Bioupcycling Technology for Sustainable Plastic Waste Management

Subjects: Biotechnology & Applied Microbiology

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Effective plastic bioupcycling processes that can act as a drive to increase waste removal from the environment and valorize post-consumer plastic streams, thus accelerating the implementation of a circular (plastic) economy.

bioupcycling plastic waste bioconversion recycling fermentation biotechnology

1. Bioupcycling

To improve the circularity of the plastic sector, post-consumer plastic waste should be recycled at the highest possible level. However, currently implemented recycling technology mainly transforms the plastic waste into lower-value products (downcycling) or, at best, into the same level. There is an urgent need for new strategies that help improving the techno-economic feasibility of the recycled plastics (still not competitive with virgin fossil ones [12]) or that lead to improved value/properties of the new plastics (defined as “upcycling”) [13]. Besides biotechnological recycling, new upcycling processes are now extensively studied and under development. The possibility to convert post-consumer PET to polyhydroxyalkanoate (PHA) by enzymatic depolymerization and subsequent bacteria fermentation [1], for instance, allows researchers to obtain a bio-material with good technical substitution potential, novel properties (depending on the co-polymer) and biodegradability. From this perspective, post-consumer plastic can be upcycled rather than only recycled. Moreover, biological methods have the advantage that they can be applied to contaminated plastic waste (i.e., food or soil) and do not require previous separation of the different fractions. Furthermore, the high selectivity of enzymes could allow for a stepwise removal of specific components of the mixed-plastic waste, facilitating the downstream processing; thus, it can go beyond the limits of mechanical and chemical recycling [2].

Recent research in the biodegradation of plastic waste allowed for the establishment of plastic biodepolymerization processes for some plastic types, thus paving the path for greener plastic recycling processes [3][4][5][6][7][8][9][10].

Both natural and engineered enzymes for plastic depolymerization have been extensively studied [3][11][12][13][14][15][16]. Even though the cost of the enzymes is still of concern, a recent study on enzymatic recycling of PET has determined that it should only contribute to 4% of the overall operating costs [17]. The authors highlighted that an enzyme-based recycling process can be cost-competitive, and the constant development of enzyme performance and optimization of the process remains an opportunity to further improve the economic viability of this process. Once bioprocesses for bulk enzyme production have been established, the enzymatic degradation of plastic is a

promising technology that will be implemented in the near future. Depolymerization enzymes can be utilized in multiple ways, including free enzymes, immobilized enzymes, extracellular enzymes of whole-cell biocatalysts, surface enzymes, and/or in the form of enzyme cocktails [18]. Moreover, synthetic biology can be applied to improve the catalytic activity of enzymes through protein engineering, e.g., direct evolution and rational protein design [19]. Plastic-degrading enzymes in microorganisms can evolve from their natural activity on recalcitrant biopolymers such as lignocellulose, chitin, and cutin. Polyester plastics could, for instance, be depolymerized by hydrolytic enzymes produced by bacteria or fungi, such as cutinases, esterases, lipases, ureases, and proteases, as they have hydrolyzable ester bonds in their backbone (Satti and Shah, 2020). An excellent example is the bacterial enzyme polyurethane hydrolases (PUase), capable of hydrolyzing polyurethane (PU) [20], or the well-known PETase and MHETase from *Ideonella sakaiensis* 201-F6, able to hydrolyze PET [15]. For plastics with a non-hydrolyzable C–C backbone, such as polyolefins (PE and PP), oxidative enzymes play a significant role in introducing active functional groups into the backbone, which consequently can undergo biodegradation [21]. Alkane hydroxylases from isolated *Pseudomonas* sp. E4 was also reported to play an important role in PE biodegradation [22]. This enzyme acts on the hydroxylation of C–C bonds to release primary or secondary alcohols, which are then further oxidized to ketones or aldehydes, and finally to carboxylic acids [23]. Thus, the identification and optimization of efficient enzymes represents a prerequisite for the development of bioupcycling processes. In fact, the enzymes are not going to use the polymers as a carbon source, as would happen with a microbial cell that degrades the polymers. This allows to fully recover the monomers for the subsequent upcycling step, for instance through the contribution of fermentation processes [24].

So, in conclusion, the established knowledge on biodepolymerization is expected to boost the development of new biocatalytic plastic upcycling processes, to produce value-added products and/or generate new (and more renewable) plastics with better properties, out of conventional plastic wastes. In this sense, the cost of renewable plastic production (which is still too high compared to conventional fossil-based plastics and related to feedstocks' price and fluctuation) could be alleviated. Hence, renewable plastic will become more economically viable for general commodities, once large-scale production is reached. At present, bioupcycling with endogenous or engineered metabolic pathways has been demonstrated as a proof of concept and is a hot topic for researchers worldwide. The most relevant published studies have been reviewed in the sub-section below.

2. Bioupcycling of Polyethylene Terephthalate (PET)

PET is a petrochemical-based plastic produced on multimillion tons worldwide. It is a polyester made of the repeating units of ethylene glycol (EG) and terephthalic acid (TA). It is cheap and has a very low permeability to gas and moisture, making it an ideal material for single-use plastic bottles. The demand of PET worldwide is around 29 Mt in 2022 [25], while in Europe it reached 4.1 Mt in 2020 [26]. However, even if PET has a higher recycling rate than other plastic types (50% in Europe and 23% in the US for PET bottles), 69% of recycled PET was used for lower-grade applications such as trays, film, strapping, or fiber [27]. From these statistics, the PET management system clearly does not show a very high level of circularity; therefore, there is a clear need for new

upcycling approaches to valorize PET into higher-value products or, at least, keep the material at the same level in the value chain.

Various upcycling strategies have been reported to valorize PET waste, including biotechnological processes and bioupcycling (**Table 1**). It typically combines depolymerization and fermentation/bioconversion strategies to produce new valuable products. Chemical processes (e.g., hydrolysis, alcoholysis, glycolysis, aminolysis, ammonolysis, and hydrogenolysis [28]) and thermal processes (e.g., hydrothermal liquefaction, pyrolysis, and microwave irradiation) are generally used for the depolymerization of PET. Interestingly, a considerable achievement through enzymatic or microbial degradation has also been reported, especially on PET, that can lead to the development of more sustainable bioupcycling processes. Since PET monomers are linked through hydrolyzable ester bonds, an increasing number of PET hydrolyzing (and/or surface modifying) enzymes have been reported recently. One of the major findings was, for instance, the discovery of *Ideonella sakaiensis* [15], a bacterium that can grow on PET as the only carbon source, thanks to the synergy of its enzymes *Is*PETase and MHETase that break down PET to mono-(2-hydroxyethyl)terephthalic acid (MHET), di-(2-hydroxyethyl)terephthalic acid (BHET), and finally to EG and TA. Subsequently, several studies reported the improvement of the mesophilic *Is*PETase through protein engineering, for example by increasing the hydrolytic activity [29] or the thermal stability, using rational protein engineering [3][13][30].

In order to boost enzyme production, several studies have been investigating the addition of signal peptides to the *Is*PETase gene to enhance its secretion during heterologous expression in *Escherichia coli* [31]. The use of heterogeneous immobilized biocatalysis through the use of different binding modules and linkers has also been extensively investigated [32][33]. Some of the most effective enzymes that act on PET hydrolysis are thermostable cutinases such as *Humicola insolens* cutinase (HiC) [4], *Thermobifida fusca* cutinase (TfCut2) [16], and leaf-branch compost cutinase (LCC) [34]. Improving their hydrolysis activity has also been reported using various methods, for example, by enhancing electrostatic interaction between TfCut2 and PET by cationic surfactant additive-based approach, and showed impressive biodegradation of PET by 97% within 30 h [5]. In general, the design of thermostable hydrolases has been intensively investigated during the last years [35] and resulted in the development of promising processes for enzymatic depolymerization. One of the biggest breakthroughs so far is probably represented by the study by Tournier and colleagues that engineered the LCC cutinase and increased its optimal reaction temperature up to the glass transition temperature (Tg) of PET [11]. Such bioprocesses can represent a valuable and sustainable alternative to thermochemical depolymerization and are laying the basis for the further conversion of plastic waste-derived monomers.

Bioupcycling post-consumer PET (or even polyolefins) to PHA has been gaining attention since this approach promotes the valorization of plastic waste into renewable biopolymers. PHA is a general term for microbial polyester of R-3-hydroxyalkanoic acids [36]. It is a promising substitute for several petroleum-based plastics, due to its superior thermal processability, biodegradability, and biocompatibility properties [37][38]. Kenny et al. reported using a two-step chemo-biotechnological process for upcycling PET to PHA [36]. First, the PET waste was hydrolyzed by pyrolysis at 450 °C at a feed rate of 1 kg/h, obtaining 77% of solid fraction (TA, oligomers, benzoic acid and others), 18% gas fraction (CO_2, CO, H_2, ethene, and others), and 6.3% liquid fraction (EG, acetic

aldehyde and others). Second, the TA was dissolved in NaOH to generate sodium terephthalate, which was used as the sole carbon source to grow a PHA-accumulating strains. *Pseudomonas putida* GO16, *P. putida* GO19, and *P. frederiksbergensis* GO23 were found to consume sodium terephthalate and accumulated PHA up to 23–27% of CDW. GO19 was the most efficient at converting TA to PHA, with a productivity of 8.4 mgPHA/L/h. The research group continued to develop the bioprocess to enhance PHA production from TA by co-feeding with waste glycerol (WG) [39]. The fermentation was designed to have two distinct phases: the biomass growth phase and the PHA production phase. They found that when *P. putida* GO16 was fed with WG only during the growth phase (0–24 h) and WG and TA during the PHA production phase (24–48 h), the highest total PHA production was achieved (5.30 g/L). This strategy promoted a 2.0-fold higher PHA production than feeding with TA alone. This study showed that bioprocess engineering strategies are key to develop highly efficient bioupcycling of plastic waste.

Tiso et al. reported the bioupcycling of PET to PHA and hydroxyalkanoyloxyalkanoate (HAA) by using a combination of enzymatic hydrolysis and whole-cell biocatalyst [1]. PET was hydrolyzed by LCC, a polyester hydrolase capable of efficient PET depolymerization to TA and EG. *Pseudomonas umsongensis* GO16 KS3 was found to consume both TA and EG (the latter at a 3.5-fold lower rate than TA) within 23 h of cultivation, in a 5 L bioreactor producing PHA. The PHA production only reached 0.15 g/L, or 7% of CDW. Interestingly, HAA was produced from TA only, while EG was exclusively used for growth. A maximum HAA concentration of 35 mg/L was achieved with a production rate and yield of 5 mg/L/h and 0.01 gHAA/gTA, respectively [1]. Despite the low performance, this represents a highly interesting approach with potential industrial applications (once optimized). In fact, HAA can be directly polymerized with 4,4′-methyl diphenyl diisocyanate and butanediol (BDO) to form biopoly(amide urethane) (bio-PU).

Another study reported the bioconversion of TA, a monomer of PET, to muconic acid (MA), which is currently used to produce adipic acid (AA), an important monomer for various plastics [40]. PET was first depolymerized by microwave radiation at 230 °C for 50 min to TA and EG. Then, the *E. coli* strain CTL-1 (expressing TphAabc, TphB, and AroY, which is responsible for converting TA to catechol, via 1,2-dihydroxy-3,5-cyclohexadiene-1,4-dicarboxylate (DCD) and protocatechuic acid (PCA)) and the strain MA-1 (expressing CatA, which is a catechol 1,2-dioxygenase responsible for converting catechol to MA), were combined for MA synthesis. The MA concentration reported was 2.7 mM, accounting for a 85.4% molar yield (MA/TA). In the same study, gallic acid (GA), pyrogallol, vanillic acid (VA), and glycolic acid (GLA) were also produced from engineered stains, using TA. In addition, GLA was produced by EG-fermenting *Gluconobacter oxydans* KCCM 40109.

The recent study by Sadler and Wallace (2021) showed the development of a one-pot bioprocess to convert TA from PET waste into a value-added molecule, vanillin. PET from a post-consumer plastic bottle was firstly hydrolyzed to TA by semi-purified LCC at 72 °C. Then, the reaction was cooled down and the engineered strain, *E. coli* RARE_pVanX, was added to perform the bioconversion. *E. coli* RARE_pVanX was constructed with two plasmids that were encoded for different enzymes (terephthalate 1,2-dioxygenase, dihydroxy-3,5-cyclohexadiene-1,4-dicarboxylic acid dehydrogenase, carboxylic acid reductase, and catechol O-methyltransferase), which convert TA to vanillin via intermediates (PCA, VA, and dihydroxybenzaldehyde). The process optimization was performed by screening the protein-expression media (M9-glucose supplemented with L-Met and n-butanol (nBuOH)),

increasing *E. coli* cell membrane permeability to TA (addition of 1% *v*/*v* n-BuOH), adjusting pH (5.5) and temperature (22 °C), and mitigating the toxicity of vanillin by in situ product removal, using oleyl alcohol. The final production of vanillin reached 789 µM (119 mg/L) or 79% conversion. [41].

The chemo-biological upcycling of PET to the multifunctional coating material, catechol, was also reported. First, PET waste was glycolyzed to a mixture of PET oligomers. Then, an enzymatic hydrolysis of the glycolyzed products was performed, turning the mixture without previous purification into TA, by *Bacillus subtilis* esterase (Bs2Est). The catechol production from TA was consequently conducted, using a catechol biosynthesis strain (obtained through the combination of the TA degradation module and catechol biosynthesis module in *E. coli*). The final titer of catechol was 5.97 mM, accounting for 99.5% conversion by mol of TA. Catechol shows great functions as a coating material without the need for an adherence layer, and its antibacterial activity is comparable to chitosan [42].

Table 1. PET bioupcycling.

Depolymerization Strategy	Depolymerization Products Used as a Feedstock for Fermentation Step	Fermentation Strategy	Products from Fermentation	Titer	Productivity	Yield	Ref.
Hydrolytic pyrolysis at 450 °C	Solid product mixture (terephthalic acid (TA), oligomers, benzoic acid, and others)	Fermentation in shake flask containing 4.2 g/L of PET-derived sodium terephthalate and 67 mg/L of nitrogen at 30 °C for 48 h by *Pseudomonas putida* GO16	medium chain length PHA (mclPHA)	0.25 g/L	8.4 mgPHA/L/h	0.27 gPHA/gCDW	[36]
Hydrolytic pyrolysis at 450 °C	Solid product mixture (TA, oligomers, benzoic acid, and others)	Fermentation in shake flask containing 4.2 g/L of PET-derived sodium terephthalate and 67 mg/L of nitrogen at 30 °C for 48 h by *P. putida* GO19	mclPHA	0.25 g/L	8.4 mgPHA/L/h	0.23 gPHA/gCDW	[36]
Hydrolytic pyrolysis at 450 °C	Solid product mixture (TA, oligomers, benzoic acid, and others)	Fermentation in shake flask containing 4.2 g/L of PET-derived sodium terephthalate	mclPHA	0.27 g/L	4.4 mgPHA/L/h	0.24 gPHA/gCDW	[36]

Depolymerization Strategy	Depolymerization Products Used as a Feedstock for Fermentation Step	Fermentation Strategy	Products from Fermentation	Titer	Productivity	Yield	Ref.
		and 67 mg/L of nitrogen at 30 °C for 48 h by *P. putida* GO23					
Pyrolysis	TA	Fed-batch fermentation in 19.5 L-stirred tank reactor with controlled pH of 6.9 and dissolved oxygen (DO) level above 40% at 30 °C for 48 h by *P. putida* GO16 supplied with TA as the sole growth and PHA substrate	mclPHA	2.61 g/L	0.05 g/L/h	0.30 gPHA/gCDW	[39]
Pyrolysis	TA	Fed-batch fermentation in 19.5 L-stirred tank reactor with controlled pH of 6.9 and DO level above 40% at 30 °C for 48 h by *P. putida* GO16 supplied with waste glycerol (WG) as growth substrate and TA as PHA substrate	mclPHA	5.22 g/L	0.11 g/L/h	0.36 gPHA/gCDW	[39]
Pyrolysis	TA	Fed-batch fermentation in 19.5 L-stirred tank reactor with controlled pH of 6.9 and DO level above 40% at 30 °C for 48 h by *P.*	mclPHA	5.30 g/L	0.11 g/L/h	0.35 gPHA/gCDW	[39]

Depolymerization Strategy	Depolymerization Products Used as a Feedstock for Fermentation Step	Fermentation Strategy	Products from Fermentation	Titer	Productivity	Yield	Ref.
		putida GO16 supplied with TA as the sole growth and PHA substrate					
Pyrolysis	TA	Fed-batch fermentation in 19.5 L-stirred tank reactor with controlled pH of 6.9 and DO level above 40% at 30 °C for 48 h by *P. putida* GO16 supplied with WG as growth and PHA substrate and TA as PHA substrate only	mclPHA	4.98 g/L	0.10 g/L/h	0.35 gPHA/gCDW	[39]
Pyrolysis	TA	Fed-batch fermentation in 19.5 L-stirred tank reactor with controlled pH of 6.9 and DO level above 40% at 30 °C for 48 h by *P. putida* GO16 supplied with WG and TA as both growth and PHA substrates	mclPHA	4.42 g/L	0.09 g/L/h	0.36 gPHA/gCDW	[39]
Enzymatic degradation by recombinant leaf-branch compost cutinase (LCC)	TA, ethylene glycol (EG), mono-(2-hydroxyethyl)terephthalic acid (MHET), di-(2-hydroxyethyl)terephthalic acid (BHET)	Fermentation in 5 L-stirred tank reactor with controlled pH of 7.0 and DO level above 20% at 30 °C for 28 h *Pseudomonas umsongensis*	mclPHA	0.15 g/L	NA	0.014 gPHA/gSubstrate	[1]

Depolymerization Strategy	Depolymerization Products Used as a Feedstock for Fermentation Step	Fermentation Strategy	Products from Fermentation	Titer	Productivity	Yield	Ref.
		GO16 KS3 supplied with hydrolyzed PET at the amount to yield 40 mM of TA and EG and limited inorganic nutrient					
Enzymatic degradation by recombinant LCC	TA, EG, MHET, BHET	Fermentation in shake flask containing Delf medium with diluted (1:20) hydrolyzed PET (TA and EG concentration of 15–18 mM) at 30 °C for 24 h by *P. umsongensis* GO16 KS3 pSB01	Hydroxyalkanoyloxy-alkanoate (HAA)	35 mg/L	5 mg/L/h	0.01 gHAA/gTA	[1]
Microwave radiation for 50 min at 230 °C	TA	Bioconversion by metabolically engineered *E. coli* strain PCA-1 and HBH-2 to convert TA to intermediate protocatechuic acid (PCA), and then to gallic acid (GA), at 30 °C and 250 rpm for 24 h in 50 mM Tris buffer (pH 7.0) containing 2% (*w*/*v*) glycerol	GA	2.7 mM	NA	0.925 M_{GA}/M_{TA}	[40]
Microwave radiation for 50 min at 230 °C	TA	Bioconversion by metabolically engineered *E. coli* strain PG-1a to convert	Pyrogallol	1.1 mM	NA	0.327 $M_{Pyrogallol}/M_{TA}$	[40]

Depolymerization Strategy	Depolymerization Products Used as a Feedstock for Fermentation Step	Fermentation Strategy	Products from Fermentation	Titer	Productivity	Yield	Ref.
		TA to intermediate PCA, GA, and then pyrogallol, at 30 °C and 250 rpm for 6 h in 50 mM Tris buffer (pH 7.0) containing 2% (*w*/*v*) glycerol					
Microwave radiation for 50 min at 230 °C	TA	Bioconversion for 6 h by metabolically engineered *E. coli* strain CTL-1 and MA-1 to convert TA to intermediate catechol, and then to muconic acid (MA)	MA	2.7 mM	NA	0.854 M_{MA}/M_{TA}	[40]
Microwave radiation for 50 min at 230 °C	TA	Bioconversion using double-catalyst VA-2a system for 48 h by metabolically engineered *E. coli* strain PCA-1 and OMT-2^{His} to convert TA to intermediate PCA and then to vanillic acid (VA), in 50 mM Tris buffer (pH 7.0) containing 10% (*w*/*v*) glycerol, 10 g/L yeast extract, 20 g/L peptone, and 2.5 mM L-methionine	VA	1.4 mM	NA	0.416 M_{VA}/M_{TA}	[40]
Microwave radiation for 50	EG	Bioconversion by *Gluconobacter*	Glycolic acid (GLA)	NA	NA	0.986 M_{GLA}/M_{EG}	[40]

Depolymerization Strategy	Depolymerization Products Used as a Feedstock for Fermentation Step	Fermentation Strategy	Products from Fermentation	Titer	Productivity	Yield	Ref.
min at 230 °C		*oxydans* KCCM 40109 using 10.7 mM of EG from PET hydrolysate as a feedstock, at 30 °C and 220 rpm in shake flask at the working volume of 1 L					
-	EG (mock substrate to study upcycling of PET-derived monomer)	Fermentation in shake flask containing 10% (*v*/*v*) EG in 250 mM potassium phosphate buffer (pH 7.0) at 30 °C with gentle stirring and aeration at 1 VVM for 120 h by *Pichia naganishii* AKU 4267	GLA	105 g/L	NA	0.880 M_{GLA}/M_{EG}	[43]
-	EG (mock substrate to study upcycling of PET-derived monomer)	Fermentation in shake flask containing 10% (*v*/*v*) EG in 250 mM potassium phosphate buffer (pH 7.0) at 30 °C with gentle stirring and aeration at 1 VVM for 120 h by *Rhodotorula* sp. 3Pr-126	GLA	110 g/L	NA	0.922 M_{GLA}/M_{EG}	[43]
-	EG (mock substrate to study upcycling of PET-derived monomer)	Fermentation in shake flask containing 100 mM of EG in nitrogen limiting M9 medium	mclPHA	NA	NA	0.32 gPHA/gCDW and 0.06 gPHA/gEG	[44]

Depolymerization Strategy	Depolymerization Products Used as a Feedstock for Fermentation Step	Fermentation Strategy	Products from Fermentation	Titer	Productivity	Yield	Ref.
		(0.132 g/L of $(NH_4)_2SO_4$) at 30 °C for more than 72 h by *P. putida* MFL185 (engineered strain that has the *tac* promoter inserted before the native glycolate oxidase operon and harbor overexpression)					
-	EG (mock substrate to study upcycling of PET-derived monomer)	Anaerobic fermentation of 50 mM EG at 30 °C by acetogenic bacterium *Acetobacterium woodii*	Acetate	10.4 mM	3.6 µmol/mg/h	NA	[45]
-	EG (mock substrate to study upcycling of PET-derived monomer)	Anaerobic fermentation of 50 mM EG at 30 °C by acetogenic bacterium *A. woodii*	Ethanol	12.0 mM	4.8 µmol/mg/h	NA	[45]
Enzymatic degradation by semi-purified LCC (pH 10.0) at 72 °C for 48 h	PET hydrolysate	Bioconversion using metabolically engineered *E. coli* RARE_pVanX to convert TA to intermediate protocatechuate (PC), and then to vanillin using optimized condition: M9-glucose supplemented	Vanillin	300–400 µM	NA	NA	[41]

3. Bioupcycling of Polyurethanes (PU)

PU is a general term used for a class of polymers typically derived from the polycondensation of (poly)isocyanates (−NCO) and polyols (exothermic reactions) [48]. There are three main types of PU: polyester, polycaprolactone, and

Depolymerization Strategy	Depolymerization Products Used as a Feedstock for Fermentation Step	Fermentation Strategy	Products from Fermentation	Titer	Productivity	Yield	Ref.
		with L-Met and nBuOH as a protein expression media, pH 5.5, room temperature for 24 h, in situ product removal (ISPR) by oleyl alcohol					[49]
	[50]						[26]
	[51]						[26]
-	TA (mock substrate to study upcycling of PET-derived monomer)	Bioconversion using metabolically engineered *E. coli* RARE_pVanX to convert TA to intermediate PC and then to vanillin using optimized condition: M9-glucose supplemented with L-Met and nBuOH as a protein expression media, pH 5.5, room temperature for 24 h, ISPR by oleyl alcohol	[52] [53] [54] Vanillin [55][56]	789 µM	NA	0.79 $M_{vanillin}/M_{TA}$	[41]
Chemical glycolysis at 200 °C for 3 h [57]	Mixture of BHET, MHET, and PET oligomers at 84.8, 7.7, and 8.7%, respectively	Enzymatic hydrolysis of the glycolyzed products (the mixture) into TA by *Bacillus subtilis* esterase (BsEst) (2 U/mL at 30 °C and 1000 rpm), following by producing	Catechol	5.97 mM	NA	0.995 $M_{Catechol}/M_{TA}$ [59]	[42]
	[49]		[58]				
	[60]						
	[61]						

in many [26]. In many PU % of PU ly 5% is ge scale nders to nes, until recycling monia or onsumer partners, n of two Poland-chemical chemical gradation a more class of ase-type chemical osity; (3) rate than reported to utilize he main d mixed-ses, and such as *Aspergillus tubingensis* [62], *Penicillium* sp. [63], *Cladosporium cladosporioides* complex [64], or fungal communities [65] were also reported for their ability to degrade PU.

Besides BDO and AA, other PU degradation products are EG, 2,4′-toluenediamine (TDA), and 4,4′-methylenedianiline (MDA). They can be used to synthesize new PU or other polyesters, e.g., PHA, polybutylene succinate (PBS), poly(1,3-propylene succinate-*ran*-1,4-butylene succinate) (PPBS), and poly(1,3-propylene adipate-*ran*-1,4-butylene adipate) (PPBA) [52].

Last but not least, a recent study reported the upcycling processes of PU waste to rhamnolipid biosurfactants, [48] (**Table 2**). They successfully showed the growth of a defined mixed culture composed of three *Pseudomonas putida* KT2440 strains obtained by adaptive laboratory evolution on mock PU hydrolysate. The highest specific

Depolymerization Strategy	Depolymerization Products Used as a Feedstock for Fermentation Step	Fermentation Strategy	Products from Fermentation	Titer	Productivity	Yield	Ref.
		catechol from PET hydrolysates using a					0.10 g/L

Depolymerization Strategy	Depolymerization Products Used as a Feedstock for Fermentation Step	Fermentation Strategy	Products from Fermentation	Titer	Productivity	Yield	Ref.
Enzymatic degradation of polycaprolactone polyol-based PU by esterase (E3576) in 0.1 M phosphate buffer (pH 7.0). The enzyme solution was replaced every 3–4 d to overcome a loss of enzymatic activity.	6-hydroxycaproic acid (1 g/L)	-	-	-	-	-	[66]
-	Adipic acid (AA) (mock substrate to study upcycling of PU-derived monomer)	Bioconversion (at 30 °C and 200 rpm for 135 h) using metabolically engineered *P. putida* KT2440 A12.1p pPS05 to convert AA into HAA and then to rhamnolipid	Rhamnolipid	0.02 g/L	NA	0.014 gRhamnolipid/gSubstrate	[48]
-	1,4-Butanediol (BDO) (mock substrate to study upcycling of PU-derived monomer)	Bioconversion (at 30 °C and 200 rpm for 135 h) using metabolically engineered *P. putida* KT2440 B10.1 pPR05 to convert BDO into HAA and	Rhamnolipid	0.13 g/L	NA	0.088 gRhamnolipid/gSubstrate	[48]

Depolymerization Strategy	Depolymerization Products Used as a Feedstock for Fermentation Step	Fermentation Strategy	Products from Fermentation	Titer	Productivity	Yield	Ref.
		then to rhamnolipid					
-	EG (mock substrate to study upcycling of PU-derived monomer)	Bioconversion (at 30 °C and 200 rpm for 135 h) using metabolically engineered *P. putida* KT2440 Δ*gclR* ΔPP_2046 ΔPP_2662::14d to convert EG into HAA and then to rhamnolipid	Rhamnolipid	0.07 g/L	NA	0.038 gRhamnolipid/gSubstrate	[48]
-	AA + BDO + EG (mock hydrolysate to study upcycling of PU-derived monomers)	Bioconversion (at 30 °C and 200 rpm for 210 h) using mixed culture of three metabolically engineered *P. putida* KT2440 to convert the mock hydrolysate into HAA and then to rhamnolipid	Rhamnolipid	0.1 g/L	NA	0.008 gRhamnolipid/gSubstrate	[48]
	AA + BDO + EG + 2,4-toluenediamine (TDA) (mock hydrolysate to study upcycling of PU-derived monomers)	Bioconversion (at 30 °C and 200 rpm for 210 h) using mixed culture of three metabolically engineered *P. putida* KT2440 to convert the mock hydrolysate	Rhamnolipid	0.02 g/L	NA	0.002 gRhamnolipid/gSubstrate	[48]

References

Depolymerization Strategy	Depolymerization Products Used as a Feedstock for Fermentation Step	Fermentation Strategy	Products from Fermentation	Titer	Productivity	Yield	Ref.
		into HAA and then to rhamnolipid without extraction of TDA					
	AA + BDO + EG + TDA (mock hydrolysate to study upcycling of PU-derived monomers)	Bioconversion (at 30 °C and 200 rpm for 210 h) using mixed culture of three metabolically engineered *P. putida* KT2440 to convert the mock hydrolysate into HAA and then to rhamnolipid with extraction of TDA at pH 3.5	Rhamnolipid	0.07 g/L	NA	0.005 gRhamnolipid/gSubstrate	[48]

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