# Biodegradation of plastics

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Widespread studies on the biodegradation of plastics have been carried out in order to overcome the environmental problems associated with synthetic plastic waste. Recent work has included studies of the distribution of synthetic polymer-degrading microorganisms in the environment, the isolation of new microorganisms for biodegradation, the discovery of new degradation enzymes, and the cloning of genes for synthetic polymer-degrading enzymes.

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Abbreviations

| | |
|---|---|
| HB | 3-hydroxybutanoate |
| OPH | oxidized polyvinyl alcohol hydrolase |
| PCL | polylcaprolactone |
| PHA | polyhydroxyalkanoate |
| PHB | polyhydroxybutyrate |
| PLA | polylactic acid |
| PQQ | pyrroloquinoline quinone |
| PUR | polyurethane |
| PVA | polyvinyl alcohol |
| PVADH | polyvinyl alcohol dehydrogenase |

## Introduction

Approximately 140 million tonnes of synthetic polymers are produced worldwide each year. These polymers are extremely stable, and do not readily enter into the degradation cycles of the biosphere. Environmental pollution by synthetic polymers, such as waste plastics and water-soluble synthetic polymers in wastewater, has been recognized as a large problem. In order to support continued sustainable development throughout the world, this problem must be addressed. In view of this, the biodegradation of plastics has been studied extensively for the past three decades. Some types of plastic have been shown to be biodegradable, and their degradation mechanisms have progressively become clearer. This paper reviews the recent advances in this area.

## Polyesters

Polyesters are polymers in which component monomers are bonded via ester linkages. Many kinds of esters occur in nature and enzymes that degrade them, esterases, are ubiquitous in living organisms. Ester-linkages are generally easy to hydrolyze. In fact, a number of synthetic polyesters have been found to be biodegradable, and bacterial polyesters (polyhydroxyalkanoates) have been used to make biodegradable plastics. Several polyesters are now produced on a semi-commercial scale by a number of companies that make biodegradable plastics.

## Polyhydroxyalkanoates

Polyhydroxybutyrate (PHB) is a naturally occurring polyester that accumulates in bacterial cells as a carbon and energy storage compound. PHB and copolymers containing other hyroxyalkanotes, such as 3-hydroxyvalerate, are being used for the manufacture of biodegradable plastics [1••]. PHB and other polyhydroxyalkanoate (PHA) polymers are metabolized by many microorganisms, and a number of bacterial PHA depolymerases and their genes have been isolated [2]. The PHA depolymerases are serine hydrolases and their protein sequences contain four regions: a signal sequence; a catalytic domain containing the lipase box; a substrate-binding domain that acts as an adsorption site for polymer substrates; and a domain that links the catalytic and substrate-binding domains. The linking domain can comprise either a sequence enriched with threonine or a fibronectin type III like sequence. In addition, the linking region of the PHB depolymerase from *Pseudomonas stutzeri* was recently shown to have a cadherin-like sequence [3]. PHA depolymerases usually have one substrate-binding domain, although recently a PHB depolymerase with two substrate-binding domains were reported [3,4]. It is thought that the presence of two substrate-binding domains might enlarge substrate specificity or enhance adsorption of the enzyme.

In terms of substrate specificity, PHB depolymerases are able to degrade all-(*R*) chains, cyclic-(*R*) oligomers, oligolides, and polymers composed of *rac*-hydroxybutanoate; they are unable to degrade all-(*S*) or syndiotactic (*R,S*) chains. The stereoselectivity of PHB depolymerase from *Alcaligenes faecalis* T1 was studied in detail using 3-hydroxybutanoate (HB) oligomers that contained up to eight HB units with given sequences of (*R*) and (*S*) configurations along the polymer chain [5••]. Analysis of the hydrolysis kinetics and products led to a proposed model for substrate recognition. The enzyme is an *endo* esterase that recognizes the orientation of the chain relative to its active site. The binding site contains four subsites, three of which must be occupied by HB units for cleavage to occur at all, and all four of which must be occupied for cleavage to take place at the maximum rate. Furthermore, the central two subsites, between which cleavage occurs, must be occupied by (*R*)HB units, whereas the terminal subsites can also be occupied by (*S*)HB units.

## Polylcaprolactone

Polylcaprolactone (PCL) is a synthetic polyester that can easily be degraded by microorganisms. Like PHB-degrading bacteria, PCL-degrading bacteria are also widely distributed in the environment [6,7], although little is known about PCL depolymerases. PCL is degraded by lipases and esterases [8]. One type of PCL-degrading enzyme includes lipases that degrade glycerides [9]. PHB

depolymerases did not hydrolyze PCL [10]. On the other hand, Nishida and Tokiwa [11] showed that some fungal phytopathogens degrade PCL and indicated the possibility that their cutinases, which degrade cutin (the structural polymer of the plant cuticle) may act as PCL depolymerases. Murphy *et al.* [12] provided several lines of evidence to show that the PCL depolymerase of the fungal pathogen *Fusarium* is in fact the cutinase: a cutinase-negative gene-replacement mutant of a *Fusarium solani* strain lacked PCL depolymerase activity; and in wild-type strains PCL depolymerase activity was induced with cutin and PCL hydrolysate, both of which induce cutinase. The chemical structure of a PCL trimer was shown to be similar to that of two cutin monomers, which are inducers of cutinase activity. The observation that cutinase can also act as a PCL depolymerase may be cited as an example of enzyme diversion. For this to occur it may be necessary to satisfy two prerequisites: relaxed substrate specificity and relaxed induction of the enzyme.

## Polylactic acid

Several companies now produce polylactic acid (PLA) on a semi-commercial scale for use in biodegradable plastics. As PLA is absorbed in animals and humans, the use of this polymer in medicine has been extensively developed [13]. The degradation of the polymer in animals and humans is thought to proceed via non-enzymatic hydrolysis [14]. Several enzymes can degrade the polymer: proteinase K, pronase and bromelain [15]; however, few have been characterized with regards to microbial degradation of the polymer. Only a few PLA-degrading microorganisms have been identified and are not thought to be widespread within the environment. Pranamuda *et al.* [16] analyzed 45 soil samples collected from various places around Tsukuba City, Japan, but only one soil sample contained PLA-degrading microorganisms. These organisms were identified by their ability to form a clear zone in an agar plate containing PLA powder. This ratio is much lower than that observed for PCL-degrading microorganisms. A PLA-degrading actinomycete, an *Amycolatopsis* sp. strain isolated from the sample, reduced 100 mg of PLA film by ~60% after 14 days in liquid culture at 30°C [16]. In addition to this strain, a thermophilic bacterium, *Bacillus brevis*, with PLA-degrading properties has been isolated from soil [17]. This strain degraded PCL molecules in a random manner and decreased 50 mg of PLA film by ~20% after 20 days in liquid culture at 60°C. PLA-degrading enzymes of these strains have not been examined. It might be said that PLA is too persistent or recalcitrant for microbes to attack. On the other hand, in a solid waste disposal site of Japan, PLA-degrading bacteria were detected together with bacteria capable of degrading other polymers, such as PHB and PCL [18]. PLA is degraded readily in compost: $^{14}C$-labeled PLA was mineralized in compost to $CO_2$ (about 90% degradation after 90 days) [19], although involvement of microorganisms in the depolymerization of PLA is unclear.

## Polyurethane

Polyurethane (PUR) is produced by the diisocyanate polyaddition process [20]. The characteristic chain link of this polymer is the urethane bond, although it is usually only present to a minor extent. In most cases, urethane groups link polyalkylene ether (polyether PUR) and/or polyester (polyester PUR) sequences with molecular masses of between ~ 200 and 6000. Since the first study on the fungal attack of PUR, a number of reports dealing with the biodeterioration of PUR by microorganisms have been published [21••]. Biodegradation by these organisms is, however, incomplete; their growth could not be supported by PUR and required additional carbon sources and nutrients. The PUR depolymerases of these microorganisms have not been examined in detail, although most degradation is thought to be carried out by esterases. Recently, a bacterial strain, *Comamonas acidovorans* TB-35, which utilizes a polyester PUR as carbon source has been reported [22]. The strain does not utilize polyether PUR, but utilizes polyester PUR containing polydiethyleneglycol adipate as the sole source of carbon. *C. acidovorans* completely degraded 50 mg of the PUR cubic specimen in 5 mL of a mineral salt medium within seven days at 30°C; in a medium with PUR as sole source of carbon and nitrogen 48% was degraded after seven days. Diethylene glycol and adipic acid were detected as degradation products, but no metabolites were found to indicate cleavage of the urethane linkage.

The polyester PUR degrading enzyme was isolated from *C. acidovorans* [23]. The enzyme is a cell-bound esterase that can hydrolyze polyester chains in PUR to diethylene glycol and adipic acid. The enzyme can also degrade polydiethyleneglycol adipate, tributyrin, and low molecular weight PLA, but did not degrade PHB, high molecular weight PLA, and triolein. The degradation of PUR is inhibited by the presence of a detergent that does not inhibit the hydrolysis of a water-soluble ester compound, suggesting that degradation proceeds via a two-step reaction: hydrophobic adsorption to the polymer surface followed by hydrolysis of the ester bond of PUR.

The gene for the polyester PUR degrading enzyme was cloned and sequenced [24]; the gene encodes a protein of 548 amino acid residues. The deduced protein sequence contains a signal sequence, the lipase box and catalytic triad, and three hydrophobic domains which are thought to have a role in the hydrophobic adsorption of the enzyme to the polymer surface. One of the three hydrophobic domains shows sequence similarity to the substrate-binding domain of PHA depolymerases but, with this exception, there is no significant similarity between PUR depolymerase and PHA depolymerases.

## Polyvinyl alcohol

Polyvinyl alcohol (PVA) is a vinyl polymer in which the mainchains are joined by only carbon–carbon linkages. The linkage is the same as those of typical plastics, such as

polyethylene, polypropylene and polystyrene, and water-soluble polymers, such as polyacrylamide and polyacrylic acid. Among the vinyl polymers produced industrially, PVA is the only one known to be mineralized by microorganisms.

PVA is water-soluble but also has thermoplasticity. In addition to its use as a water-soluble polymer (e.g. as a substituent for starch in industrial processes), it can also be molded in various shapes, such as containers and films. PVA can therefore be used to make water-soluble and biodegradable carriers, which may be useful in the manufacture of delivery systems for chemicals such as fertilizers, pesticides, and herbicides.

In 1973, it was shown for the first time that PVA is completely degraded and utilized by a bacterial strain, *Pseudomonas* O-3, as a sole source of carbon and energy [25]. However, as shown in an extensive screening of the PVA-degrading microorganisms from environmental samples [26], PVA-degrading microorganisms are not ubiquitous within the environment. Since then, only several PVA-degrading bacterial strains have been reported. Almost all of the degrading strains belong to the genus *Pseudomonas* [25–29], although some do belong to other genera [30,31].

Among the PVA-degrading bacteria reported so far, a few strains showed no requirement for pyrroloquinoline quinone (PQQ) [25,27]. A requirement of PQQ for PVA degradation by bacteria was shown for the first time by Shimao *et al.* [32], and has recently been reported by other research groups for other PVA-degrading bacteria [29,30]. Shimao and colleagues [26] isolated several PVA-degrading bacterial cultures using a medium that contained PVA as sole source of carbon and energy. The cultures were all symbiotic mixed cultures composed of two bacterial strains that could not grow on PVA in isolation [34]. From a PVA-utilizing mixed culture, *Pseudomonas* sp. VM15C and *Pseudomonas putida* VM15A were isolated. Their symbiosis is based on a syntrophic interaction, as shown in Figure 1. VM15C is a PVA-degrading strain that degrades and metabolizes PVA, while VM15A excretes a growth factor that VM15C requires for PVA utilization. The growth factor was identified as PQQ [32]. Although the PQQ supplier VM15A does not degrade or utilize PVA directly in the symbiotic mixed culture, it is thought to utilize some unidentified metabolites excreted from the VM15C cells growing on PVA. Thus, if PQQ is added to the medium, the PVA-degrading strain can grow on PVA in a pure culture. The PQQ concentration needed for PVA utilization is very low: ~10 μg/L is enough for the strain to grow on PVA at almost the maximum growth rate in pure culture. In the natural environment, however, in which PVA-degrading bacteria depend on other strains for PQQ, the PQQ excreted by other bacteria might be a critical factor for PVA degradation [35,36].

Several different enzyme systems for the degradation of PVA mainchains have been reported, depending on the PVA-degrading bacteria. In theses systems, the carbon–carbon linkage of the mainchains of PVA is cleaved first by the action of either a dehydrogenase or an oxidase and this is then followed by a hydrolase or aldolase reaction (Figure 2).

The PVA-degrading strain VM15C uses this system [37••]. PQQ-dependent PVA dehydrogenase (PVADH) dehydrogenates PVA and introduces β-diketone groups into the PVA molecules. Oxidized polyvinyl alcohol hydrolase (OPH) then hydrolyzes these β-diketone groups. PQQ-dependent PVADH was first found in VM15C [38]. In this strain, PVA oxidase (similar to the secondary alcohol oxidase described below) is also present [39,40], but the oxidase is not essential for PVA utilization because a PVA oxidase-lacking mutant grows well with PVA as sole source of carbon and energy [41]. On the other hand, the PQQ-dependent PVADH is indispensable as indicated by the fact that PQQ is an essential growth factor for PVA utilization. PQQ-dependent PVADH was first identified as a membrane-bound enzyme in the PVA-degrading bacterium *Pseudomonas* sp. VM15C [38]. VM15C produces the enzyme as an apoenzyme, which then binds to exogenous PQQ to form the active holoenzyme. The PVADH activity of the enzyme is completely dependent on exogenous PQQ and explains the PQQ requirement of the strain for PVA utilization. A similar PQQ-dependent PVADH has been found in another PVA-degrading *Pseudomonas* sp. strain that requires PQQ for PVA utilization [31].

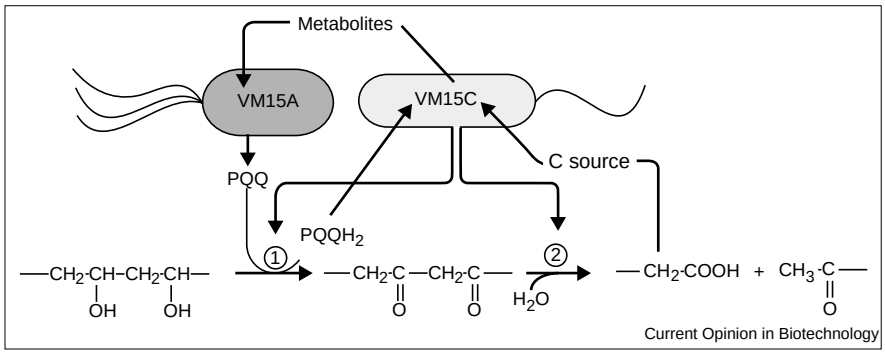
The PVADH gene, *pvaA*, has been cloned and characterized; the gene encodes a protein of 639 amino acid residues (68,045 Da) [42]. The deduced amino acid sequence contains a signal sequence, confirming that PVADH is present on the bacterial surface — an appropriate location for the degradation of PVA. A heme *c* binding site is present in the protein sequence and the enzyme-bound heme *c* of the holoenzyme is reduced by PVA dehydrogenation. The enzyme is a quinohemoprotein that uses heme *c* as another prosthetic group, in addition to the noncovalently bound PQQ. PVADH showed only low sequence similarity to other types of quinoproteins. A PQQ-binding cation, $Ca^{2+}$ or $Mg^{2+}$, was required for holoenzyme formation, and $Ca^{2+}$ was more effective than $Mg^{2+}$.

The gene for OPH, *pvaB*, was also cloned for the first time from VM15C [37••]. The gene encodes a protein of 379 amino acid residues (40,610 Da) and the deduced amino acid sequence showed a lipoprotein signal sequence and the lipase box of serine hydrolases. The enzyme is completely inhibited by phenylmethylsulfonyl fluoride. Thus, the *pvaB* product is a serine hydrolase. The protein is present as a membrane-bound protein, as suggested by the lipoprotein signal sequence.

OPH showed no significant similarity to other proteins, but did show very low similarity to a few PHA depolymerases. The genes *pvaA* and *pvaB* are clustered in the order *pvaBA*. A putative promoter and terminator

**Figure 1**

Symbiotic PVA utilization by bacteria. Two strains, *Pseudomonas putida* VM15A and *Pseudomonas* sp. VM15C, are unable to grow in isolation on PVA as sole carbon source. A mixture of the two strains is able to grow on PVA, however, in a symbiotic relationship. Strain VM15A releases the growth factor PQQ, which enables the growth of VM15C on PVA (see text for details). 1, PQQ-dependent PVA dehydrogenase; 2, oxidized PVA hydrolase.



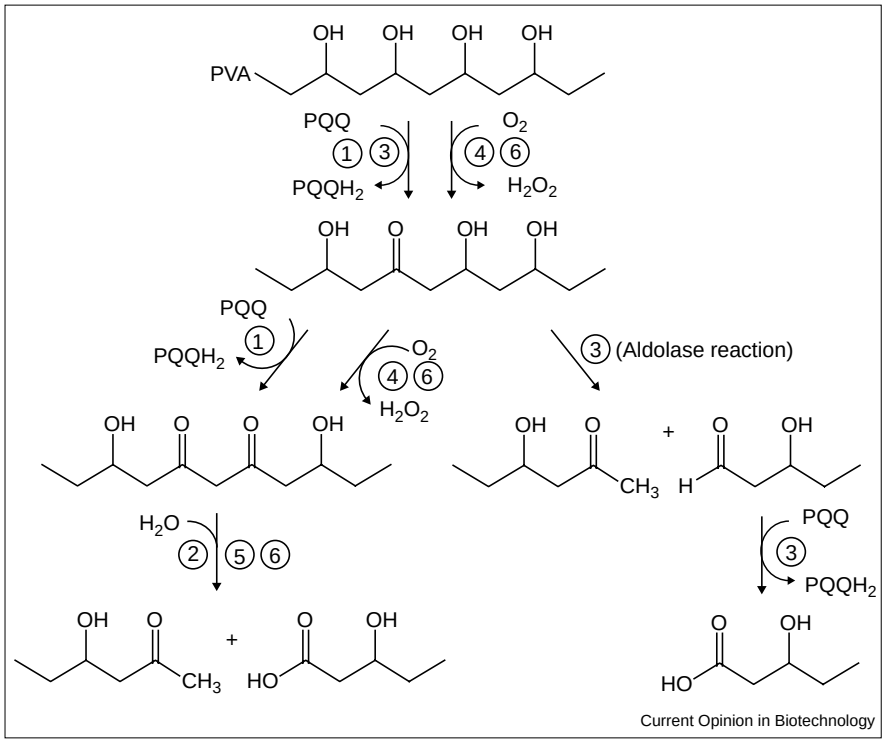

sequence are present in the operon, but nothing is yet known about the regulation of the operon. This is an area for future work.

Matsumura *et al.* [33] purified a PQQ-dependent PVADH from a PVA-degrading bacterium, *Alcaligenes faecalis* KK314. The strain was isolated from river water in Japan [30] and required PQQ for PVA utilization. Using a vinyl alcohol trimer as a model PVA compound, it was found that a β-hydroxyketone compound rather than a β-diketone compound was formed by the enzyme, and that the β-hydroxyketone compound was further degraded to a methylketone and an aldehyde by the enzyme. The aldehyde is assumed to be further dehydrogenated to a carboxylic acid by the enzyme. The degradation of the β-hydroxyketone compound also occurred with the apoenzyme and was thought to follow an aldolase-type reaction rather than a hydrolase-type reaction. Thus, the enzyme is a bifunctional enzyme having PVADH and β-hydroxyketone aldolase activities. For PVA, it is thought that the hydroxyl group of PVA is first dehydrogenated by the enzyme to the corresponding carbonyl group to form the β-hydroxyketone moiety and this is followed by an aldolase-type cleavage to produce the methyl ketone and aldehyde terminus. The aldehyde terminus may be further dehydrogenated to a carboxylic terminus.

Sakai *et al.* [27] also isolated the extracellular secondary alcohol oxidase and the β-diketone hydrolase from a *Pseudomonas* sp. The secondary alcohol oxidase catalyzes

**Figure 2**

Proposed pathway for PVA degradation by various enzyme systems. 1, PQQ-dependent PVA dehydrogenase; 2, oxidized PVA hydrolase; 3, PVA-degrading enzyme with dehydrogenase and aldolase activities; 4, secondary alcohol oxidase; 5, β-diketone hydrolase; 6, PVA-degrading enzyme with oxidase and hydrolase activity.



PVA oxidation using molecular oxygen as an electron acceptor to produce hydrogen peroxide and β-diketone groups in PVA molecules. The β-diketone group of PVA is then hydrolyzed and cleaved by the β-diketone hydrolase.

Suzuki [43] purified a PVA-degrading enzyme from a culture supernatant of *Pseudomonas* sp. O-3. As with secondary alcohol oxidase, the enzyme catalyzed PVA oxidation using oxygen as an electron acceptor and produced hydrogen peroxide. However, in this case, the PVA mainchains were cleaved without the need for another enzyme [44]. The enzyme also hydrolyzed diketone compounds, but not monoketone compounds. Thus the PVA-degrading enzyme is a bifunctional enzyme with both oxidase and hydrolase activities.

In addition, a small number of acetate groups are present in the sidechains of PVA. An esterase catalyzing hydrolysis of these acetate groups was isolated from a PVA-degrading bacterium [45].

## Nylon and polyethylene

High molecular weight nylon-66 membrane was found to be degraded significantly by lignin-degrading white rot fungi grown under ligninolytic conditions with limited glucose or ammonium tartrate [46]. The characteristics of a nylon-degrading enzyme purified from a culture supernatant of white rot fungal strain IZU-154 were identical to those of manganese peroxidase, but the reaction mechanism for nylon degradation was suggested to differ from that reported for manganese peroxidase [47]. Nylon-6 fibers are also degraded by the enzyme. Drastic and regular erosion observed on the nylon surface suggests that the nylon is degraded to soluble oligomers. High molecular weight polyethylene is also degraded by lignin-degrading fungi under nitrogen-limited or carbon-limited conditions, and by manganese peroxidase partially purified from a strain of *Phanerochaete chrysosporium* [48].

## Conclusions

These studies on the biodegradation of plastics have increased our knowledge of degrading microbes, degradation enzymes and their genes, and polymer structures for biodegradation. This knowledge is useful for the molecular design of biodegradable polymers and for the molecular evolution and breeding of degradation enzymes and microbes. Our increased understanding will contribute towards the development of biotechnology for polymer waste treatment.

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