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Waste to plastic by enzymes and bacteria

From plastic waste to PHA using enzymes and the bacterium P. putida

The continuing demand for plastic products, the lack of appropriate recycling processes and the ubiquitous pollution of the environment with plastic waste pose a global challenge. In 2018, approximately 358 million tonnes of plastics were produced worldwide. In many countries without a functioning disposal system in place, waste ends up in landfills or even in the countryside and ultimately in the sea. Depending on environmental conditions, it takes hundreds of years for conventional plastics to decompose. The EU has set targets for 2020 that require 50 % of PET plastics, for example in beverage bottles, to be recycled, and 70 % of the polyurethane (PU) foams used in building & construction. At present, however, less than 30 % of PET waste and less than 5 % of PU are treated. It requires considerable efforts to convert the traditional value chain to a sustainable one based, among other things, on fully biodegradable plastics such as polyhydroxyalkanoates (PHA).

Under the EU HORIZON 2020 program, two projects are currently receiving funding to research new, sustainable polymer recycling methods. The projects: P4SB (From Plastic Waste to Plastic Value using *Pseudomonas putida*, grant agreement No 633962) [1] and MIX-UP (MIXed plastics biodegradation and UPcycling using microbial communities, grant agreement No 870294) [2], have been carried out by international, multidisciplinary consortiums formed for this purpose.

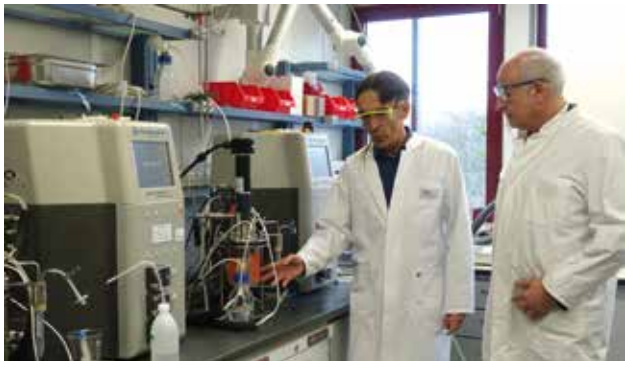
In February 2020, bioplastics MAGAZINE visited Professor Lars M. Blank, Chair of Applied Microbiology at RWTH Aachen University (Germany), who has been involved in both programs. "Our activities started back in 2012, when I was approached by Victor de Lorenzo, who was inspired by Wolfgang Zimmermann's conference presentation," Lars began, "Since the beginning of the 2000s, Wolfgang had been studying esterases, enzymes that can degrade PET." These are thermophilic (or better thermo-tolerant) cutinases that attack the ester bonds. "This means that in theory polymers containing such ester bonds can be degraded by these enzymes", Lars went on. That was when the projects on PET and PU recycling described in this article were initiated.

P4SB

The main objective of P4SB is the biotransformation of non-sustainable plastic waste (e.g. PET and PU) into sustainable value-added alternative bioplastics such as polyhydroxyalkanoates (PHA).

This is done through the utilisation of the tools of contemporary Synthetic Biology to bring about the sustainable and environmentally friendly bioconversion of fossil oil-based plastic waste into fully biodegradable counterparts by means of deeply engineered, whole-cell bacterial catalysts. These tools were used to design tailor-made enzymes for the bio-**de**-polymerisation of PET and PU, but also for the custom design of a *Pseudomonas putida* Cell Factory capable of metabolizing the resulting monomers. P. putida (a gram-negative, rod-shaped, saprotrophic soil bacterium) underwent deep metabolic surgery to channel these diverse substrates efficiently into the production of PHA and derivatives. In addition, synthetic downstream processing modules based on the programmed cell lysis facilitated the release and recovery of the bioplastic from the bacterial biomass. These industry driven objectives helped to address the market need for novel routes to valorise the gigantic plastic waste streams in the European Union and beyond, with direct opportunities for SME and industry partners of P4SB spanning the entire value chain from plastic waste via Synthetic Biology to biodegradable PHA. As a result the project has anticipated a completely biobased process reducing the environmental impact of plastic waste by establishing it as a novel bulk second generation carbon source for industrial biotechnology, while at the same time opening new opportunities for the plastic recycling industry and helping to achieve the ambitious recycling targets set by the European Union for 2020. P4SB finished in March 2019.

Professor Blank (left) explains a laboratory fermenter from Eppendorf facilitating research on plastic hydrolysates as carbon and energy source for microbes to Michael Thielen



Professor Blank shows a fermenter, in which plastics monomers are converted by P. putida into the bioplastic PHA.

MIX-UP

The subsequent project MIX-UP started in January 2020 and investigates sustainable alternatives to mechanical and chemical recycling especially focusing on mixed plastic waste. The project is coordinated by Professor Lars M. Blank, (RWTH Aachen). Other RWTH Aachen institutions involved in the project are the Chair of Biotechnology, the Institute of Technical and Macromolecular Chemistry, and the Aachen Process Engineering department (AVT). In addition, ten other European institutions and four high-profile partners from China participate in the project, including Tsinghua University, Beijing, a strategic partner of RWTH Aachen University.

MIX-UP will use plastic mixtures of five of the most important fossil-based plastics and future biodegradable plastics for microbial transformation processes. The enzymatic, microbial degradation of mechanically pre-treated plastic waste will be combined with the subsequent microbial conversion into high-quality chemicals and polymers.

Known plastics-degrading enzymes will be optimized with respect to high specific binding capacities, stability and catalytic efficacy for a broad spectrum of plastic polymers under high salt concentrations and temperatures. New enzymes with activities on recalcitrant polymers will be isolated. The project also seeks to optimize the production of these enzymes and develop enzyme cocktails tailored to specific waste streams.

It is, for example, known, that the bacteria *Ideonella sakaiensis* cleaves PET in a first step by the enzyme *PETase* to mono(2-hydroxyethyl)terephthalic acid (MHET) and in a second step by the enzyme *MHETase* into the monomers of the plastic, ethylene glycol and terephthalic acid. Another example is the bacterium *Thermobifida fusca*, using the enzyme *cutinase* [3]. Similar processes work with other plastics and the resulting monomers are for example butanediol (BDO), adipic acid etc. "Certainly, chemists can produce PET directly from clean ethylene glycol and terephthalic acid fractions," Lars explained, "but in many cases we find mixed waste streams, which cannot be decomposed into such clean fractions."

The released plastic monomers (Lars calls this *hydrolysate*) will be selectively used by stable microbiomes as substrates to produce, among other products, value-added chemicals or again microbial polyesters (PHA) [4]. The processes of bacteria such as *P. putida* creating PHA as energy storage and the subsequent extraction of the PHA from the bacteria is well known and has been the subject of numerous articles in bioplastics MAGAZINE. Any remaining material recalcitrant to enzymatic activity will be recirculated into the process after a physico-chemical treatment.

The technologies developed and used in P4SB and MIX-UP thus open-up the opportunity to increase recycling rates and to add value to the previously inadequately processed waste streams of unsorted plastics. "One example was a colleague from Indonesia", Lars added, "who was so

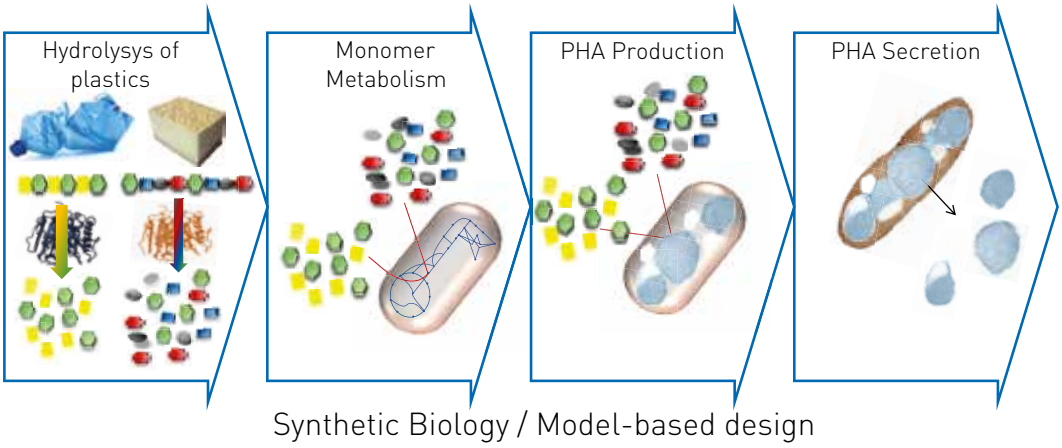


Fig.1: The The P4SB Workflow (Source: P4SB)

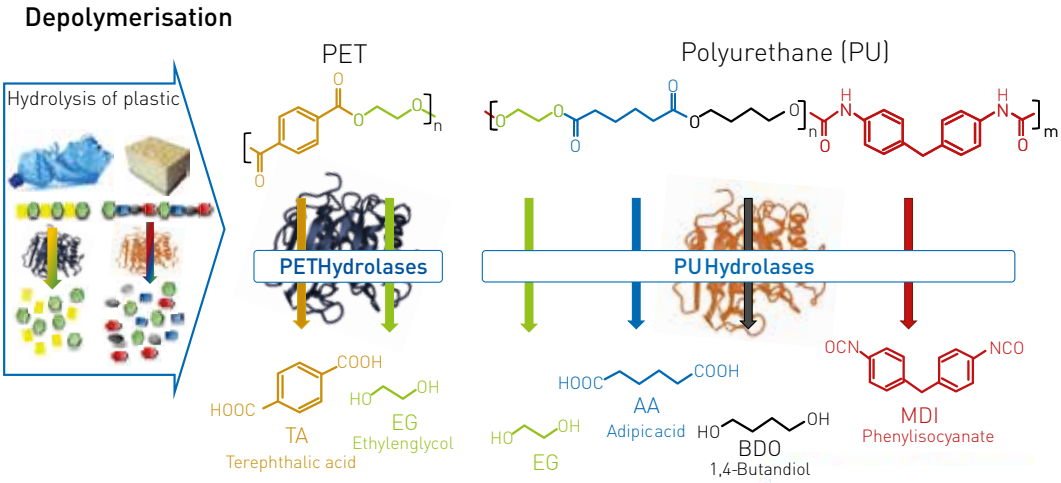


Fig.2: The hydrolysis of PET and PU into the monomers (Source: IAMB, RWTH AAachen)

frustrated about the waste on the beaches in his country. He got himself a scholarship and is now working in our project." Indonesia's coasts are almost four times as long as those of China, so virtually the entire population lives near the coast. And many countries around the world do not recycle or incinerate waste to energy at all. However, as Lars Blank pointed out, this all works well and fast with e.g. thin PET films (and other polyesters including textile fibers), but is difficult, and up to now not yet possible for bottles with significant ratio of crystalline material. "But for bottles it is not that necessary, as bottles can be and are very well recycled, as the example of e.g. Germany shows," he concluded. MT

www.p4sb.eu | www.mixup-eu.de

Rerences:

[1] N.N.: What is the Unique Selling Proposition (USP) of P4SB? https://www.p4sb.eu/about-p4sb.html

[2] N.N.: Adding Value to Plastic Waste Streams, https://www.rwth-aachen.de/cms/root/Die-RWTH/Aktuell/Pressemitteilungen/November/~elvrd/Die-Werte-der-Abfallstroeme-von-Kunststo/?lidx=1

[3] Wei R., Zimmermann W.: Microbial enzymes for the recycling of recalcitrant petroleum-based plastics: How far are we? 2017Microbial Biotechnology 10(6) DOI: 10.1111/1751-7915.12710

[4] Blank LM, Narancic T, Mampel J, Tiso T, O'Connor K.. Biotechnological upcycling of plastic waste and other non-conventional feedstocks in a circular economy. Curr Opin Biotechnol. 2019 Dec 24;62:212-219

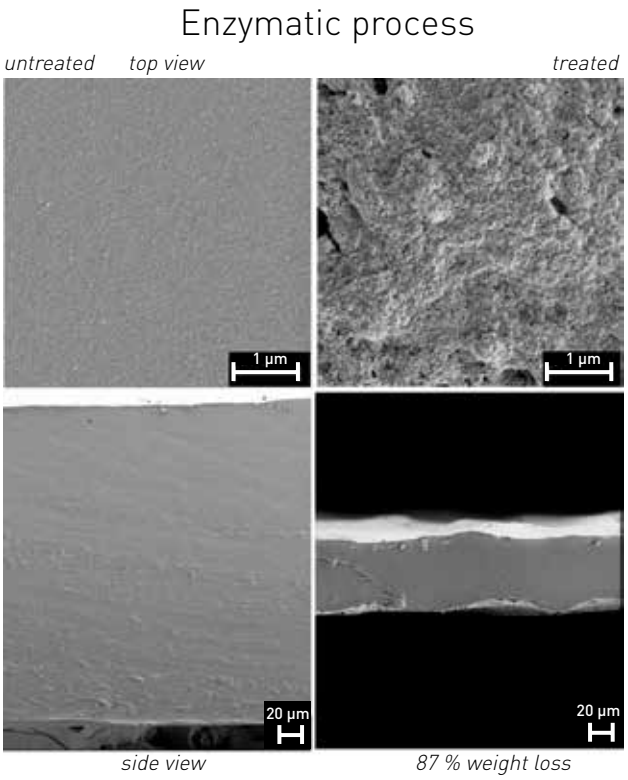


Fig. 3: Enzymatic degradation of thin PET film

(Sources:
Wei et al., (2017) Microb Biotechnol 10:1308-1322;
Zimmermann, Billig (2011) Adv Biochem Eng Biotechno/125:97-120;
Alisch et al (2004) Biocatal. Biotrans. 22:347)

What are enzymes?

Enzymes are proteins that act as catalysts in all living organisms – microorganisms, plants, animals, and humans. As catalysts, enzymes serve as compounds that increase chemical reactions in biological systems. Enzymes are affected by a number of conditions, such as temperature and pH (acidity), and are subject to inhibition by various means. Enzymes are classified by the type of reaction they catalyse and the substance (called a substrate) they act upon. It is customary to attach the suffix "ase" to the name of the principle substrate upon which the enzyme acts.

Source: www.amano-enzyme.com/about-enzymes/

How are enzymes produced industrially?

Bacteria, yeasts or moulds are cultivated in closed steel tanks - the fermenters. Starch or other sugars are added to the liquid culture medium. The microbes grow and flourish and ideally release the enzymes into the medium. The proteins are then harvested: The medium is concentrated and filtered. Most enzyme preparations are sold as powders.

Source: https://biooekonomie.de/enzyme-die-supertalente-der-bioindustrie

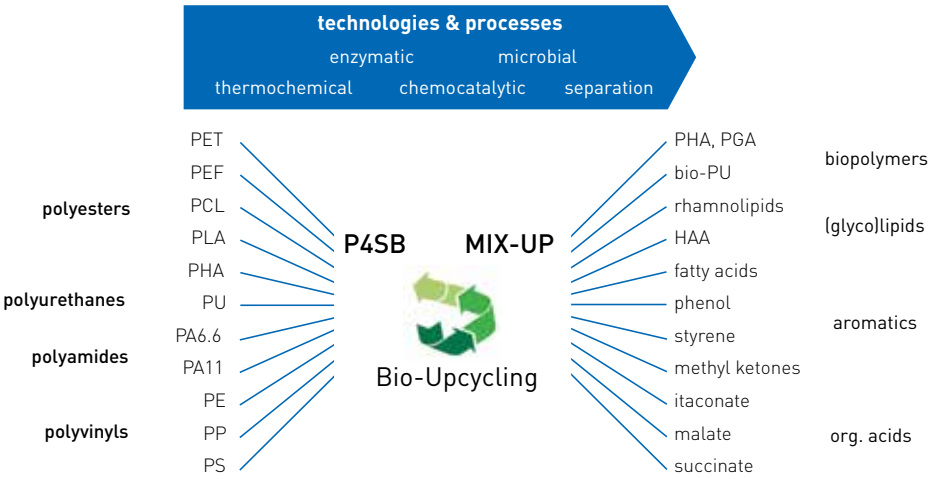


Fig. 4: The Vision

(Sources:
Wierckx et al., Plastic waste as a novel substrate for industrial biotechnology, Microbial Biotechnology, 2015, 8: 900 – 903;

Wierckx et al., Plastic biodegradation: challenges and opportunities, 2018, DOI: 10.1007/978-3-319-44535-9_23-1;

Blank et al., Biotechnological upcycling of plastic waste and other non-conventional feedstocks in a circular economy. Curr Opin Biotechnol. 2019;62:212-219)