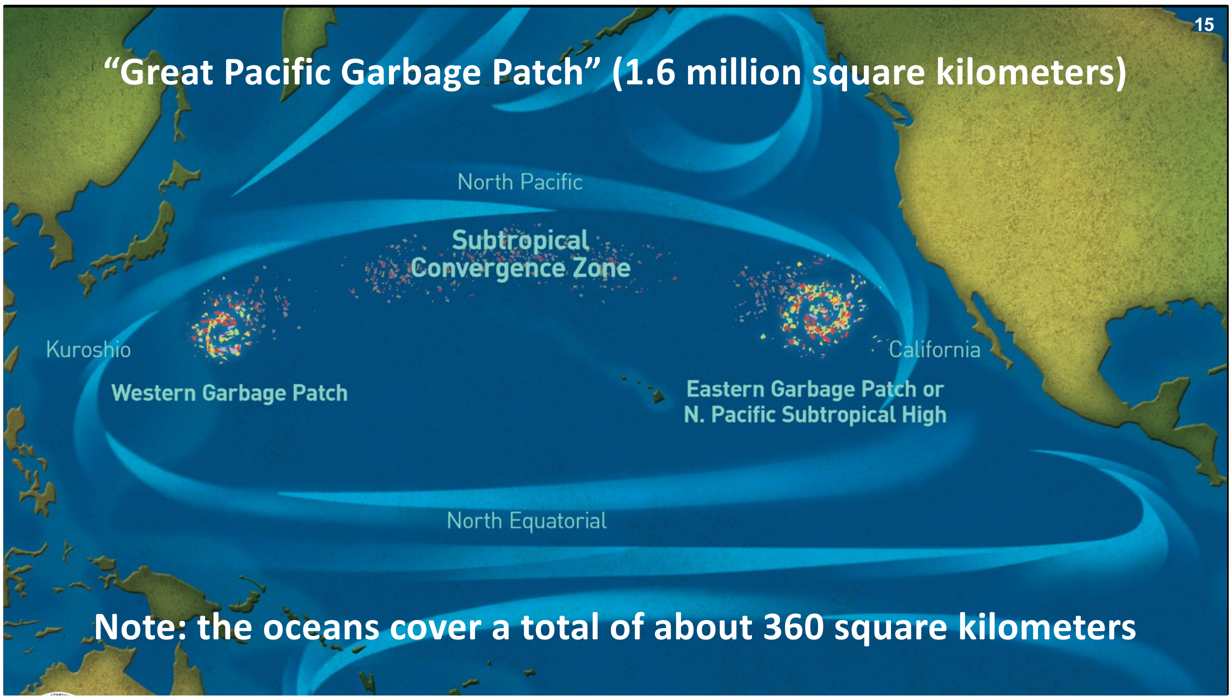
Source: https://education.nationalgeographic.org/resource/great-pacific-garbage-patch/
Source: https://www.visualcapitalist.com/cp/visualized-ocean-plastic-waste-pollution-by-country/
Source: https://theoceancleanup.com/great-pacific-garbage-patch/

Great Pacific Garbage Patch

Definiiton of "gyre": a circular or spiral motion or form; especially, a giant circular oceanic surface current. The Great Pacific Garbage Patch (gyre #1 in the diagram) is the largest of the five offshore plastic accumulation zones in the world's oceans. The Great Pacific Garbage Patch is a collection of marine debris in the North Pacific Ocean. Also known as the Pacific trash vortex, the garbage patch is actually two distinct collections of debris bounded by the massive North Pacific Subtropical Gyre. It covers an estimated surface area of 1.6 million square kilometers, an area three times the size of France.

Plastic Accumulation

It is estimated that 1.15 to 2.41 million tonnes of plastic are entering the ocean each year from rivers. More than half of this plastic is less dense than the water, meaning that it will not sink once it encounters the sea. The stronger, more buoyant plastics show resiliency in the marine environment, allowing them to be transported over extended distances. They persist at the sea surface as they make their way offshore, transported by converging currents and finally accumulating in the patch. Once these plastics enter the gyre, they are unlikely to leave the area until they degrade into smaller microplastics under the effects of sun, waves, and marine life. As more and more plastics are discarded into the environment, microplastic concentration in the Great Pacific Garbage Patch will only continue to increase.



Source: https://education.nationalgeographic.org/resource/great-pacific-garbage-patch/
Source: https://www.visualcapitalist.com/cp/visualized-ocean-plastic-waste-pollution-by-country/
Source: https://theoceancleanup.com/great-pacific-garbage-patch/

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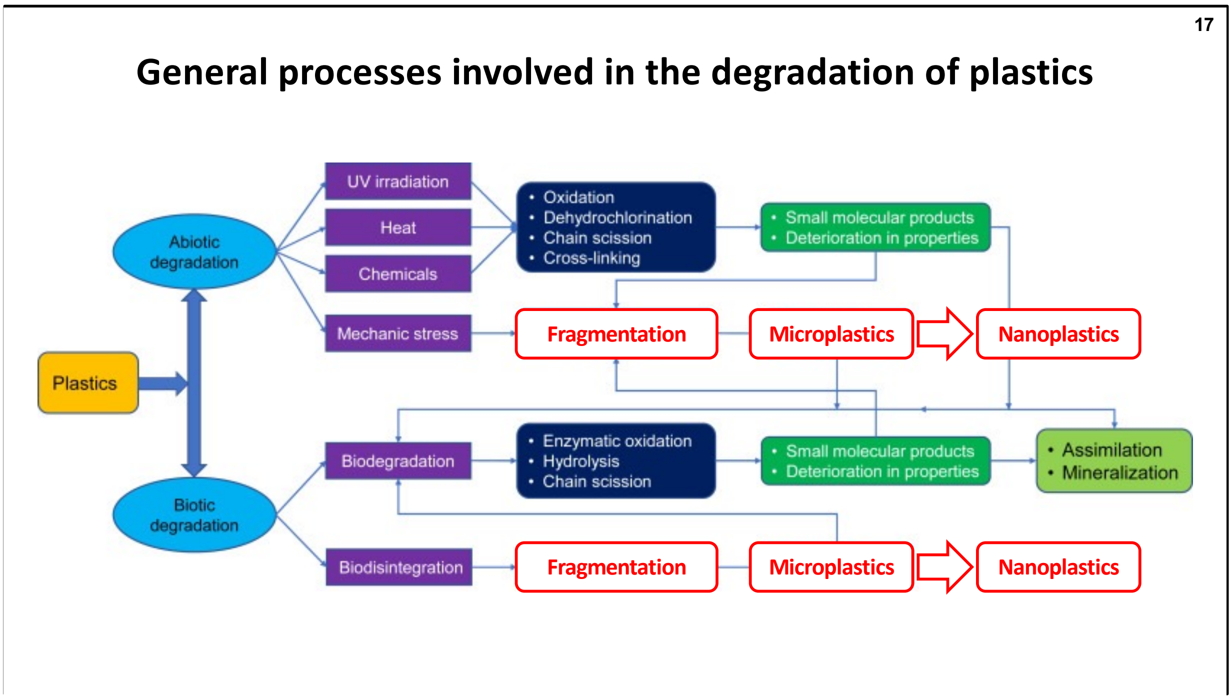
Microplastics: the smaller they get, the bigger the problem gets…

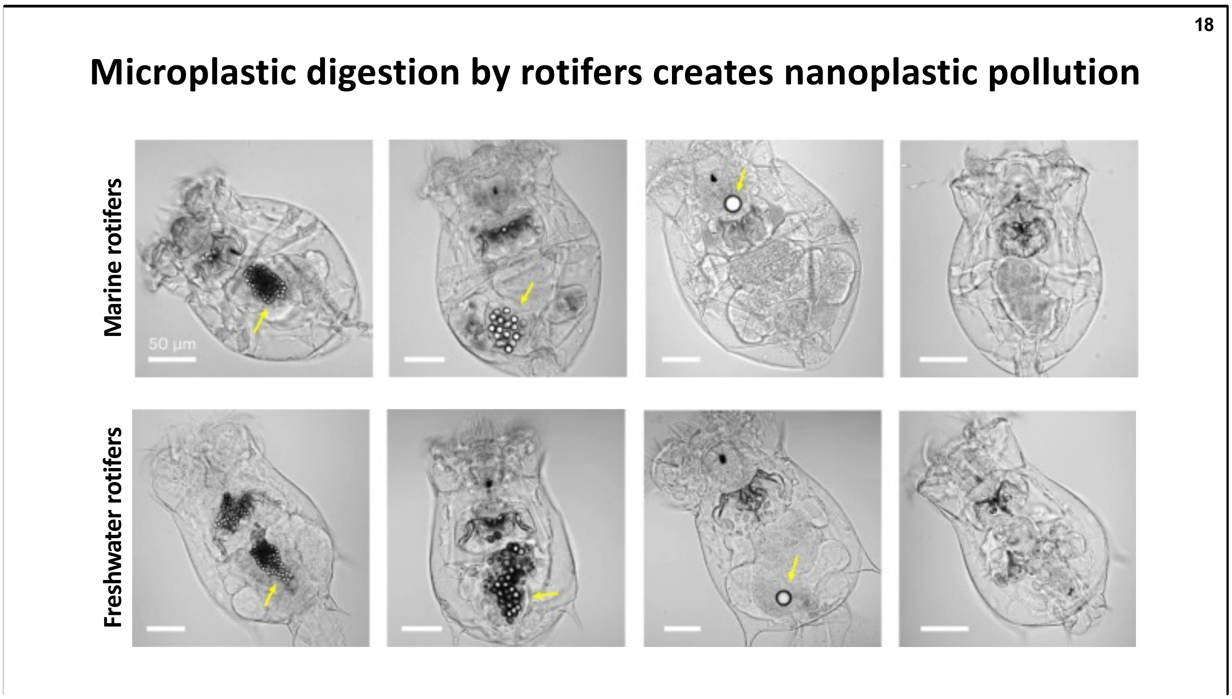
Source: https://researchoutreach.org/blog/omnipresent-microplastic/
Source: https://www.theseacleaners.org/news/microplastics-in-human-blood-and-lungs-the-urgent-need-for-scientific-research/

Microplastics have the ability to accumulate and concentrate toxic substances from the surrounding environment, such as persistent organic pollutants and heavy metals. As marine organisms consume microplastics, these pollutants can be transferred and magnified through the food chain.



Source: Zhang K, Hamidian AH, Tubić A, Zhang Y, Fang JKH, Wu C, Lam PKS (2021) Understanding plastic degradation and microplastic formation in the environment: A review. *Environ Pollut* 274: 116554. PMID: 33529891.

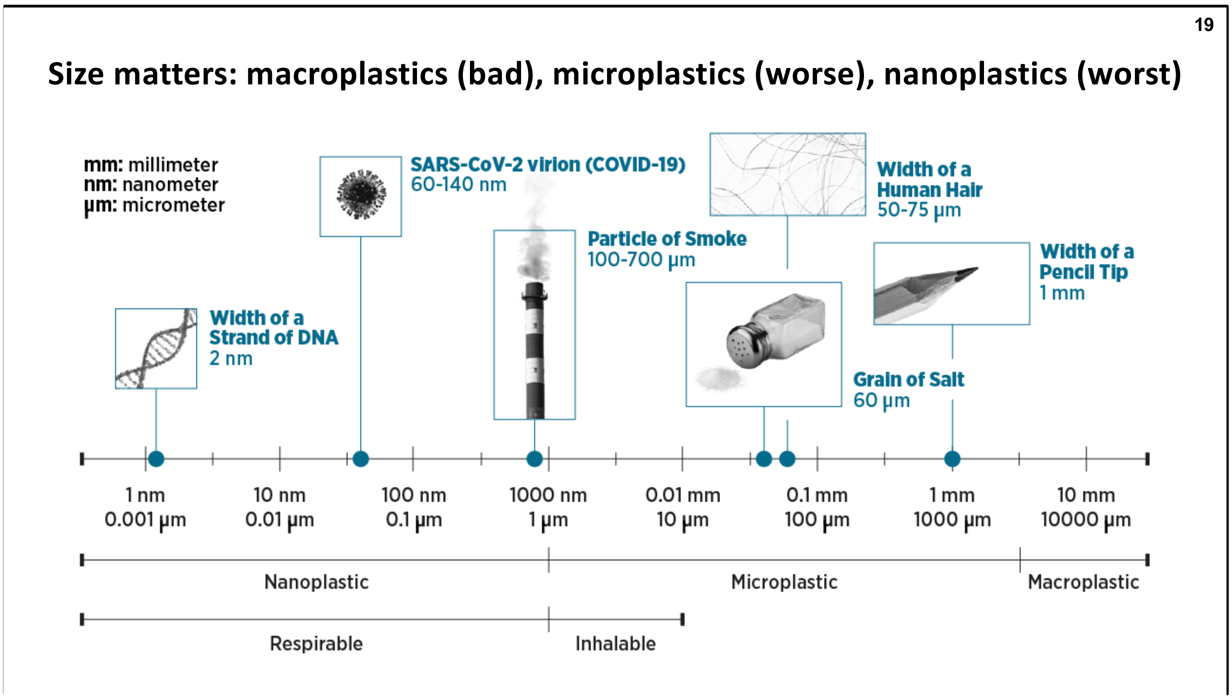
Figure 1. A schematic diagram showing the general processes involved in the degradation of plastics.



Source: Zhao J, Lan R, Wang Z, Su W, Song D, Xue R, Liu Z, Liu X, Dai Y, Yue T, Xing B (2023) Microplastic fragmentation by rotifers in aquatic ecosystems contributes to global nanoplastic pollution. *Nature Nanotechnology* PMID: 37945989.
Source: https://phys.org/news/2023-11-zooplankton-ocean-freshwater-rapidly-escalating.html

Figure 1: Ingestion of polystyrene microplastics of different sizes by marine and freshwater rotifers as imaged and counted by optical microscopy. Uptake of microplastics by marine rotifers (top row) and freshwater rotifers (bottom row). The sizes of the exposed polystyrene microplastics were 5, 10, 20, or 30 µm (left to right). The yellow arrows point to the ingested polystyrene microplastics.

During the global plastic-carbon cycle, microplastics (1 µm–5 mm) and nanoplastics (<1 µm) are the last two stages of plastic debris before degradation to molecules. Microplastics have attracted much concern, mainly due to their risks to ecosystems and human health. Nanoplastics, distinguished from microplastics with respect to their small size and notably elevated surface reactivity, mobility, environmental exposure and toxicity, have not received the same attention. The abundance of nanoplastics is much higher than that of microplastics because one microplastic particle could be theoretically fragmented into 10^{14} nanoplastic particles after sufficient environmental exposure. This fragmentation process commonly requires hundreds of years, with the assistance of physical wear, chemical oxidation, biofouling heat and sunlight irradiation. However, calculation and prediction of nanoplastic abundance would be modified if there is another fragmentation pathway that rapidly generates them worldwide. The role of aquatic organisms in the biological fragmentation of microplastics and their contribution to global nanoplastic pollution are poorly understood. Here we present a biological fragmentation pathway that generates nanoplastics during the ingestion of microplastics by rotifers, a commonly found and globally distributed surface water zooplankton relevant for nutrient recycling. Both marine and freshwater rotifers could rapidly grind polystyrene, polyethylene and photo-aged microplastics, thus releasing smaller particulates during ingestion.

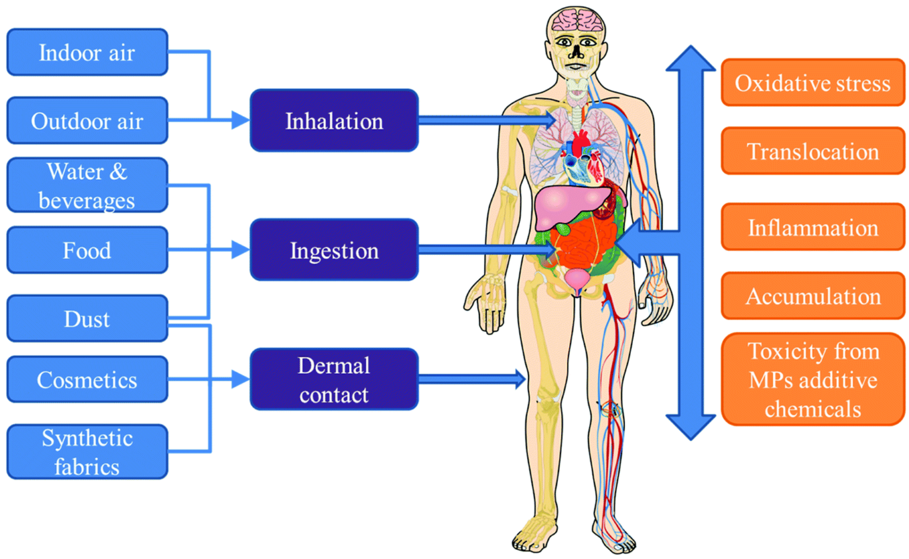


Source: https://www.ciel.org/breathing-plastic-the-health-impacts-of-invisible-plastics-in-the-air/

Microplastics are plastic particles less than 5 millimeters (mm) in diameter, about the size of an orange seed. Airborne microplastics, however, are much smaller; even a 500 µm particle is considered large. When plastic fragments are below 1 µm, they are less than 1/100th of the thickness of a human hair. Particles this small are called nanoplastics and cannot be seen by the naked eye.

Fundamentally, atmospheric microplastics come from human creation and use of plastics. Microplastics fall into categories based on their origin. Primary microplastics are intentionally produced at microscale for a specific use (such as agrochemicals or pharmaceuticals). In contrast, secondary microplastics result from the mechanical, chemical, and physical fragmentation of larger (macro) plastics, which can include “legacy” plastics disposed of in the environment decades ago. Every stage of the plastics life cycle, from the extraction of feedstocks to production, transport, use, disposal, and remediation, emits both primary and secondary microplastics and other hazardous substances.

Routes of entry of micro- and nano-plastics into the human body



Source: https://pubs.rsc.org/en/content/articlehtml/2022/em/d1em00301a

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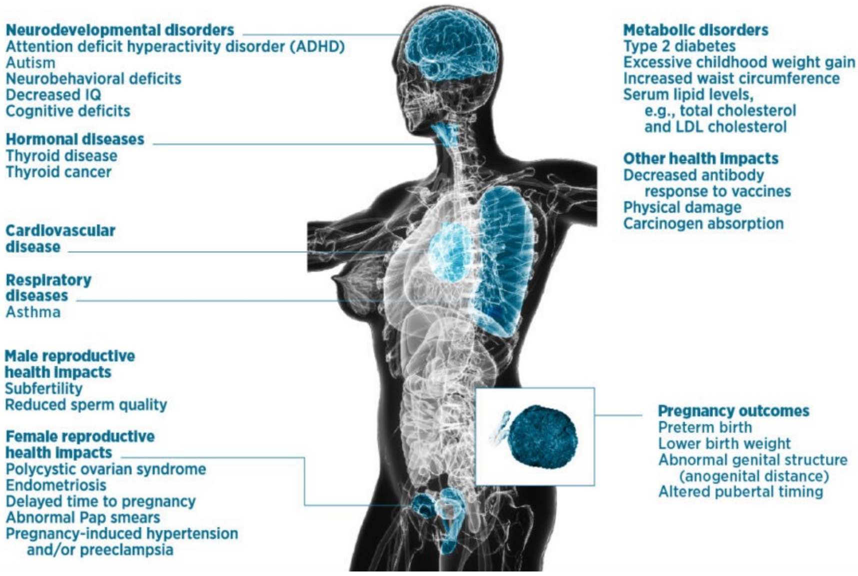
Impact of microplastics and nanoplastics on human health



Source: https://www.ciel.org/breathing-plastic-the-health-impacts-of-invisible-plastics-in-the-air/
Source: Campanale C, Massarelli C, Savino I, Locaputo V, Uricchio VF (2020) A detailed review study on potential effects of microplastics and additives of concern on human health. *Int J Environ Res Public Health* 17(4): 1212 PMID: 32069998.
Source: https://www.grida.no/resources/15023
Source: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7068600/

Potential solutions to plastic waste

1. Stop making and using plastic!
   - Oops! Too late. There's already a lot of plastic "out there"… ☹
   - Anyway, fuhgettaboutit! Because the stuff is just too useful… ☹

2. Recycle all plastic waste!
   - Oops! Impossible… ☹

3. Switch to biodegradable plastics!
   - Good idea! But there's already a lot of plastic "out there"… 😐

4. Engineer enzymes to break down plastic waste *ex situ*!
   - Good idea! But there's already a lot of plastic "out there"… 😐

5. Engineer microbes to break down plastic waste *in situ*!
   - Good idea! Hey, that just might work! ☺

Solution 1: Stop making, using, and discarding plastic stuff!

Oops! Too late! There’s already a lot of plastic “out there”… ☹
Anyway, fuhgettaboutit! Because plastic is just too useful… ☹

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Solution 2: "Recycle all plastic waste!"

Oops! Sadly, that won't work… ☹

Source: Enck J, Dell J (2022) Plastic recycling doesn't work and will never work. *The Atlantic (*30 May 2022).

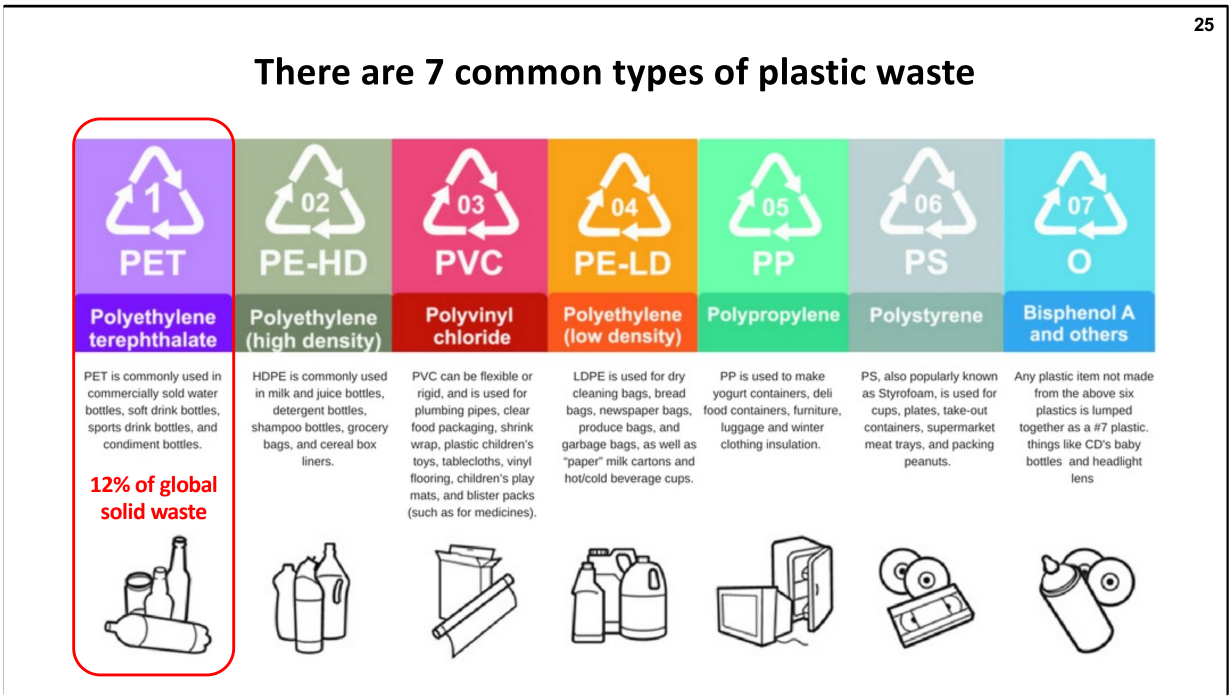
Although some materials can be effectively recycled and safely made from recycled content, plastics cannot. Plastic recycling does not work and will never work. The United States in 2021 had a dismal recycling rate of about 5 percent for post-consumer plastic waste, down from a high of 9.5 percent in 2014, when the U.S. exported millions of tons of plastic waste to China and counted it as recycled, even though much of it wasn't. Recycling in general can be an effective way to reclaim natural material resources. The U.S.'s high recycling rate of paper (68 percent) proves this point. The problem with recycling plastic lies not with the concept or process but with the material itself.

1. There are thousands of different plastics, each with its own composition and characteristics. They all include different chemical additives and colorants that cannot be recycled together, making it impossible to sort the trillions of pieces of plastics into separate types for processing.

2. Unlike metal and glass, plastics are not inert. Plastic products often include toxic additives and readily absorb chemicals. They are generally collected in curbside bins filled with possibly dangerous materials such as plastic pesticide containers. According to a report published by the Canadian government, toxicity risks in recycled plastic prohibit "the vast majority of plastic products and packaging produced" from being recycled into food-grade packaging.

3. Another problem is that plastic recycling is simply not economical. Recycled plastic costs more than new plastic because collecting, sorting, transporting, and reprocessing plastic waste is exorbitantly expensive. The petrochemical industry is rapidly expanding, which will further lower the cost of new plastic.

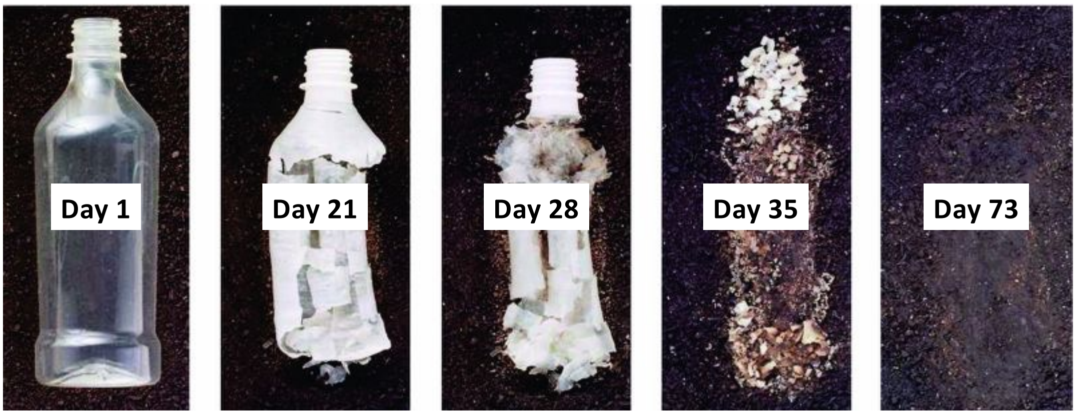
4. Recycling plastic is more energy-intensive (and thus produces more CO_2) than making plastic *de novo* from petrochemicals.



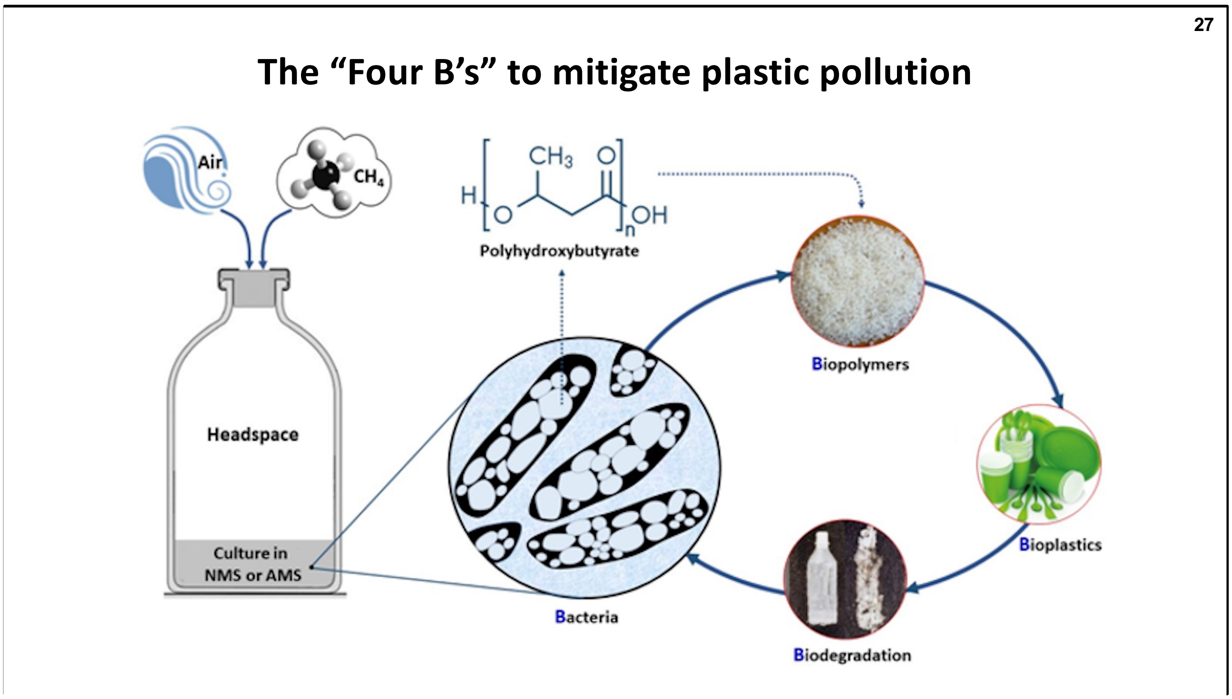
Source: https://plasticoceans.org/7-types-of-plastic/
Source: https://www.chemistryworld.com/news/plastic-eating-bacteria-show-way-to-recycle-plastic-bottles-sustainably/9556.article

Polyethylene terephthalate (PET) is the most common thermoplastic polymer resin of the polyester family and is used in fibres for clothing, containers for liquids and foods, thermoforming for manufacturing, and in combination with glass fiber for engineering resins.

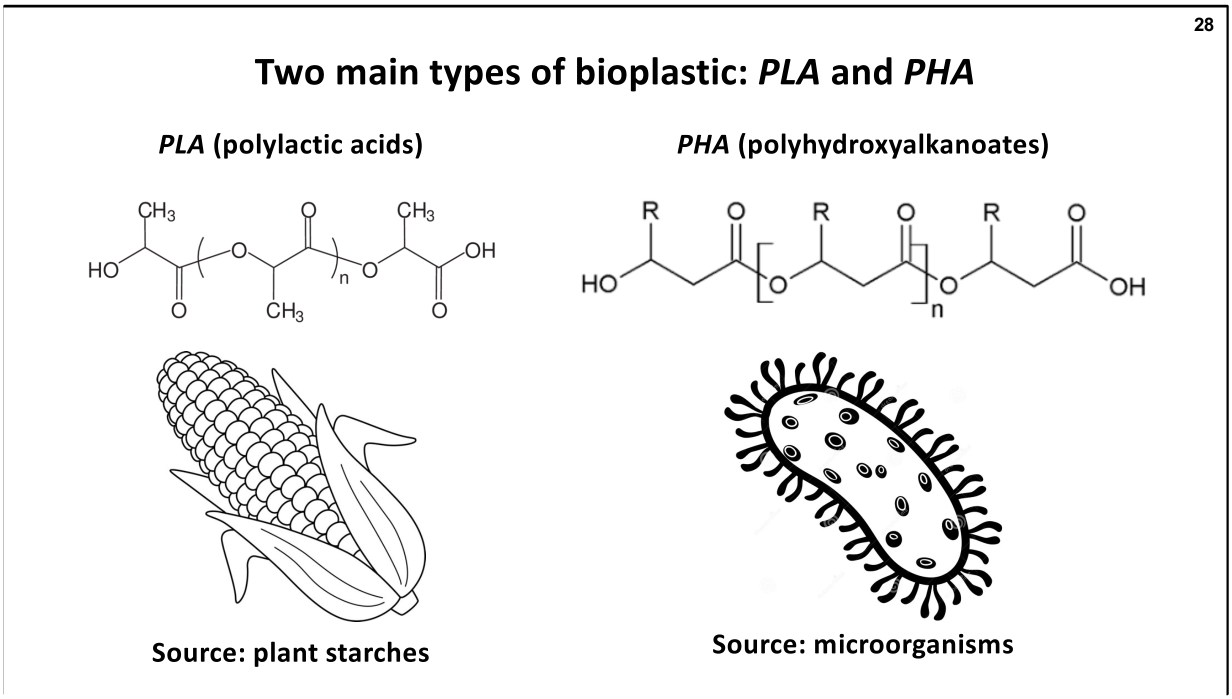
Solution 3: "Switch to biodegradable plastics!"



Good idea! But there's already a lot of plastic "out there"… 😐



Source: Sharma HK, Sauvageau D, Stein LY (2022) Optimization of methane feed and N:C ratio for biomass and polyhydroxybutyrate production by the alphaproteobacterial methanotroph *Methylocystis* sp. Rockwell. *Methane* 1(4): 355-364 doi:10.3390/methane1040026.



Source: https://news.climate.columbia.edu/2017/12/13/the-truth-about-bioplastics/

There are two main types of bioplastics:

PLA (polylactic acid) is typically made from the sugars in corn starch, cassava or sugarcane. It is biodegradable, carbon-neutral and edible. To transform corn into plastic, corn kernels are immersed in sulfur dioxide and hot water, where its components break down into starch, protein, and fiber. The kernels are then ground and the corn oil is separated from the starch. The starch is comprised of long chains of carbon molecules, similar to the carbon chains in plastic from fossil fuels. Some citric acids are mixed in to form a long-chain polymer (a large molecule consisting of repeating smaller units) that is the building block for plastic. PLA can look and behave like polyethylene (used in plastic films, packing and bottles), polystyrene (Styrofoam and plastic cutlery) or polypropylene (packaging, auto parts, textiles). Minnesota-based NatureWorks is one of the largest companies producing PLA under the brand name Ingeo.
See: https://bioplasticsnews.com/2019/07/02/all-you-need-to-know-about-pla/

PHA (polyhydroxyalkanoate) is made by microorganisms, sometimes genetically engineered, that produce plastic from organic materials. The microbes are deprived of nutrients like nitrogen, oxygen and phosphorus, but given high levels of carbon. They produce PHA as carbon reserves, which they store in granules until they have more of the other nutrients they need to grow and reproduce. Companies can then harvest the microbe-made PHA, which has a chemical structure similar to that of traditional plastics. Because it is biodegradable and will not harm living tissue, PHA is often used for medical applications such as sutures, slings, bone plates, and skin substitutes; it is also used for single-use food packaging.
See: https://bioplasticsnews.com/polyhydroxyalkanoates-or-pha/

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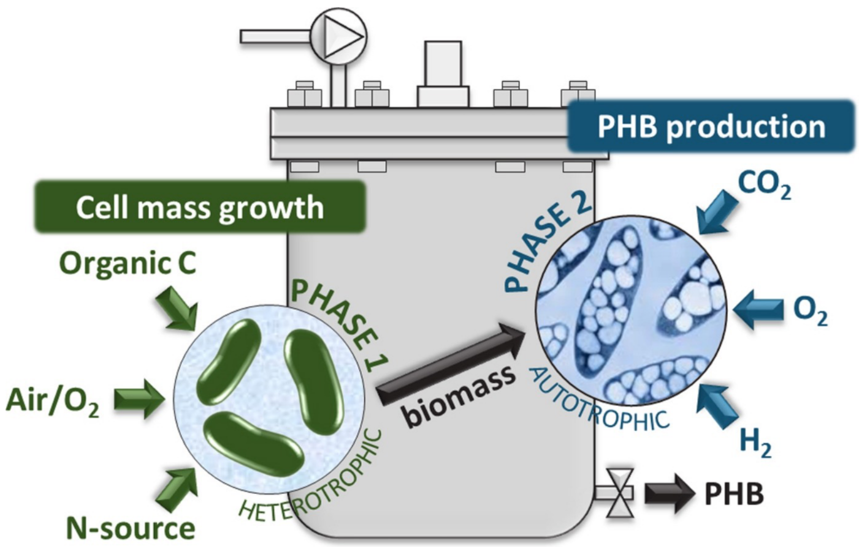
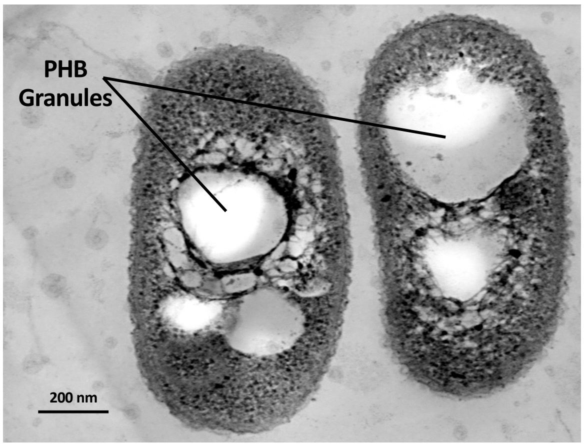
Industrial PHB production by bacteria (*Cupriavidus necator*)



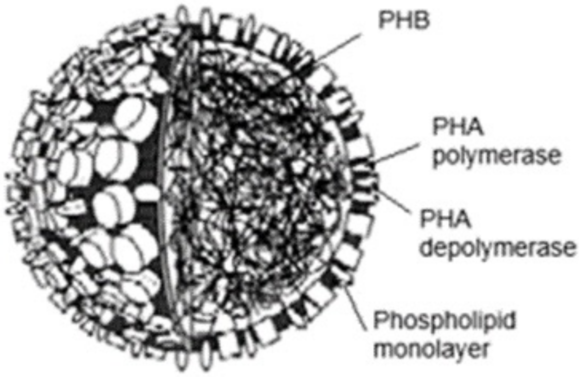
Source: Garcia-Gonzalez L, Mozumder MSI, Dubreuil M, Volcke EIP, De Wever H (2015) Sustainable autotrophic production of polyhydroxybutyrate (PHB) from CO_2 using a two-stage cultivation system. *Catalysis Today* 257(2): 237-245 doi: 10.1016/j.cattod.2014.05.025.

Cupriavidus necator is a hydrogen-oxidizing ("knallgas" bacterium) capable of growing at the interface of anaerobic and aerobic environments. It can easily adapt between heterotrophic and autotrophic lifestyles. Both organic compounds and hydrogen can be used as a source of energy. *C. necator* can perform aerobic or anaerobic respiration by denitrification of nitrate and/or nitrite to nitrogen gas. When growing under autotrophic conditions, *C. necator* fixes carbon through the reductive pentose phosphate pathway. It is known to produce and sequester polyhydroxyalkanoate (PHA) plastics, such as polyhydroxybutyrate (PHB), when exposed to excess amounts of sugar substrate. PHA can accumulate to levels around 90% of the cell's dry weight.

Carbon storage in PHB granules in *Cupriavidus necator*

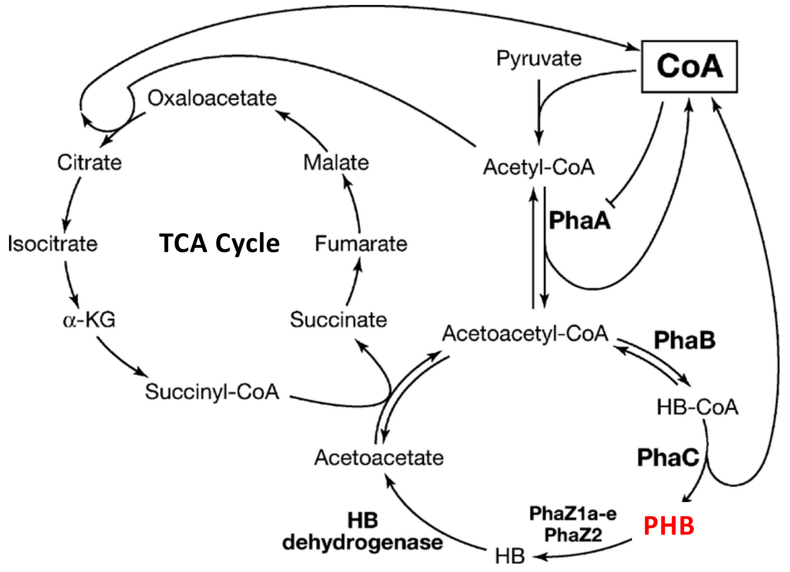


Structure of PHB granule



Source: https://www.intechopen.com/chapters/39415
Source: Muhammadi S, Afzal M, Hameed S (2015) Bacterial polyhydroxyalkanoates – eco-friendly next generation plastic: production, biocompatibility, biodegradation, physical properties and applications. *Green Chemistry Letters and Reviews* 8: 3-4, 56-77, doi: 10.1080/17518253.2015.1109715.

Connection between central metabolism and PHB production

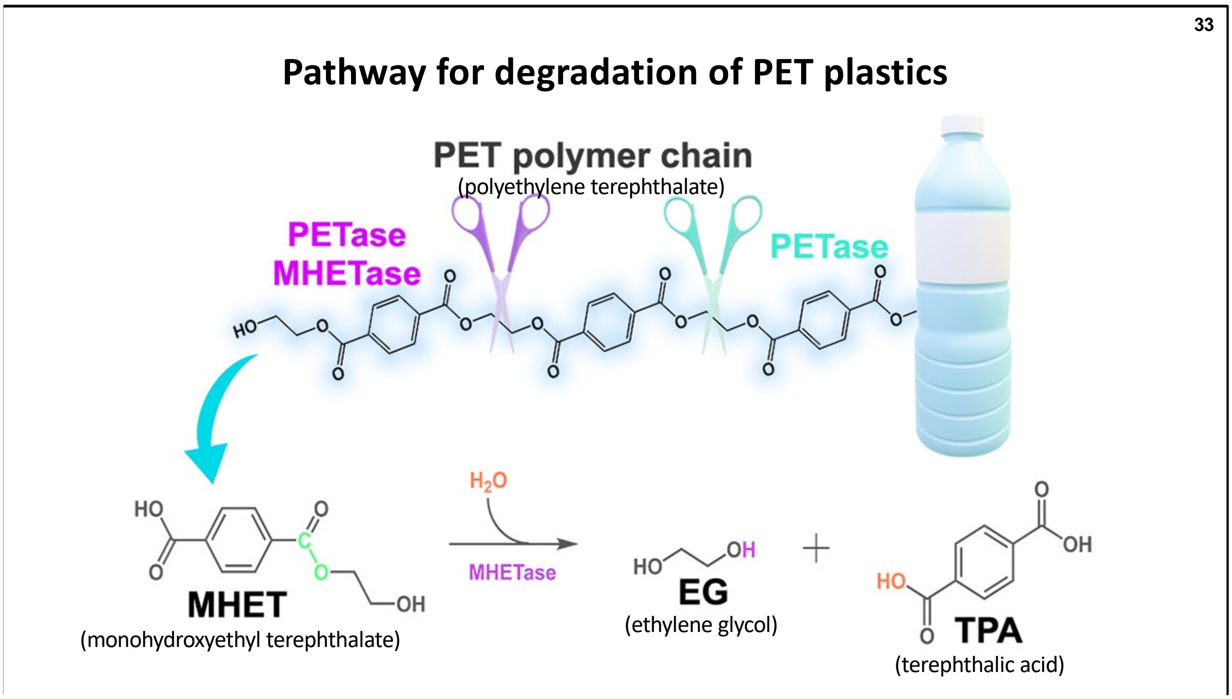


Source: Suriyamongkol P, Weselake R, Narine S, Moloney M, Shah S (2006) Biotechnological approaches for the production of polyhydroxyalkanoates in microorganisms and plants - a review. *Biotechnology Advances* 25(2): 148-175 PMID: 17222526.

Source: https://education.nationalgeographic.org/resource/great-pacific-garbage-patch/
Source: https://www.visualcapitalist.com/cp/visualized-ocean-plastic-waste-pollution-by-country/
Source: https://theoceancleanup.com/great-pacific-garbage-patch/

Great Pacific Garbage Patch
Definiiton of "gyre": a circular or spiral motion or form; especially, a giant circular oceanic surface current. The Great Pacific Garbage Patch (gyre #1 in the diagram) is the largest of the five offshore plastic accumulation zones in the world's oceans. The Great Pacific Garbage Patch is a collection of marine debris in the North Pacific Ocean. Also known as the Pacific trash vortex, the garbage patch is actually two distinct collections of debris bounded by the massive North Pacific Subtropical Gyre. It covers an estimated surface area of 1.6 million square kilometers, an area three times the size of France.

Plastic Accumulation
It is estimated that 1.15 to 2.41 million tonnes of plastic are entering the ocean each year from rivers. More than half of this plastic is less dense than the water, meaning that it will not sink once it encounters the sea. The stronger, more buoyant plastics show resiliency in the marine environment, allowing them to be transported over extended distances. They persist at the sea surface as they make their way offshore, transported by converging currents and finally accumulating in the patch. Once these plastics enter the gyre, they are unlikely to leave the area until they degrade into smaller microplastics under the effects of sun, waves, and marine life. As more and more plastics are discarded into the environment, microplastic concentration in the Great Pacific Garbage Patch will only continue to increase.



Source: Pinto AV, Ferreira P, Neves RPP, Fernandes PA, Ramos MJ**,** Alexandre L. Magalhães AL (2021) Reaction mechanism of MHETase, a PET degrading enzyme. *ACS Catalysis* 11, 16: 10416–10428 doi: 10.1021/acscatal.1c02444.
Source: https://www.acs.org/pressroom/presspacs/2023/november/plastic-eating-bacteria-turn-waste-into-useful-starting-materials-for-other-products.html.

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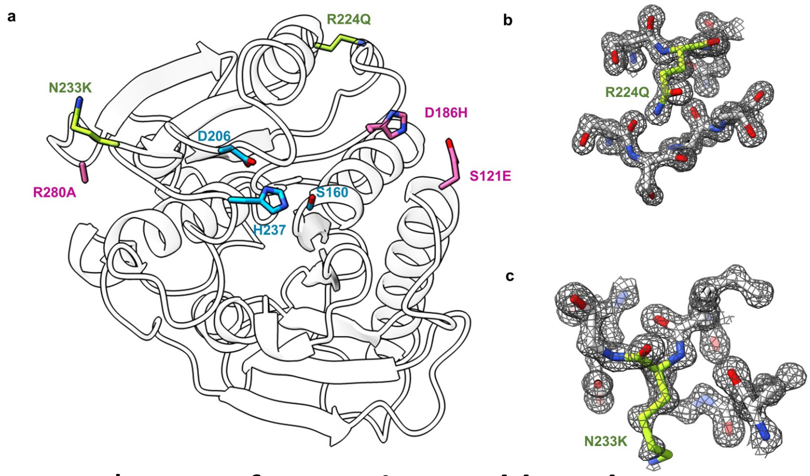
Machine learning-aided engineering of “FAST-PETase”*



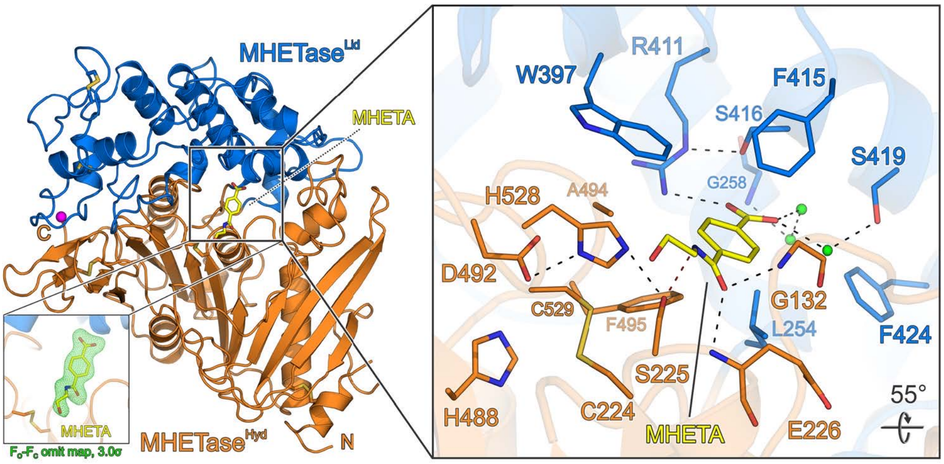
***FAST = fast, active, stable, tolerant**

Source: Lu H, Diaz DJ, Czarnecki NJ, Zhu C, Kim W, Shroff R, Acosta DJ, Alexander BR, Cole HO, Zhang Y, Lynd NA, Ellington AD, Alper HS (2022) Machine learning-aided engineering of hydrolases for PET depolymerization. *Nature* 604(7907): 662-667 PMID: 35478237.
Source: https://earth.org/plastic-eating-enzyme/

Extended Data Figure 3. X-ray crystal structure of FAST-PETase. (A) Overall crystal structure of FAST-PETase. Catalytic triads (S160, D206, H237) are shown in blue sticks. Mutations originating from or shared with ThermoPETase (S121E, D186H, R280A) are shown in pink sticks, and completely novel mutations predicted by MutCompute are shown in green-yellow sticks. (B,C) 2Fo-Fc map (contoured at 1.5 σ) shown as grey mesh superimposed on the stick models of novel mutation sites (B) R224Q, (C) N233K. The source of wild-type PETase was *Ideonella sakaiensis*.

Abstract: Plastic waste poses an ecological challenge and enzymatic degradation offers one, potentially green and scalable, route for polyesters waste recycling. Polyethylene terephthalate (PET) accounts for 12% of global solid waste, and a circular carbon economy for PET is theoretically attainable through rapid enzymatic depolymerization followed by repolymerization or conversion/valorization into other products. Application of PET hydrolases, however, has been hampered by their lack of robustness to pH and temperature ranges, slow reaction rates and inability to directly use untreated postconsumer plastics. Here, we use a structure-based, machine learning algorithm to engineer a robust and active PET hydrolase. Our mutant and scaffold combination (FAST-PETase: functional, active, stable and tolerant PETase) contains five mutations compared to wild-type PETase (N233K/R224Q/S121E from prediction and D186H/R280A from scaffold) and shows superior PET-hydrolytic activity relative to both wild-type and engineered alternatives between 30 and 50 °C and a range of pH levels. We demonstrate that untreated, postconsumer-PET from 51 different thermoformed products can all be almost completely degraded by FAST-PETase in 1 week. FAST-PETase can also depolymerize untreated, amorphous portions of a commercial water bottle and an entire thermally pretreated water bottle at 50 ºC. Finally, we demonstrate a closed-loop PET recycling process by using FAST-PETase and resynthesizing PET from the recovered monomers. Collectively, our results demonstrate a viable route for enzymatic plastic recycling at the industrial scale.

Structure-guided variants of MHETase with enhanced activity



Source: Palm GJ, Reisky L, Böttcher D, Müller H, Michels EAP, Walczak MC, Berndt L, Weiss MS, Bornscheuer UT, Weber G (2019) Structure of the plastic-degrading *Ideonella sakaiensis* MHETase bound to a substrate. *Nature Communications* 10(1): 1717 PMID: 30979881.
Source: Qi X, Yan W, Cao Z, Ding M, Yuan Y (2021) Current advances in the biodegradation and bioconversion of polyethylene terephthalate. *Microorganisms* 10(1): 39 PMID: 35056486.

Figure 1. Structure of *Ideonella sakaiensis* MHETase bound to a nonhydrolyzable MHET analog. The structure explains substrate specificity and reveals an induced-fit substrate-binding mode. (A) Co-structure of MHETase bound to MHETA (yellow), α/β-hydrolase domain ($MHETase^{Hyd}$) in orange, lid domain ($MHETase^{Lid}$) in marine blue. Inset, bottom left—refined F_O–F_C-omit electron density map (green) contoured at 3σ for MHETA. MHETA of the refined final structure is shown as sticks. (B) Close-up view on MHETA (yellow) bound to the active site of MHETase. The source of wild-type MHETase was Ideonella sakaiensis.

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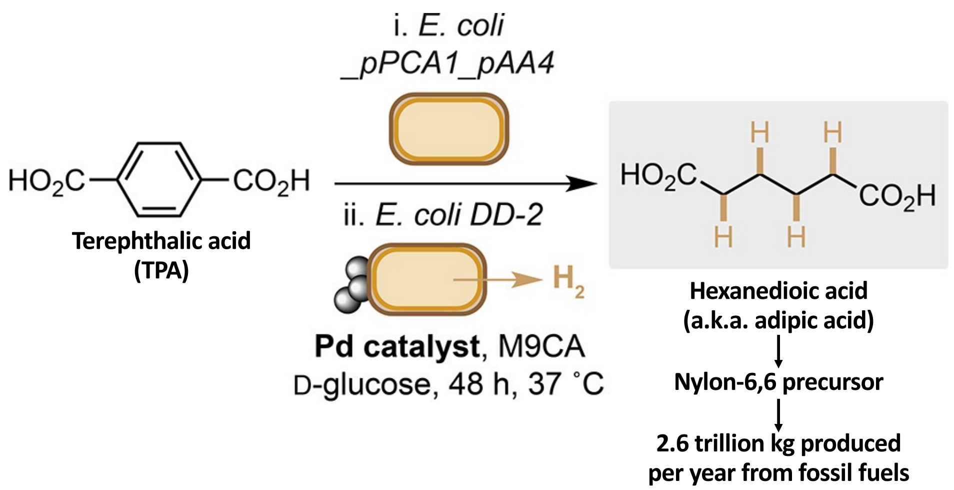
Bioconversion of TPA (from PET waste) to adipic acid in a "one-pot process" using genetically engineered bacteria



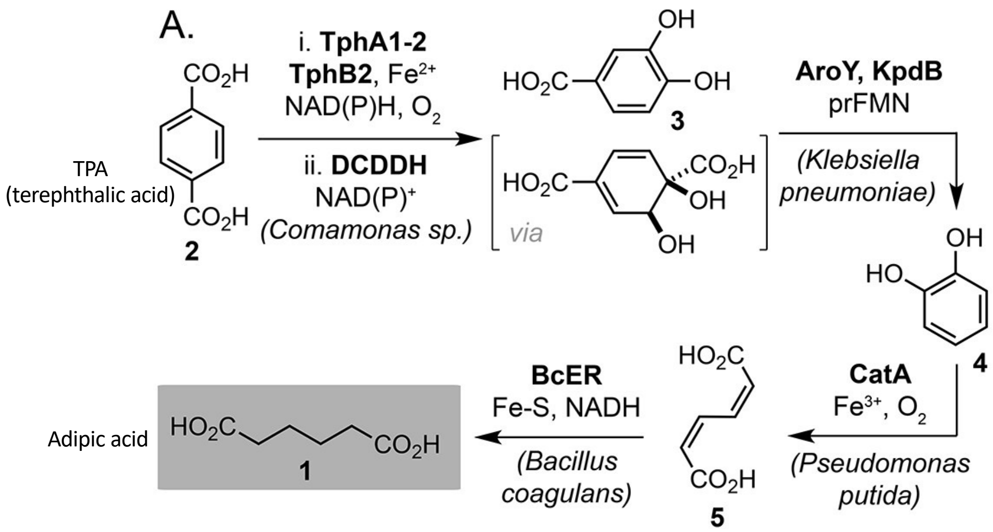
Source: Marcos Valenzuela-Ortega M, Suitor JT, White MFM, Hinchcliffe T, Wallace S (2023) Microbial upcycling of waste PET to adipic acid. ACS Central Science doi: 10.1021/acscentsci.3c00414.

Abstract: Microorganisms can be genetically engineered to transform abundant waste feedstocks into value-added small molecules that would otherwise be manufactured from diminishing fossil resources. Herein, we report the first one-pot bio-upcycling of PET plastic waste into the prolific platform petrochemical and nylon precursor adipic acid in the bacterium *Escherichia coli*. Optimizing heterologous gene expression and enzyme activity enabled increased flux through the *de novo* pathway, and immobilization of whole cells in alginate hydrogels increased the stability of the rate-limiting enoate reductase BcER. The pathway enzymes were also interfaced with hydrogen gas generated by engineered *E. coli* DD-2 in combination with a biocompatible Pd catalyst to enable adipic acid synthesis from metabolic *cis*,*cis*-muconic acid. Together, these optimizations resulted in a one-pot conversion to adipic acid from terephthalic acid, including terephthalate samples isolated from industrial PET waste and a post-consumer plastic bottle.

Source: https://www.acs.org/pressroom/presspacs/2023/november/plastic-eating-bacteria-turn-waste-into-useful-starting-materials-for-other-products.html.

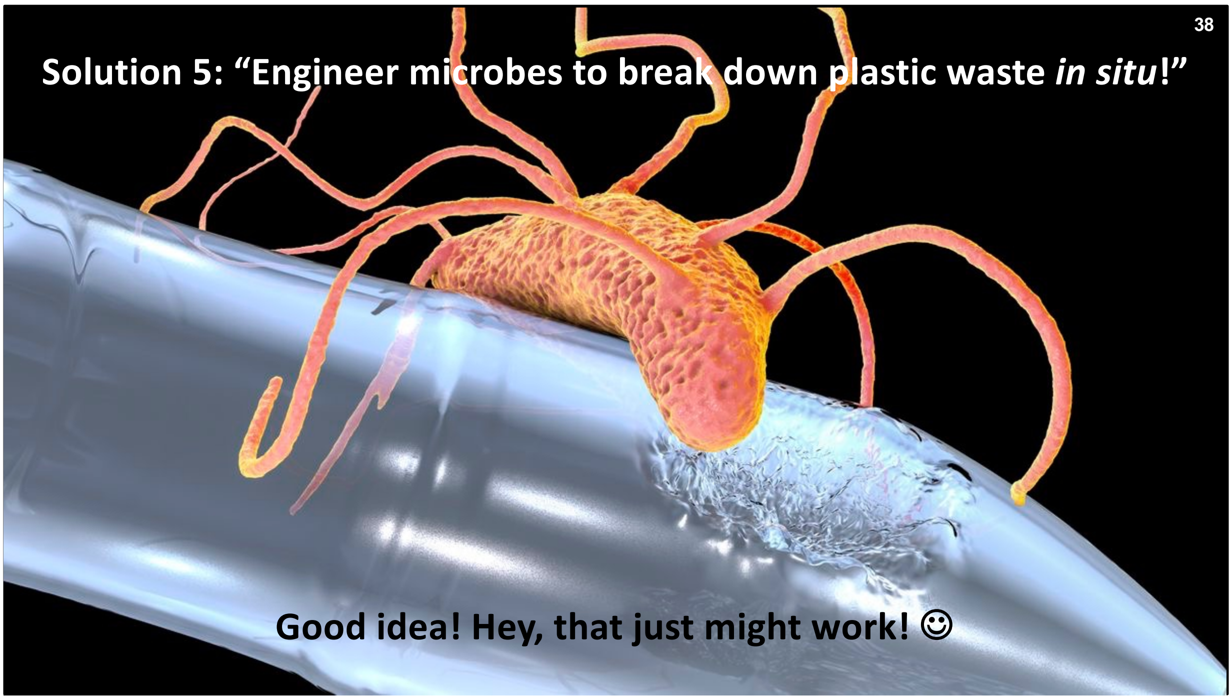
The researchers sought to expand *E. coli*'s biosynthetic pathways to include the metabolism of terephthalic acid into adipic acid, a feedstock for many everyday products that's typically generated from fossil fuels using energy-intensive processes. The team developed a new *E. coli* strain that produced enzymes that could transform terephthalic acid into compounds such as muconic acid and adipic acid. Then, to transform the muconic acid into adipic acid, they used a second type of *E. coli*, which produced hydrogen gas, and a palladium catalyst. In experiments, the team found that attaching the engineered microbial cells to alginate hydrogel beads improved their efficiency, and up to 79% of the terephthalic acid was converted into adipic acid. Using real-world samples of terephthalic acid from a discarded bottle and a coating taken from waste packaging labels, the engineered *E. coli* system efficiently produced adipic acid. In the future, the researchers say they will look for pathways to biosynthesize additional higher-value products.

Synthetic pathway for conversion of TPA to adipic acid

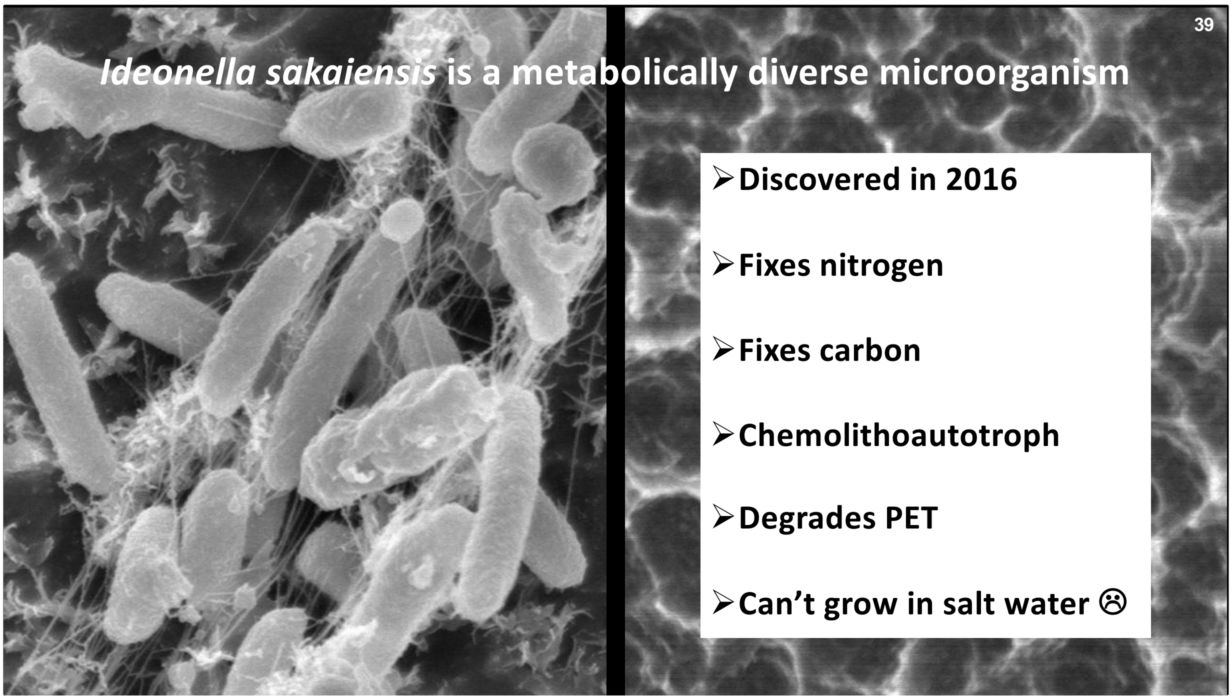


Source: Marcos Valenzuela-Ortega M, Suitor JT, White MFM, Hinchcliffe T, Wallace S (2023) Microbial upcycling of waste PET to adipic acid. ACS Central Science doi: 10.1021/acscentsci.3c00414.

Figure 2. Initial pathway construction and whole-cell activity. (A) The *de novo* biosynthesis pathway to adipic acid from TPA (terephthalic acid).

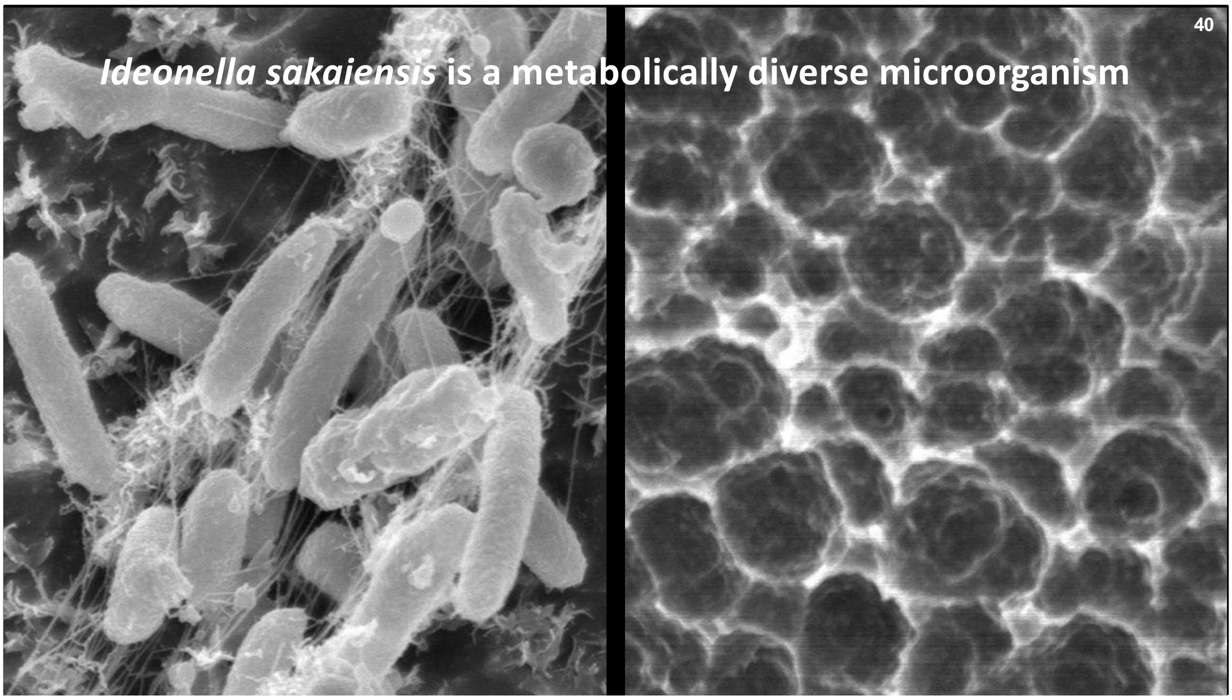


Source: https://www.livescience.com/plastic-eating-bacteria



Source: Yoshida S, Hiraga K, Takehana T, Taniguchi I, Yamaji H, Maeda Y, Toyohara K, Miyamoto K, Kimura Y, Oda K (2016) A bacterium that degrades and assimilates poly(ethylene terephthalate). *Science* 351(6278): 1196-1199 PMID: 26965627.
Source: https://www.wsj.com/articles/new-species-of-bacteria-eats-plastic-1457636401

Ideonella sakaiensis is a Gram-negative, aerobic, non-spore forming, rod-shaped bacterium. First identified in 2016 by a team of researchers led by Kohei Oda of Kyoto Institute of Technology and Kenji Miyamoto of Keio University after collecting a sample of PET-contaminated sediment at a plastic bottle recycling facility in Sakai, Japan.



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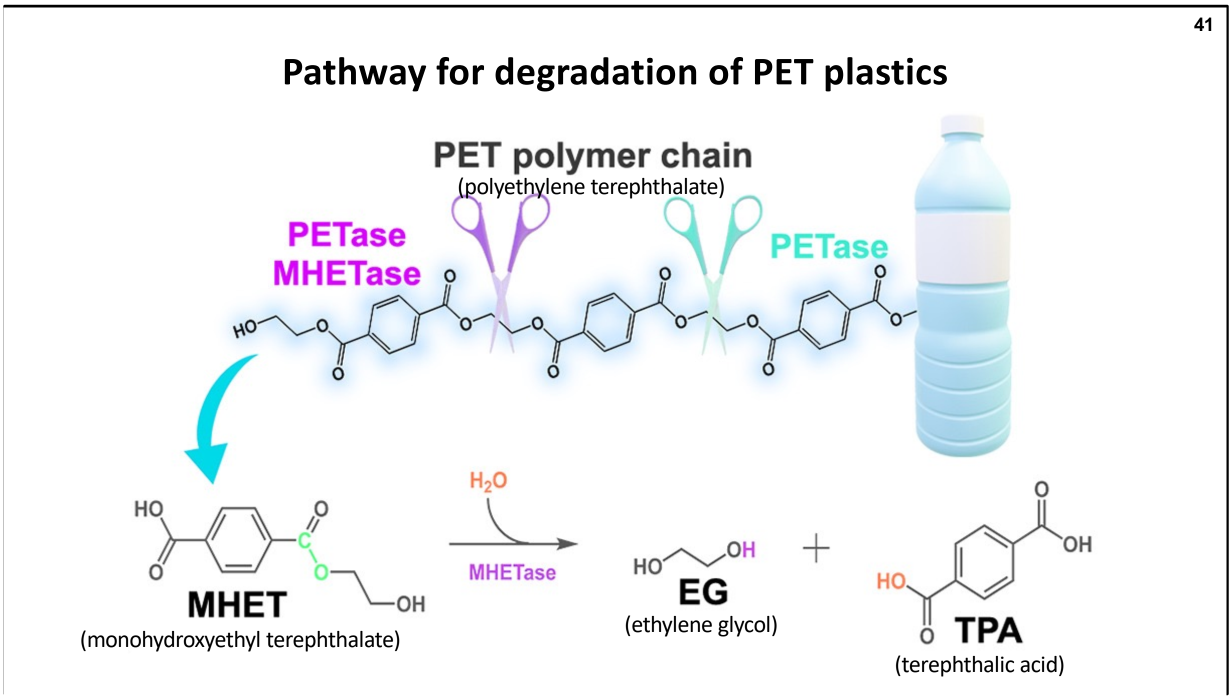
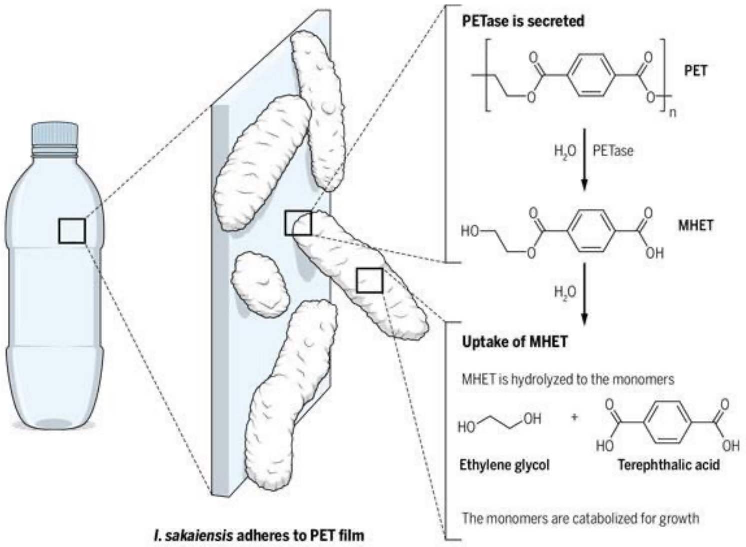
Left panel: *Ideonella sakaiensis* is a Gram-negative, aerobic, non-spore forming, rod-shaped bacterium. First identified in 2016 by a team of researchers led by Kohei Oda of Kyoto Institute of Technology and Kenji Miyamoto of Keio University after collecting a sample of PET-contaminated sediment at a plastic bottle recycling facility in Sakai, Japan.

Right panel: A piece of PET after partial digestion by *Ideonella sakaiensis*. The pitted areas indicate places where digestion has taken place. In the absence of bacteria, the surface of the PET is almost perfectly smooth.



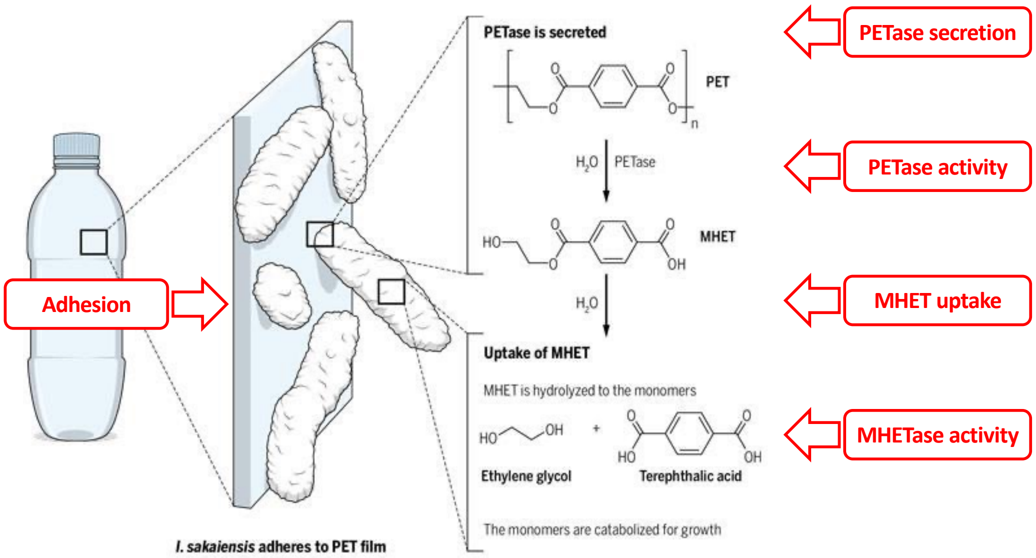
Source: Pinto AV, Ferreira P, Neves RPP, Fernandes PA, Ramos MJ**,** Alexandre L. Magalhães AL (2021) Reaction mechanism of MHETase, a PET degrading enzyme. *ACS Catalysis* 11, 16: 10416–10428 doi: 10.1021/acscatal.1c02444.
Source: https://www.acs.org/pressroom/presspacs/2023/november/plastic-eating-bacteria-turn-waste-into-useful-starting-materials-for-other-products.html.

Ideonella sakaiensis is a plastic (PET)-eating bacterium



Source: https://www.chemistryworld.com/news/plastic-eating-bacteria-show-way-to-recycle-plastic-bottles-sustainably/9556.article

Engineering *Ideonella sakaiensis* to enhance PET biodegradation



Source: https://www.chemistryworld.com/news/plastic-eating-bacteria-show-way-to-recycle-plastic-bottles-sustainably/9556.article

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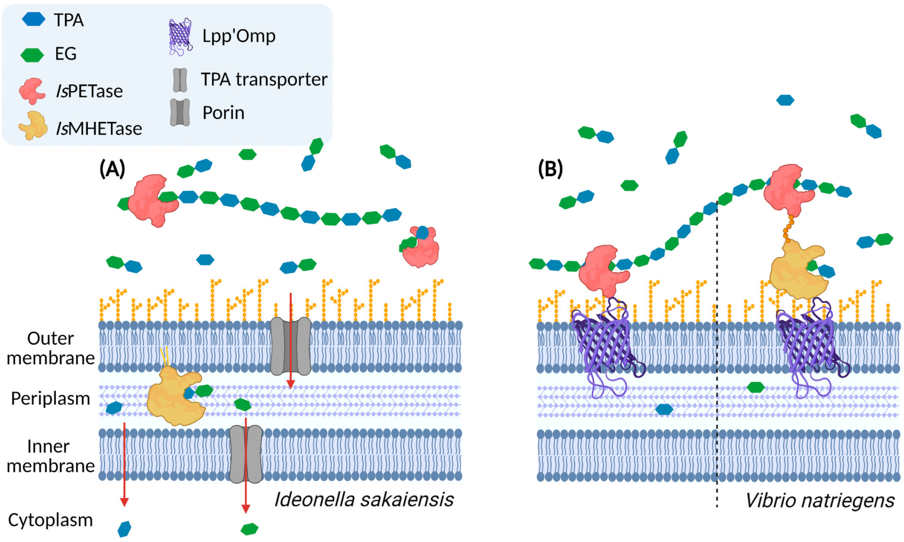
Engineering *Vibrio natriegens* to degrade PET in salt water



Source: Li T, Menegatti S, Crook N (2023) Breakdown of polyethylene therepthalate microplastics under saltwater conditions using engineered *Vibrio natriegens*. *AIChE Journal* doi: 10.1002/aic.18228.

Figure 1. Proposed PET complete hydrolysis pathway by strain Is29. *Ideonella sakaiensis* PETase is secreted to the cell exterior under the guidance of a signal peptide, then depolymerizes PET to produce MHET as the major product. The PET hydrolysis products diffuse through an outer membrane porin into the periplasm. MHET is further hydrolyzed by the outer membrane anchored lipoprotein IsMHETase into TPA and EG. (B) Engineered IsPETase and IsMHETase displayed on the outer surface of engineered *Vibrio natriegens* strains by surface anchors.

Source: https://new.nsf.gov/news/genetically-modified-bacteria-break-down-plastics

Genetically modified bacteria break down plastics in saltwater

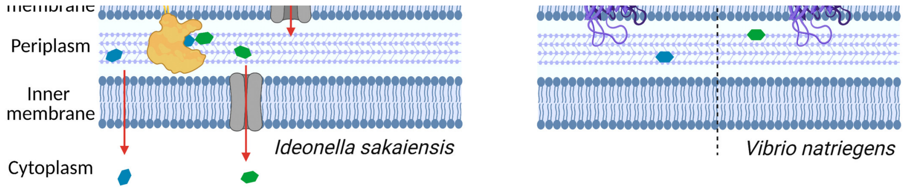
Researchers have genetically engineered a marine microorganism to break down plastic in saltwater. The modified organism can break down polyethylene terephthalate (PET), a contributor to microplastic pollution in oceans that is used in everything from water bottles to clothing. The researchers worked with two species of bacteria. The first, *Vibrio natriegens*, thrives in saltwater and reproduces very quickly. The second, *Ideonella sakaiensis*, produces enzymes that enable it to break down and metabolize PET. The researchers took the DNA from *I. sakaiensis* and incorporated its genetic sequence into a plasmid. By introducing the plasmid containing the *I. sakaiensis* genes into *V. natriegens* bacteria, the researchers were able to get *V. natriegens* to produce the desired enzymes on the surfaces of their cells. The researchers then demonstrated that *V. natriegens* could break down PET in a saltwater environment at room temperature. This is the first genetically engineered organism that we know of that is capable of breaking down PET microplastics in saltwater. The researchers acknowledged that additional hurdles must be addressed, but breaking down the PET in saltwater was the most challenging part of their work, according to them.

Strategies for microbial bioremediation of oceanic plastic *in situ*

1. **Transfer PET-degrading enzymes to a salt water-tolerant organism.**

2. **Engineer *Ideonella sakaiensis* to tolerate oceanic conditions (salt water).**

3. **Isolate PET-degrading species from PET "garbage patches" in the ocean.**



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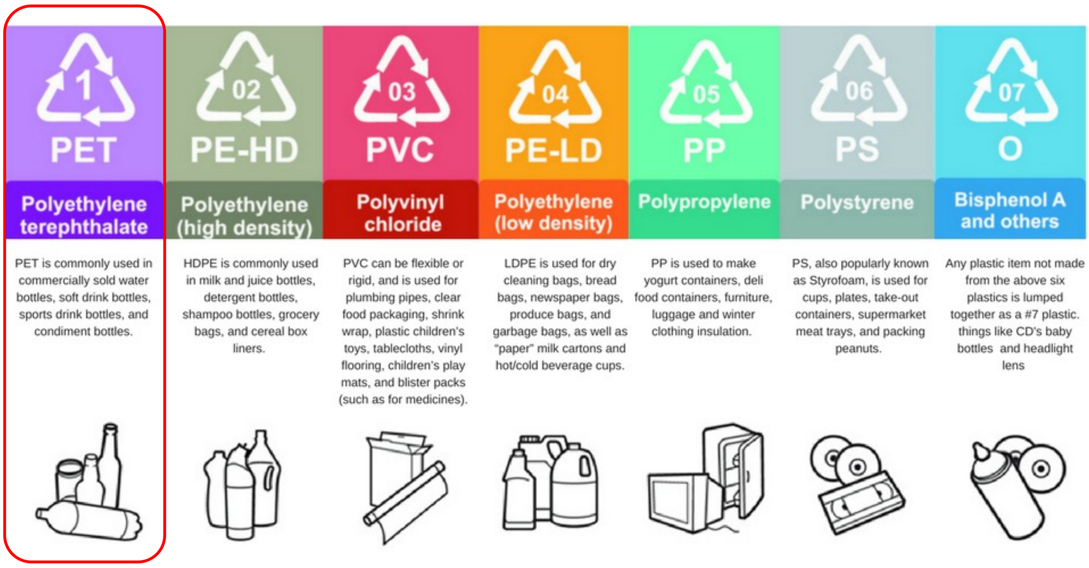
So far so good for PET, but what about all those other plastics?



Source: https://plasticoceans.org/7-types-of-plastic/