MIXed Plastics Biodegradation and UPcycling Using Microbial Communities: The EU Horizon 2020 Project MIX-UP

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Abstract

This article introduces the EU Horizon 2020 research project MIX-UP, “Mixed plastics biodegradation and upcycling using microbial communities”. The project focuses on the ambitious vision to change the traditional linear value chain of plastics to a sustainable, biodegradable based one. In MIX-UP, plastic mixtures containing five of the top six fossil-based recalcitrant plastics (PE, PUR, PP, PET, and PS), along with upcoming biobased and biodegradable plastics (bioplastics) such as PHA and PLA, will be used as feedstock for microbial transformations. The generated new workflow increases recycling quotas and adds value to present poorly recycled plastic waste streams. Consecutive controlled enzymatic and microbial degradation of mechanically pre-treated plastics waste combined with subsequent microbial conversion to polymers and value-added chemicals by mixed cultures. Through optimization of known plastic-degrading enzymes by integrated protein engineering, high specific binding capacities, stability, and catalytic efficacy towards a broad spectrum of plastic polymers under high salt content and temperature conditions will be achieved. Another focus lies in the search and isolation of novel enzymes active on recalcitrant polymers. MIX-UP will also enhance the production of enzymes and formulate enzyme cocktails tailored to specific waste streams. In vivo and in vitro application of these cocktails enables stable, self-sustaining microbiomes to convert the released plastic monomers selectively into value-added products, key building blocks, and biomass. Any of the remaining material recalcitrant to the enzymatic activity will be recirculated into the process by physicochemical treatment. The Chinese-European MIX-UP is a multidisciplinary and industry-participating consortium to address the market need for novel sustainable routes to valorize plastic waste streams. MIX-UP realizes a circular (bio) plastic economy and will contribute where mechanical and chemical plastic recycling show limits.

Background

General global plastic waste situation

Due to their benefits as a functional material, their extreme durability, longevity, low weight and low price, synthetic plastics, including polyethylene (PE), polystyrene (PS), polypropylene (PP), polyvinyl chloride (PVC), polyurethane (PUR), and polyethylene terephthalate (PET), have become ubiquitous not only in work and social environments but also in natural systems as contaminants. Plastic pollution has become a global threat, affecting all ecosystems, even remote ones like the pole regions, uninhabited atolls or deep ocean basins [1–6]. The global scale of plastics production increased by 15% in the last five years, reaching 359 million metric tons (Mt) in 2018 [7]. China and the European Union (EU) account for 30% and 17%, ranking first and third globally of all the world’s plastic production, respectively [7]. Highest in plastic waste generation, in 2016, were the United States with 42 Mt followed by the EU (30 Mt), India (26 Mt) and China (22 Mt) [8]. All nations worldwide are struggling to manage the current volumes of plastic waste, making a highly efficient waste management system increasingly important. A significant unintended drawback of the existing plastic economy is its linearity. Of all plastics produced globally, 83% has not been reused due to a lack of proper recycling technologies, and of the recycled 10%, only 15% has been reused more than once [9]. Seven main plastic polymers account for 92% of all primary produced plastic ever made (1950–2015: 8,300 Mt). The largest groups are the polyolefins, with PE (36%), and PP (21%), PVC (12%), followed by PET, PUR, or PS (less than 10% each) [7, 9]. Biobased plastics (bioplastics) with increasing volumes emerged as non-fossil alternatives on the last decennium's plastic markets (Fig. 1B). Persistent biomass-derived plastic materials, non-biodegradable bioplastics derived from renewable resources represent 57% of all the bioplastics (2 Mt), including biobased PET, polyamides (PA), and PE [10].

Effective plastic recycling poses a significant challenge for sustainability, as a plastic polymer currently degrades each time it is recycled [11]. Technological solutions as part of a circular economy can form only part of more radical changes required in human behaviors like throw-away mentality symbolized by single-use consumer plastics or unnecessary packaging. Multilevel mitigation strategies to reduce the waste of natural resources started with policymakers banning and placing levies on single-use plastic consumer products to stimulate sustainable alternatives and changed consumer behavior. Upcycling plastic waste from fossil sources in an open-loop process to biodegradable plastic and chemicals to valorise post-consumer plastic should be part of a rethinking towards a circular economy [12–14]. Essential for a circular economy is the intense utilization of every side stream to minimize waste production or redundant CO_2 release. Available recycling concepts are often not cost-competitive and produce polymers of lower quality. The biotechnological recycling supplemented with physicochemical techniques to tackle the more recalcitrant plastic polymers may promote new waste management strategies. Promising new value-chains for plastic waste and the increasing demand for recycled plastics by the multinational brand owners, driven mainly by the rising consumer awareness concerning sustainability issues, shall urge the private sector to invest in a circular economy. The partners of the Mix-Up consortium envision a better plastic future built on the '6 R' principles (rethink, refuse, reduce, reuse, recycle, replace) [15]. In the 2019 position paper "A circular economy for plastics", the European Commission explained its vision for a circular plastics system. The plastics should be produced using renewable energy and feedstocks. The plastic products should be designed to be used, reused, repaired and recycled (mechanically, chemically, biologically) so that the material streams in society are fully circular, keeping high value without posing risks for human health nor the environment [16].

Chemical and mechanical plastic waste recycling

Crude oil and other fossil resources are the cost-effective lifeblood of the chemical industry and have been extensively used as energy and carbon feedstock for almost 90% of its products. One of the last boosts has been the shale gas-driven multi-billion investments into the U.S. chemical industry, leading to an acceleration of virgin plastics manufacturing [17]. For decades, the traditional economy of high-income countries followed the "take-make-dispose" strategy creating economic value by manufacturing and selling as many products as possible. The envisioned global transition to a circular economy initiated the founding of initiatives like, e.g., *The Global Plastics Alliance* or *Alliance to End Plastic Waste* [18, 19]. It activated private investors' investment or development banks into recycling technology to recover and create value from plastic waste.

The only widely applied large-scale technology to treat solid plastic waste is mechanical recycling. Limitations for mechanical recycling are temperature-sensitive plastics, composites, and thermosets that cannot be liquidized at high temperatures [20]. Differences in mechanical behavior and thermal properties of all the different plastics require thorough sorting, washing to remove organic residues and shredding of the collected wastes. The melted and remolded polymers are often blended with virgin plastics to correct for lost properties. Two of the most prominent commodity plastics, PET and PE, with annual EU market shares of about 8% and 30%, respectively, mainly used in packaging, are the only ones recovered by mechanical recycling [7, 20].

Chemical recycling has emerged as an alternative, promising technology to valorize plastic waste. Plastic wastes can be gasified into synthesis gas. Solvolytic processes may convert polymers into monomers and oligomers, subsequently re-polymerized after purification by, e.g. precipitation combined with filtration [21]. Pyrolysis (thermolysis) and hydrogenolysis using advanced catalysts can selectively produce gases, fuels, or waxes. The latter requires selective and efficient catalysts, preserving critical functional groups [20]. High-energy costs but low costs for competing virgin monomers from fossil-based feedstocks often make chemical recycling commercially unattractive [22]. Effective recycling processes within circular approaches should not only produce monomers for later polymerization (“bottle to bottle”), but rather focus on value-added products or intermediates for alternative supply chains. The upcycling of PE into long-chain alkyl aromatics ready to be sulfonated to make surfactants was reported [23]. Others described the synthesis of intermediary cyclic acetals, which are useful as solvents, fuel additives or monomers for polymers [24]. The greatest challenge is the chemical recycling of commingled plastic waste, as even small amounts of the various polymer contaminants may change the properties of the end-product. Therefore, chemical recycling requires often the use of pure waste feedstocks obtained only after resource-intensive sorting. The use of suitable compatibilizers for upcycling recovered polymer mixtures can overcome this problem [25–27].

Another feasible approach for PET/polylactate (PLA) polyester mixtures is using a molecular ruthenium catalyst for selective hydrogenolysis to separate the differing sorts of monomeric diols, and methanol, respectively, at varying reaction conditions (temperature, solvent) [28]. Alternatively, using pyrolysis oil in a naphtha cracker might close the carbon loop, but much of the beneficial molecular structure and plastic properties are lost in this option.

Microbial and enzymatic plastics biotransformation

Facing the unabated growth of global plastic production and considering the shortcomings of traditional mechanical and chemical recycling technologies, biological depolymerisation and conversion technologies have been increasingly discussed, complementing end-of-life plastic treatment options. With a view to the economic circularity, selective removal of polymer-building blocks using enzymatic treatments under mild conditions and the ability to the selective recovery of monomers from mixed plastic substrates would be a real improvement [29–31]. Building blocks of plastic polymers can, in general, be divided into different major groups as (i) monomers with vinyl groups to produce PS, PE or

PVC; (ii) bifunctional monomers with terminal hydroxyl, amine, or carboxyl groups to obtain polyesters or polyamides; (iii) diisocyanates for PUR [32]. In recent years, considerable progress concerning plastic polymers with hydrolysable groups in their backbones, as PET, PA, or PUR were reported, obtained mainly by polyaddition or polycondensation. Several studies described the ability of microorganisms and enzymes to degrade these plastics [33–46]. Typical enzymes are cutinases, lipases, and carboxylesterases [47]. The main challenge of enzymatic degradation is the fraction of plastic polymers based on persistent and robust chemical groups, which resist hydrolysis with common biological enzymes that are highly recalcitrant even under conditions favouring microbial processes. These polymers (e.g., PE, PP, PS, PVC) obtained by chain polymerization comprise the major part of the plastic waste market and are generally considered non-biodegradable. The polymers possess extensive inert C-C backbone structures, are completely devoid of functional groups and might be only degraded by high-energy redox reactions [47]. Only a few enzymes have been reported to reduce the molar mass of PE and PS. Alkane hydroxylase AlkB, a hydroquinone peroxidase, laccases, and a laccase mediator system demonstrated C-C-bond cleavage via autooxidation mediated by putative radical mechanisms thought to occur randomly, generating a large diversity of short-chain scission products [4, 47–54]. In addition to the description of enzymatic activities towards PE and PS, several reports described their mineralization to CO_2 by insect larvae and their enteric microbiome. The latter potentially benefitting from the combined mechanical pretreatment and enzymatic hydrolysis [55–60]. Recently, biodegradation of PVC in the gut of *Tenebrio molitor* larvae has been described [61]. No biodegradation has been demonstrated so far for the highly recalcitrant polymer PP.

Mixed cultures in industrial applications

The application of microbial consortia in traditional foods, such as bread, soy sauce, cheese and wine, have been recorded for centuries. These bioprocesses were realized with naturally occurring mixed cultures. Mixed cultures were gradually replaced by pure cultures in fermentation processes to avoid contaminations by food spoilers or pathogenic microbes. Pure cultures have been the workhorses for biotechnological processes to produce bulk products like amino acids, antibiotics, enzymes or organic acids. Fermentations based on pure cultures usually require strict aseptic conditions, purified substrates, high operational energy costs, and gain in addition to the targeted product high concentrations of by-products in the form of biomass and potentially of organic acids or alcohols. The traditional strategy of consolidated bioprocessing integrates all bioconversion reactions in one step-bioprocesses using metabolically engineered whole-cell biocatalysts hosting all required functional genes in one consolidated strain.

Compared with the competing fossil-based chemical production, industrial biotechnology lacks cheap, readily available feedstocks to produce bulk biobased chemicals using highly specialized whole-cell biocatalysts as pure cultures. The main drawback for using lignocellulose, molasses, sludge or organic wastes as feedstock in pure-culture fermentations is the heterogeneity of the feedstocks, non-aseptic conditions and the high costs for substrate pre-treatments. Although mixed cultures as industrial microbiomes are well established in the fields of biofuels (biogas, bio-hydrogen, butanol-production), biobased chemicals, and biopolymers, the emphasis in industrial biotechnology still lies on pure cultures [62–65]. The specific advantages of mixed culture fermentation compared with pure culture are i) the possibility of utilizing cheaper or mixed substrates (*e.g.* organic waste, lignocellulose, raw glycerol); ii) the synergies of different enzymatic systems and combination of metabolic pathways of various microorganisms that can result in more efficient utilization of substrates and a narrow production spectrum contributing to product purification; iii) shorter development times for mixed-culture design compared with deep-genetic engineering to create universal “superbugs”, and iv) cost reduction, due to the high microbial diversity with non-sterile requirements [66]. An alternative for the latter is the use of robust extremophilic strains able to produce the target compounds (e.g., PHA) under simplified process conditions, in open unsterile, continuous fermentation facilities where most other organisms are unable to proliferate. The extremophiles based process seems to be suitable for simple growth on mixed degradation products, including fatty acids, plastic monomers and food wastes [67–70]. In mixed cultures and consortia exist in addition to intraspecies interactions, e.g., quorum sensing, interspecies interactions between cells of the different species. Metabolite effects like mutualism, synergy, and competition for nutrients in an ecological niche might affect metabolisms and the yield of fermentation target products [71–74].

From Plastic Waste to Plastic value using* Pseudomonas putida *Synthetic Biology in MIX-UP

MIX-UP can, in part, build on the success of P4SB (grant no: 633962), an H2020 project in which several of the MIX-UP partners already worked together on plastic waste valorisation. The innovation radar has ranked P4SB as one of the top ten EU Biotechnology projects [75]. The main outcomes of P4SB regarding plastic hydrolysis are engineered PET degrading enzymes with significantly increased PET hydrolysing activity [76]. Furthermore, PUR hydrolases were identified [38]. In terms of monomer metabolism, *P. putida* strains for growth on all PET and PUR monomers tested could be isolated. However, efficient growth could not be achieved on all monomers. Subsequently, via genetic engineering, the P4SB partners could generate recombinant *P. putida* strains capable of efficient catabolism of ethylene glycol, terephthalic acid, and 1,4-butanediol [12, 13, 65, 77]. For the valorisation of plastic monomers, besides PHA synthesis, hydroxy alkanoyl oxy-alkanoic acids (HAA) synthesis has been successfully established. PHA synthesis could be shown on all PET and PUR plastic monomers [13].

Project aim, concept, and approach

The core aim of MIX-UP project is to establish mixed plastic waste as standard second-generation feedstock for industrial biotechnology – plastic waste as a valuable resource. The bioconversion of unsorted, mixed plastic waste into value-added, sustainable biomaterials using heavily engineered enzyme mixtures for depolymerization and mixed microbial cultures as whole-cell biocatalysts for biosynthesis is the way to achieve this goal as a contribution to the transition towards a low-fossil carbon circular bioeconomy (Fig. 1A).

The main idea of MIX-UP is to showcase a novel approach to the circularity of the plastic life cycle. The overall concept is depicted in Fig. 2. MIX-UP will develop and use engineered polymer hydrolyzing and oxidizing enzymes to depolymerise the mechanically sheared mixed plastic waste (*e.g.* marine litter, household) into their monomeric components (biotic plastics depolymerization). These enzymes will be expressed in mixed microbial cultures, synthesized in an optimized production reactor (enzyme production) or as envisioned in a subsequently consolidated bioprocess with simultaneously implemented whole-cell biocatalysts biodegradation. The released metabolites, plastic monomers, and oligomers from the various plastics types will be transferred to the bioreactor (mixed culture). Here the plastic derived feedstock is fed to dedicated microbial communities converting the substrate into central metabolites, which provide afterwards the building blocks for the synthesis of novel polymers (e.g. HAA,

PHAs), products (biosurfactants) or building blocks for chemo-catalysis (Fig. 2). The approach follows the bow-tie structure of metabolism [78]. Finally, MIX-UP will tackle downstream processing and recovery of the product by, for example, conditional release of the intracellular products and separation. The recalcitrant process residues will be separated and subjected to chemical transformation, also cracking persistent ester bonds, synthesizing valuable chemicals, and closing the cycle by subsequent re-entering of the bioprocesses. The entire bioprocess will be optimized, performing metabolic engineering in an integrated manner by considering the upstream (strain/microbiome development, protein engineering), midstream (fermentation), and downstream (recovery and purification) processes altogether.

MIX-UP targets the engineering of a new-to-nature biological route to convert mixed plastic waste to value-added bio-products, which will enable the recycling industry a qualitatively new dimension. Furthermore, when successful, mixed plastic wastes can be established as novel second-generation carbon sources for bio-products, aiding to solve the conflict of food *vs.* fuel that is pervasive in contemporary Industrial Biotechnology. Thus, through a combination of metabolic engineering of mixed cultures, intensive protein engineering and bioprocess-optimization, MIX-UP will enable new value chains within the framework of a sustainable knowledge-based bio-economy across sectors, including materials, chemicals, and environmental technologies. That will ultimately benefit the economy, environment, and society at large. The project has already produced a large number of publications that are available at the MIX-UP website www.mix-up.eu.

Abbreviations

EU: European Union; MIX-UP: Mixed plastics biodegradation and upcycling using microbial communities; PP: polypropylene; PE: polyethylene; PUR: polyurethane; PET: polyethylene terephthalate; PS: polystyrene; PA: polyamide; PLA: polylactate; PHA: polyhydroxyalkanoate; PBS: polybutylene succinate; TPS: thermoplastic starch; PVC: polyvinyl chloride; HAA: hydroxylalkanoyloxy-alkanoic acids; Mt: million metric tons; P4SB: From plastic waste to plastic value using *Pseudomonas putida* synthetic biology; H2020: Horizon 2020; *P. putida*: *Pseudomonas putida.*

Declarations

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Authors' contributions

HB and LMB compiled the manuscript and wrote the introductory section parts of the manuscript. All authors read and approved the final manuscript.

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Ethics declarations

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

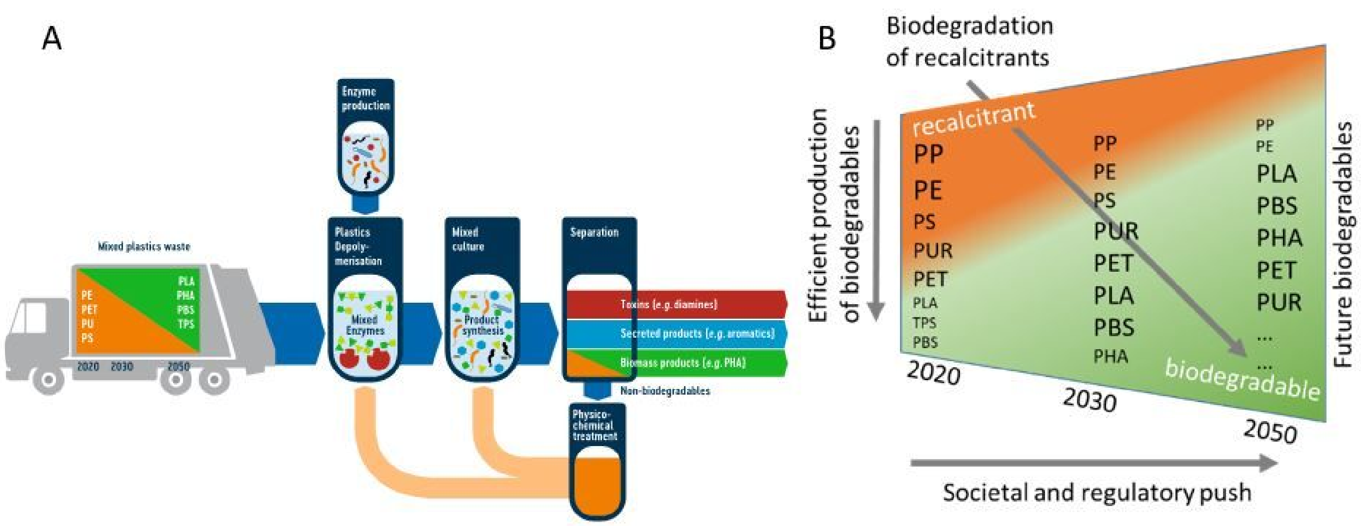

Figure 1

A) Schematic overall concept of the proposed MIX-UP-project; B) The MIX-UP ambition to make the majority of the vast plastics biodegradable.

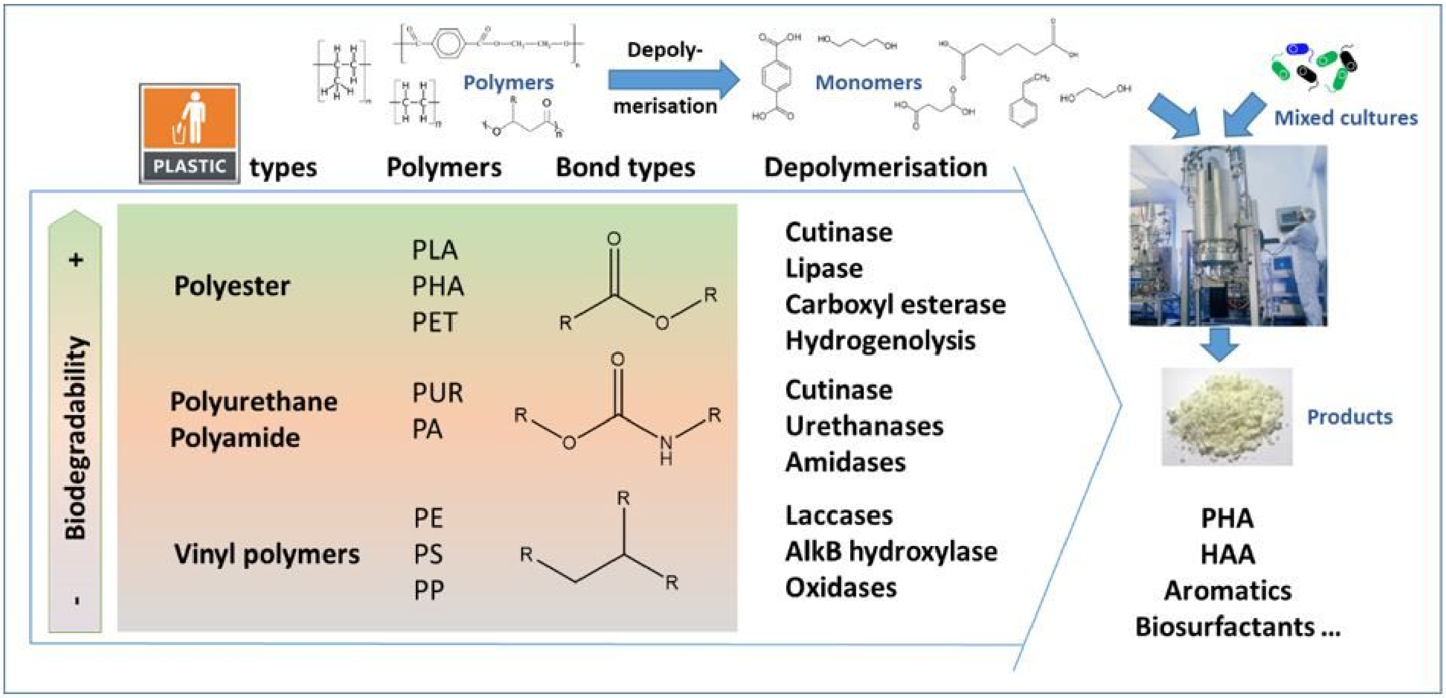

Figure 2

MIX-UP overview.