Biodegradation of Polyolefins

Subjects: Microbiology

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Polyolefins, including PE, PP and PS, are composed of C–C and C–H bonds, which are more stable against degradation than ester bonds. During polyolefin biodegradation, C–C and C–H bonds are oxidized. Many types of microorganisms have been isolated from sea water, compost and activated sludge with the capacity for polyolefin biodegradation.With the development of synthetic biology and metabolic engineering strategies, engineered strains could be used to degrade plastics.

Keywords: polyolefins ; polyethylene ; biodegradation

1. Isolated Microorganisms

Numerous of microorganisms with the capacity for polyolefin degradation, including bacteria, fungi and microbial consortia, have been isolated from the environment, such as soil containing plastic waste, the ocean and the guts of plastic-eating worms. These microorganisms are capable of utilizing polyolefins as sole carbon source or can generate depolymerases involved in polyolefin degradation. Therefore, the screening of these microorganisms is vital for the further degradation of polyolefins. Polyolefin-degrading bacteria can be identified by the following procedure. Soil samples collected from an area containing polyolefin waste was mixed with water and shaken [1]. In order to isolate strains from waxworm guts, worm gust need to be isolated and suspended in water [2]. Then, a portion of the suspension was transferred to polyolefin-containing medium. After incubation for several days, the polyolefin-degrading strains would be isolated through gradient dilution the of medium or collecting the strains on the agar media on which PE fragments were spread [2][3][4].

1.1. Single Bacteria

At present, most single strains that are capable of degrading polyolefins are bacteria, which can form biofilms on the surface of polyolefins or destroy the surface of polyolefins. An increased rate of PE degradation was detected after LDPE was pretreated with UV light [5]. Changes in the molecular weight and molecular number of LDPE were also detected after incubation with *R. ruber* C208 [6]. It was reported that *R. ruber* C208 is also capable of degrading PS, achieving a weight loss of 0.8% after 8 weeks [7]. Moreover, *R. rhodochrous* ATCC 29672 exhibited the ability to degrade PP based on the characterization of changes in the metabolic activity of bacteria, such as ATP content, ADP/ATP ratio and cell viability [8].

Biodegradation should not only be determined by weight loss. Chemical properties, as well as changes in Mw (molecular weight) and Mn (molecular number), which can be characterized Fourier transform infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), nuclear magnetic resonance (NMR) and gel permeation chromatography (GPC) analyses, are also vital for the measurement of the degree of polyolefin degradation. After the biodeterioration stage, the nature and occurrence of functional groups on the surface of polyethylene substrates are changed, which can be studied by FTIR [9] spectroscopy, XPS [3] and NMR [10]. After the biofragmentation stage, the length of polyolefins is shorted. The result of biofragmentation can be proven by using GPC to measure changes in Mw and Mn [11]. After the biofragmentation stage, degradation products (such alkanes, alkenes, etc.) can be determined by GC-MS (gas chromatography mass spectrometry) [12]. Moreover, the result of bioassimilation can be determined through measurement of dry biomass weight of polyethylene-containing media. Finally, the percent of mineralization can be analyzed by CO_2 measurement [13]. Furthermore, scanning electron microscopy (SEM) is another vital method used to characterize the surface features of polyolefins.

As previously reported, some waxworms can chew and eat plastics [3][14][15][16][17]. *Enterobacter asburiae* YT1 and *Bacillus* sp. YP1 were isolated from the guts of waxworms with the capability of degrading PE. Over 28-day incubation of the two strains on PE films, the physical properties (tensile strength and surface topography), chemical structure (hydrophobicity and appearance of carbonyl groups), Mw (accompanied by the formation of daughter products) and weight loss were detected [3]. Kyaw et al. [18] incubated *Pseudomonas aeruginosa* PAO1 with LDPE films. After

exposure to *P. aeruginosa* PAO1, the LDPE sample turned into a mixture of long-chain fatty acids, esters, hydrocarbons, oxygenated chemical compounds predominantly containing aldehydes, ketones, esters and ether groups, unsaturated fatty acids and certain unknown compounds. Given that the structure of polyolefins is similar to that of alkanes, some strains with an ability to degrade alkanes also have an effect on PE degradation. *Alcanivorax borkumensis*, a bacterial strain isolated from the sea that can utilize alkane as carbon source [19] was found to induce a weight loss of 3.5% in 7 days [20].

1.2. Microbial Consortia

A mixed microbial consortium consisting of two *Bacillus* sp. and two *Paenibacillus* sp. was isolated from a landfill site. After incubation with the mixed microbial consortium for 30 days, the weight and the mean diameter of the PE sample were reduced 16.7% and 22.8%, respectively [21]. A microbial consortium consisting of *Lysinibacillus xylanilyticus* and *Aspergillus niger* was found to be capable of degrading PE [22]. After UV irradiation, the mineralization percentage increased from 15.8% to 29.5% after incubation for 126 days in soil, and according to FTIR and XRD, the chemical properties also improved. Skariyachan et al. [23] isolated a microbial consortium comprising *Brevibacillus* sps. and *Aneurinibacillus* sp. from waste landfills. During a 140-day incubation of the two strains on PE samples, weight loss for LDPE and HDPE strips reached 58.2% and 46.6% respectively, and weight loss for LDPE and HDPE pellets were 45.7% and 37.2%, respectively. As with a single bacterium, UV pretreatment aided in the degradation of polyolefin samples. In addition, the result of GC-MS analysis indicated that PE samples were degraded into cis-2-chlorovinylacetate, tri-decanoic acid and octadecanoic acid. Muenmee et al. [24] pretreated HDPE, LDPE, PP and PS samples with UV for 200 h, then mixed the plastics together. To simulate a landfill environment, the plastics mixture was placed in simulated lysimeters with a synthetic landfill gas (60% CH_4:40% CO_2) and a microbial consortium composed of *Methylocyctis* sp., *Methylocella* sp., *Methylobactor* sp., *Methyloccus capsulatus.*, *Nitrosomonas* sp., *Nitrosomonas europaea, Nitrobacter winogradskyi*, *Nitrobacter hamburgensis*, *Burkholderia* sp., *Pseudomonas* sp. and *Xanthobacter* sp. After a reaction period of 3 months under semi-aerobic landfill conditions where different aeration rates were supplied, the plastic samples were degraded into hydrocarbon and oxygenated compounds, such as aliphatic alkanes, alkenes, alcohols and esters. The degradation products of different plastic types, such as the degraded products of HDPE, are mainly alkanes ($C_{24}H_{50}$, $C_{32}H_{66}$), alkenes ($C_{15}H_{30}$, $C_{19}H_{38}$) and some alcohols ($C_{15}H_{32}O$, $C_{20}H_{42}O$) were found, while for LDPE, only one alcohol ($C_{11}H_{24}O$) was found.

2. Engineered Strains

2.1. Hydrolases Capable of Polyolefin Degradation

Most the enzymes capable of degrading PE are oxidoreductases. As shown in **Table 1**, well-known identified enzymes with the ability to oxidize polyolefins include laccase, manganese peroxidase, alkane hydroxylase and soybean peroxidase. Furthermore, a PS-degrading enzyme called hydroquinone peroxidase was identified from *Azotobacter beijerinckii* HM121. However, no PP-degrading enzymes have been identified to date. The result of enzymatic degradation of PE is that oxidation groups are introduced into the PE chain, which means polyolefins cannot be oxidized into monomers as the only act of these enzymes. Most PE-degrading enzymes can only perform terminal oxidation (the terminal carbon in polyolefins be oxidized) and subterminal oxidation (the carbon adjacent to the terminal carbon in polyolefins be oxidized) of PE. For example, laccase and manganese peroxidase can perform terminal oxidation, and the AlkB family can degrade n-alkanes, the main component of polyethylene, through either terminal or subterminal hydroxylation reactions [25]. Therefore, an ideal polyolefin-degrading enzyme has high hydroxylation activity against any carbon in the carbon chain so as to achieve the efficient transformation of polyolefin to its oligomer or monomer.

Laccases (EC 1.10.3.2), which belong to the so-called blue-copper family of oxidases, can catalyze the oxidation of a wide range of phenols and arylamines. Laccases, which are glycoproteins, have been reported in higher plants, fungi and bacteria. A laccase was purified from *R. ruber* C208 with the ability to degrade LDPE. As laccases contain four copper ion bonding sites, copper markedly affects their induction and activity, resulting in PE degradation. mRNA quantification by RT-PCR revealed a 13-fold increase in laccase mRNA levels in copper-treated cultures compared with an untreated control. The addition of copper to C208 cultures containing PE enhanced the biodegradation of PE by 75% [1][26]. A laccase mediator system (LMS) is composed of laccase and some small-molecule compounds that are easily oxidized by laccase, such as HBT, ABTS and DMP. In the process of LMS oxidation, laccase oxidizes the mediator first; then, the oxidized mediator oxidizes the substrate. It was reported that in the presence of a mediator, laccase can oxidize some substrates that it cannot oxidize alone. HBT, which has been used for PE degradation, reacts with non-phenolic models by a radical mechanism involving hydrogen atom abstraction [27]. Some researchers treated polyethylene with LMS using HBT (0.2 mM) as a mediator; after 3 days, the polyethylene membrane exhibited no elongation, and its relative tensile

strength decreased by about 60%, which is higher than in the absence of HBT (20%) [28]. Johnnie et al. used laccase from *Trichoderma viride* fungus and 1-HBT to degrade LDPE. After incubation for 10 days, the weight loss of LDPE came to 2.3% [29].

Cytochrome P450 (CYP, P450), a member of a superfamily of heme–thiolate proteins, is distributed in most living organisms. There are more than 300,000 P450 genes. However, no P450 genes were found in *E. coli*, which means that *E. coil* is a good chassis for the heterologous expression of P450 genes. P450 enzymes can identify multiple substrates and catalyze diverse reactions, such as C–H hydroxylation; C=C double-bond epoxidation; heteroatom oxygenation; O-, N- and S-dealkylation; aromatic coupling; and C–C bond cleavage [30]. Because other PE-degrading enzymes can only perform terminal oxidation and subterminal oxidation of PE, the application of an ideal P450 enzyme that can cleave PE into short chains would contribute to the biodegradation of PE.

Alkane, which is composed of C–C bonds and C–H bonds, has a similar structure to that of PE. Therefore, alkane monooxygenase enzymes are potential candidates for the degradation of PE. One alkane hydroxylase, namely AlkB, has been reported to degrade PE [30]. AlkB, which was first identified in alkane-consuming *Pseudomonas* species isolated from oil-contaminated areas, is a membrane-bound, non-heme di-iron monooxygenase [31].

Manganese peroxidase was purified from a lignin-degrading fungus: *P. chrysosporium*. Manganese peroxidase was first identified as a lignin-degrading enzyme. It was reported that the addition of Mn (II) to nitrogen- or carbon-limited culture medium enhanced PE degradation [32].

2.2. Engineered Chassis for Polyolefin Biodegradation

As shown in **Table 1**, the microorganisms that are capable of secreting polyolefin biodegrading enzymes are not model organisms, which means that they are difficult to genetically engineer. Two model organisms, *E. coli* and *Y. lipolytica,* have been applied to the expression and secretion of polyolefin-biodegrading enzymes to date [33][34][35]. The heterologous expression of polyolefin-degrading enzymes in model organisms can efficiently increase the expression level of polyolefin-degrading enzymes through genetic engineering in model organisms.

E. coli*:E. coli* is one of the most widely used model microorganisms for production of recombinant proteins. As a model microorganism, *E. coli* has advantages in many aspects, such as a simple genetic background, ease of genetic modification and simple growth conditions. Engineered *E. coli* has been used to express alkane hydroxylase to degrade LMWPE. The *alkB* gene, which was cloned from *Pseudomonas* sp. E4, was introduced into *E. coli* BL21. After incubation for 80 days at 37 °C with engineered *E. coli,* 19.3% of the LMWPE was degraded [13]. The rubredoxin and rubredoxin reductase could help alkane monooxygenase to transfer electron. If the rubredoxin and rubredoxin reductase are co-expressed with alkane monooxygenase, the conversion rate can be increased. Researchers fused and expressed *alkB* with its coenzyme genes—*rubA1, rubA2 and rub*—in *E. coli* BL21. The result indicated that 30.5% of the carbon of LMWPE-1 degraded into CO_2 after 78 days [36]. Another study revealed that an alkane-1-monooxygenase (AlkB) in *Acinetobacter johnsonii* JNU01 degraded PS, and this finding was later confirmed by recombinant *alkB* in *E. coli* BL21 [37]. These researchers also expressed *alkB2* in *E. coli,* and the result indicates that *alkB2* was more efficient for low-molecular-weight PE biodegradation than *alkB1* [33]. A laccase gene isolated from a marine fungus was expressed in *E. coli* and showed PE degradation ability [38].

Many recent studies have shown that engineered *E. coli* can be used for laccase heterologous expression and secretion [39][40][41]. Ihssen et al. [34] expressed five novel bacterial laccase-like multicopper oxidases (LMCOs) of diverse origin. However, a potential issue with laccase expression in *E. coli* is that it is easy for *E. coli* to form inclusion bodies when expressing extracellular enzymes. Mo et al. [35] expressed three laccases from three different organisms, namely Lac1326 from marine sediment samples, fungal tvel5 laccase from *Trametes versicolor* and bacterial BPUL laccases from *Bacillus pumilus* for the purpose of degrading β-estradiol. The result of Western blot analysis indicates that laccase was detected both in vivo and in vitro in *E. coli,* which means that some laccase stayed in inclusion bodies instead of being secreted in vitro. Given that laccase has been widely used for the biodegradation of PE [10][29][42][43], the heterologous expression of laccase in *E. coli* is a potential method for the biodegradation of PE.

Yarrowia lipolytica*:Y. lipolytica* is a Crabtree-negative ascomycete yeast with good protein secretion capacities. Compared to other yeasts, *Y. lipolytica* lacks α-1,3-mannosyltransferase, a factor that limits the amount of excessive mannosylation of secreted heterologous glycoproteins and constitutes a valuable asset for the production of therapeutic proteins [44]. *Y. lipolytica* W29 is a wild-type strain with a remarkable characteristically high secretion level of proteins [45]. *Y. lipolytica* W29 ura302 was obtained through genetic convention of URA3 into ura3-302 in *Y. lipolytica* W29, and it was able to utilize sucrose and molasses as a carbon source under the control of XPR2 promoter. After the genetic

convention of XPR2 into xpr2-322 and AXP1 into axp1-2, which indicates inactivation of alkaline extracellular protease and acid extracellular protease that would degrade foreign extracellular protein, a strain with high heterologous protein production capability called *Y. lipolytica* Po1f was obtained. A new strain called *Y. lipolytica* Po1g that carries a pBR322 docking platform was obtained through the integration of PINA300′ plasmid in *Y. lipolytica* Po1f [46]. *Y. lipolytica* Po1g was induced with a YLEX kit for expression/secretion of heterologous proteins [47]. A laccase from the white-rot fungus *Trametes versicolor* was expressed in *Y. lipolytica* Po1g for the biodegradation of PE. Compared to the yeast secretion signal, the native secretion signal showed higher enzyme activity in the culture medium. The yield of laccase reached 2.5 mg/L (0.23 units/mL) [26]. Laccase has been widely used for the biodegradation of PE, and the heterologous expression of laccase in *Y. lipolytica* is a promising method of PE degradation.

Table 1. Enzymes capable of polyolefin degradation.

Plastics	Enzymes	Enzyme Source	Pretreatment	Experimental Condition	Result	Reference
PE	Laccase	*R. ruber C208*	Unpretreated LDPE film	Incubation for 30 days at 30 °C	Weight loss: 1.5–2.5%; reduction of 20% in Mw and 15% in Mn	[1]
	Manganese peroxidase	*Phanerochaete chrysosporium*	Unpretreated PE film	Incubation for 12 days at 37 °C	Mw decreased	[32]
	Soybean peroxidase	Soybean	Unpretreated HDPE film	Reaction for 2 h at 60 °C	Hydrophilicity increased	[48]
	Alkane hydroxylase	*Pseudomonas* sp. E4	Unpretreated LMWPE sheet	Incubation for 80 days at 37 °C	Weight loss: 19.3%	[13]
	Alkane hydroxylase	*Pseudomonas aeruginosa* E7(uniport Q9I0R2)	Unpretreated LMWPE film	Incubation for 50 days at 37 °C	Weight loss: 19.6–30.5%	[33]
PS	Hydroquinone peroxidase	*Azotobacter beijerinckii* HM121	Unpretreated PS film	Incubation for 20 min	Mw decreased	[49]
	Alkane hydroxylase	*A. johnsonii* JNU01	Unpretreated low-molecular-weight PS powder	Incubation for 7 days at 28 °C	Confirmed by FTIR and SEM	[26]

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