Microbial *cis*-3,5-Cyclohexadiene-1,2-diol, Its Polymer Poly(*p*-phenylene), and Applications

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Contents

Abstract This chapter describes the production of cis-3,5-cyclohexadiene-1,2-diol (DHCD) from aromatic compounds, their polymerization into poly(p-phenyelene) (or PPP), and the properties and applications of the polymer. Large-scale synthesis of DHCD has been demonstrated, and DHCD is widely used in the pharmaceutical industry, as well as in chemical industries for polymer productions. Recent study including different types of dioxygenases, strain development by recombination, and genetical modification were done to develop the process technology for commercialization of this new polymer and chemical intermediates.

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1 Introduction

Research into new methods for the production of polymers is attractive mainly for two reasons. The first is the desire to improve the environmental impact of current polymer-producing processes by the use of more environmentally compatible reagents and milder conditions. The second is that the important polymer properties, such as chirality, temperature–structure profiles, electrical characteristics, optics, and biodegradability, are being investigated. These new methods will create new markets for polymers with entirely new properties and will also serve existing markets by the introduction of more environmentally acceptable processes. One of the most attractive methods is biotechnological, specifically biocatalytic, polymer synthesis, which is economical and environmentally compatible (Dordick 1992a, b; Ballard et al. 1994).

Novel monomers may be able to be synthesized from simple starting materials by biocatalysis, and subsequently polymerized into new materials with novel properties (Ballard et al. 1994). The common soil microorganism *Pseudomonas putida* is able to synthesize *cis*-3,5-cyclohexadiene-1,2-diol (DHCD) from benzene, catalyzed by the enzyme dioxygenase. Polymerization of DHCD produces polymers which are precursors for poly(*para*-phenylene) (PPP) synthesis (Ballard et al. 1983a, b, 1988). PPP and other polyaromatics are key materials with great potential in industries demanding high performance, such as electronics and aerospace. PPP can be considered as the ultimate polyaromatic since the product is composed entirely of phenylene rings. The intractability of this structure had precluded its synthesis in a way allowing useful applications (Ballard et al. 1994).

In this chapter, we will describe the biosynthetic approach to PPP from DHCD and the strain development to produce DHCD. Properties and applications of PPP, and the development of polyaromatics will also be considered.

2 Synthetic Approaches to PPP

Polyaromatics are polymers that formed by direct linkage between carbon atoms of aromatic rings. PPP is one of the most widely researched and used polyaromatics (Fig. 1). Owing to its novel properties, such as high tensile strength, high Young's modulus, and thermostability, PPP is considered to have high potential for applications in the electronic and aerospace industries.

PPP was first synthesized by Goldschmiedt (1886). He applied the Wurtz–Fittig reaction which coupled *para*-dibromobenzene using sodium, and the product was determined to be a tridecamer (13 subunits) by elemental analysis. In 1936, the same monomer was used and a PPP containing up to 16 benzene rings was obtained (Busch et al. 1936).

Owing to the inertness of benzene, it is not an easy task to synthesize PPP using traditional methods of fabrication. However, various syntheses of PPP have still been attempted since the 1950s.

The direct polymerization of benzene is definitely one route that can be followed. This technique was developed by Kovacic and others in the 1960s

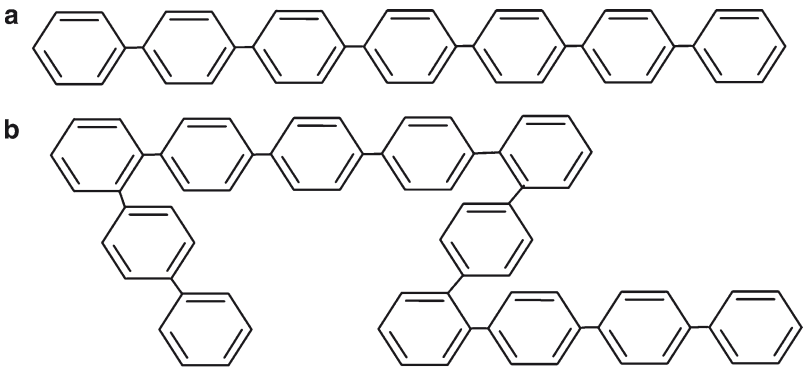
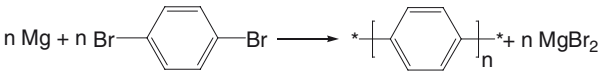


Fig. 1 Chemical structures of poly(*p*-phenylene) (PPP). **a** PPP ultimately composed of a 1,4 unit, **b** PPP containing a 1,2 unit

(Kovacic and Kyriakis 1962, 1963; Kovacic and Oziomek 1964; Speight et al. 1971). The process is known as oxidative cationic polymerization. It used $AlCl_3$ as a Lewis acid catalyst and $CuCl_2$ as an oxdidant. The polymer produced was a black material, containing a mixture of 1,2 (*ortho*) and 1,4 (*para*) units as well as chemical defects such as chlorination and intermolecular cross-links (Brown et al. 1986; Berresheim et al. 1999). The products should be more correctly defined as oligomers rather than polymers, as the chain lengths were between ten and 15 phenylene rings. And it was difficult to remove the $CuCl_2$. Nevertheless, this technique is still successful as a route to PPP (Kovacic and Jones 1987).

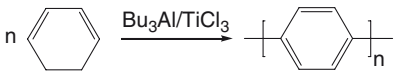
Kovacic's method was improved by Arnautov and Kobryanskii (2000) using an oxidative polycondensation route. The PPP obtained had a higher molecular weight (Sun et al. 2005).

A second synthesis option for PPP was developed by Yamamoto et al. (1978), in which *para*-dibromobenzene is polymerized using a nickel catalyst in the presence of magnesium (Scheme 1). This is an example of directly using Grignard chemistry to form a macromolecule. However, the result is still not ideal, since the growth of the chain length does not go beyond 10–12 phenylene rings.



(1)

An early attempt to use polymers of cyclohexadiene as a route to PPP was by Marvel and coworkers (Marvel and Hartzell 1959; Cassidy et al. 1965). This process involves the direct polymerization of cyclohexa-1,3-diene using a Ziegler catalyst (Scheme 2; Ballard et al. 1994).



(2)

Marvel et al. used 5,6-dibromocyclohexa-1,3-diene, which was converted into poly(5,6-dibromo-1,4-cyclohex-2-ene) and subsequently into PPP with elimination of

HBr (Berresheim et al. 1999). However, this method of synthesis has two main defects. First, the poly(cyclohexene) produced contains 1,4 and 1,2 units. Second, there are a number of bromo-substituted intermediates and thus the aromatization is incomplete.

Recently, Grubbs et al. improved this method by using trimethylsiloxy protecting groups and a nickel catalyst. The polyphenylene produced contained up to 96% of 1,4 units (Gin et al. 1991, 1992a, b, 1994a, b). Another improvement was made by Natori et al. (2006), in which soluble polyphenylene homopolymers with a controlled polymer chain structure were synthesized by the complete dehydrogenation of poly(1,3-cyclohexadiene) having 1,2 units and 1,4 units.

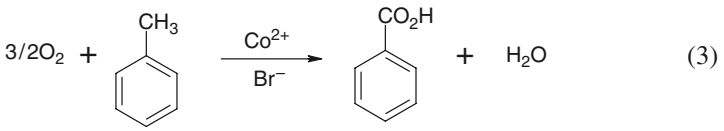
Although the route from cyclohexadiene to PPP is not mature enough, it offers an innovative approach which seeks to avoid the difficulties of direct fabrication of PPP, by creating a precursor polymer to be subsequently converted into PPP. Given this intent, one possible option is to use the appropriate derivative of cyclohexa-1, 3-diene that can be polymerized by a 1,4 addition reaction. Synthesis of such derivatives using organic chemistry has been studied but is not practical and cost-effective. However, the biological oxidation of benzene and its derivatives, which proceeds via a dihydroxylated cyclohexadiene intermediate, offers the potential for large-scale synthesis (Ballard et al. 1994).

3 Biocatalytic Production of *cis*-DHCD

Benzene *cis*-diol, namely, *cis*-DHCD, is a chiral chemical which is important for the pharmaceutical and chemical industries (Hack et al. 1994; Wahbi et al. 1996, 1997). It is one of the intermediates during biological aromatic oxidation in some microorganisms. Bacteria such as *P. putida* can be genetically modified to achieve the synthesis and highly efficient production of *cis*-DHCD.

3.1 Aromatic Oxidation in Microorganisms

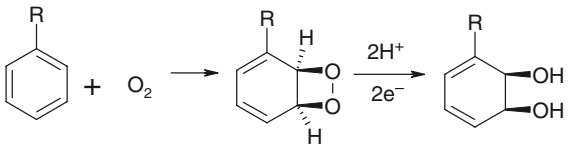
Aerobic microorganisms can utilize various aromatic compounds as carbon sources (Doelle 1975; Stanier and Ornston 1973). Oxygenase enzymes catalyze the first step of the degradation of aromatic substrates by functionalizing the benzenoid ring using O_2 as an oxdidant. The two major groups of oxygenases are monooxygenases and dioxygenases (Ballard et al. 1994).



(3)

The differences are unique between biological oxygenase reactions and classical organic chemical processes. As is shown in Scheme 3, the industrial process is used

to produce the aromatic dibasic acids from *ortho*-xylenes, *para*-xylenes, and *meta*-xylenes. A temperature between 150 and 200°C and oxygen under pressure are required. Owing to the stability of the aromatic ring, 98% of the product is benzoic acid. In contrast, oxidation by dioxygenase results in almost exclusive attack on the aromatic nucleus (Scheme 4), with the aromatic substrate dioxidized by addition of dioxygen. The product of this reaction has been shown to be *cis*-DHCD (Gibson 1990), which is without parallel in organic chemistry.



(4)

The *cis*-DHCDs are subsequently further degraded to central metabolic intermediates such as pyruvate and fumarate (Doelle 1975). This metabolism of aromatics via *cis*-DHCDs is a property of certain bacteria. Higher organisms and mammalian systems oxidize aromatics to *trans*-DHCDs with an entirely different reaction mechanism and do not provide options for synthesis of *cis*-DHCDs (Ballard et al. 1994).

3.2 Aromatic Dioxygenases

Since the 1960s, many aromatic dioxygenases have been discovered and identified from various bacterial species and they can catalyze reactions to obtain over 300 types of arene *cis*-diols (Gibson et al. 1970; Gibson and Parales 2000; Mason and Cammack 1992; Lipscomb et al. 2002; Bui et al. 2000; Reiner and Hegeman 1971; Subramanian et al. 1979, 1985). Dioxygenases can be grouped into benzene dioxygenase, toluene dioxygenase (TDO), and benzoate dioxygenase (BZDO) in terms of catalytic mechanism, enzyme structure, and reaction substrate (Bagneris et al. 2005; Costura and Alvarez 2000; Friemann et al. 2005; Kim et al. 2003; Shindo et al. 2005; Yildirim et al. 2005).

Most dioxygenases have in common that they have relatively low specificity and similar enzyme structures. Many of them can catalyze a broad range of aromatic substrates, such as benzene, toluene, naphthalene, and chlorobenzene (Quintana and Dalton 1999; Raschke et al. 2001). In general, dioxygenase enzymes contain an iron–sulfur center (Mason and Cammack 1992). For example, benzene dioxygenase enzyme from *P. putida* comprises three subunits: the first one an iron–sulfur-centered dioxygenase, the second an electron transfer protein also possessing an iron–sulfur center, and the third a flavoprotein (Axell and Geary 1973; Harpel and Lipscomb 1990; Subramanian et al. 1985; Geary et al. 1984; Ensley and Gibson 1983). Similarly, TDO is composed of three subunits: flavoprotein reductase, ferredoxin, and a terminal iron–sulfur dioxygenase (ISP_{TOL}; Butler and Mason 1997). The catalytic mechanisms are also similar: two electrons are passed from reduced NADH through subunits to dioxygen and the substrate (Fig. 2).

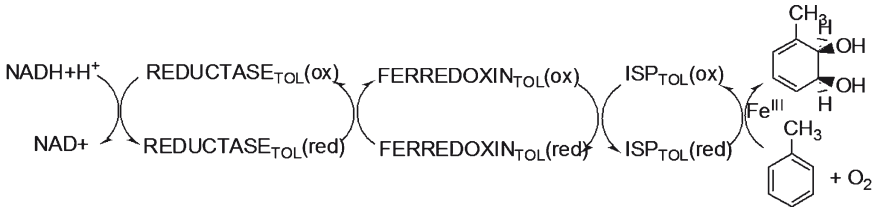
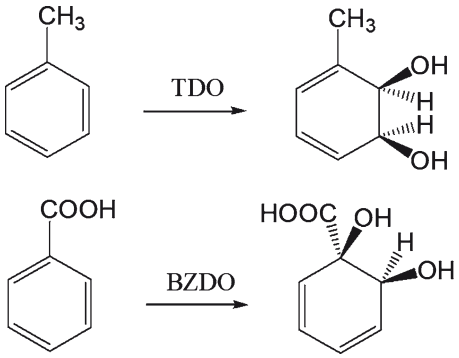


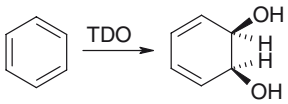
Fig. 2 Electron transportation in the toluene dioxygenase (TDO) system (Subramanian et al. 1979)

Fig. 3 Differences between dioxydation reactions catalyzed by TDO and benzoate dioxygenase (*BZDO*)



BZDO is one of the most special dioxygenases owing to its catalytic mechanism. Other dioxygenases convert aromatic rings to *cis*-diols by adding two hydroxyl groups to two or three sites (Bui et al. 2000). In contrast, BZDO, as well as its homogeneous enzyme toluate dioxygenase, catalyzes the reaction to produce *cis*-diols with hydroxyl groups at one or two sites (Yamaguchi et al. 1975; Lipscomb et al. 2002; Fig. 3).

BZDO enzyme also contains an iron–sulfur center. But it consists of two components: an oxygenase of an $(\alpha\beta)_3$ subunit structure, in which the α contains a Rieske [2Fe–2S] cluster and a mononuclear iron site, and a reductase with one FAD and one [2Fe–2S] cluster (Yamaguchi et al. 1975; Yamaguchi and Fujisawa 1978, 1980, 1982). In the process of dioxidation of benzoate by BZDO, the iron ion plays an important role (Fig. 4).



(5)

The application of bacterial strains containing these dioxygenases has used for transformation of environmental pollutant aromatic compounds to nonaromatic compounds (Cavalca et al. 2004; Parales and Haddock 2004). Moreover, the *cis*-diols produced by dioxygenases are attractive because of their inimitable chiral structures and their potentials in industrial synthesis for useful chemical products

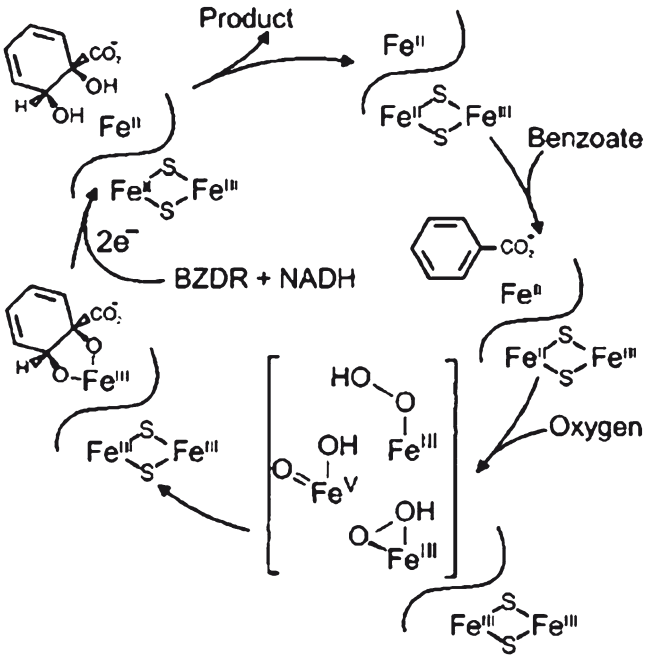


Fig. 4 Catalytic metabolism of BZDO. *BZDR* benzoate dioxygenase reductase. (Lipscomb et al. 2002)

(Boyd and Bugg 2006). The most widely researched and used is benzene *cis*-diol, or *cis*-DHCD, the reaction to produce it being catalyzed by benzene dioxygenase or TDO (Scheme 5). One of the applications of *cis*-DHCD is to synthesize the novel polymer PPP.

3.3 Synthesis of cis-DHCD

For synthesis of chiral *cis*-diols, biotransformations using bacterial strains containing dioxygenase have advantages over purely chemical reactions, including enantiospecificity, high yields, low economic cost, and environmental friendliness (Reddy et al. 1999). And there are two key issues that have to be addressed: one is the selection of an appropriate enzyme source and a bacterial strain; the other is the development of appropriate process technology (Ballard et al. 1994).

The development of appropriate process technology for using the biocatalyst and the subsequent product recovery is critical for achieving an economic and reliable process, particularly in cases such as biotransformation of benzene, where oxygen and toxic, volatile, poorly water soluble substrates are needed (Ballard et al. 1994).

Biocatalysts can be used for synthetic purposes in different forms, such as immobilized cells or enzymes, dissolved enzymes or intact cells (Lilly et al. 1990;

Lilly 1977). For the conversion of benzene to DHCD, the intact cell as a catalyst is preferred for several reasons. First, the reaction requires NADH, which can be supplied by the cell. Second, appropriate host strains have been supplied by strain development which are resistant to toxic organic substrates and can take up a range of aromatic compounds. Moreover, for the option of using dissolved enzymes as synthetic catalysts, it has been unsuccessful in isolating the three-protein enzyme complex (Yeh et al. 1977; Axcell and Geary 1975; Zamanian and Mason 1987; Mason and Cammack 1992).

Some problems are to be solved in the process of synthesis of DHCD by a growing culture during fermentation. One is the growth inhibition by aromatics and DHCDs at high concentration. Another is the recovery of DHCD downstream of the biotransformation, to dissolve the product in a nonaqueous organic solvent, as DHCD is an unstable compound in acidic aqueous media. This also requires keeping the pH above 7 to avoid degradation of DHCD to phenol, which clashes with the fact that dioxygenase activity is optimal at pH 7.

As a source of the enzyme, a microbial strain should be able to tolerate a significant concentration of benzene or other aromatic compounds. And the enzyme efficiency or a high rate of benzene oxidation is another key factor. Microorganisms used in the early studies for the metabolic pathway identification were characterized by high sensitivity to benzene (Gibson et al. 1968a, b), and are thus not suitable for cost-effective, large-scale operation. ICI isolated microorganisms from sites where levels of hydrocarbon contamination were significant. These bacteria showed rapid growth ability in benzene-saturated, aqueous solution. One of the strains, *P. putida* NCIMB 11767, was selected for development (Ballard et al. 1994). By application of chemical mutations to inactivate the DHCD dehydrogenase, a mutant strain was selected which had lost the ability to further metabolize DHCD as a carbon source. Further mutation was done to derive constitutive expression of the dioxygenase and to overcome the catabolic repression of dioxygenase synthesis by glucose. Finally, one of the strains, *P. putida* UV4, which showed a high level of DHCD accumulation from benzene after growth on glucose, was selected for process development. This catalyst was widely used for the production on a ton scale of DHCD and other substituted DHCDs from a wide range of aromatic substrates (Taylor 1982).

Recently, genetic modifications on microbial hosts have been preferred as an improvement in the cost-effective and large-scale fermentation. Natural strains containing dioxygenases are difficult to use for direct production of DHCDs owing to their long growth period, low benzene transformation efficiency, and difficulties in controlling growth conditions. New strains have been developed through genetic engineering, for example, a host strain harboring a plasmid containing dioxygenase gene and other related components to overexpress dioxygenase enzyme.

P. putida is an appropriate host strain for DHCD production. The strain mt-2 (ATCC 33015) was isolated from soils in the early 1960s by Hosokawa and others (Nozaki et al. 1963). It is able to grow on *meta*-toluate as the sole carbon source owing to its pWW0 plasmid. KT2440 is a mutant strain of mt-2, widely used as a host for *Pseudomonas* gene cloning and expression. The most important

character of KT2440 is the ability to transform and convert various aromatic compounds, utilizing benzoic acid as the sole carbon source and energy through the β-ketoadipate pathway.

P. putida KT2442 is a mutant strain of KT2440. It has the high environmental tolerance of KT2440. Since *Pseudomonas* species provide a good basis for genetic manipulation, which is necessary for strain development, KT2442 is considered to be a suitable host for DHCD synthesis. It is reported that *Pseudomonas stutzeri* 1317 (Chen et al. 2004) and *Aeromonas hydrophila* 4AK4 (Chen et al. 2001; Ouyang et al. 2005) are also able to utilize broad-range substrates and survive well in organic solvents (Jiménez et al. 2002; Chen et al. 2001, 2004). These three bacterial species can be used for highly efficient biotransformation of benzene to DHCD.

Escherichia coli JM109 harboring TDO genes has been used successfully for large-scale production of DHCD (Quintana and Dalton 1999; Qu et al. 2003), in which the TDO gene *tod* is constructed into plasmid pKST11. More recently, Ouyang et al. (2007a) constructed this *tod* gene into plasmid pSPM01, which was introduced into *P. putida* KT2442, *P. stutzeri* 1317, and *A. hydrophila* 4AK4. These three strains showed higher efficiency of DHCD production than *E. coli* JM109 (pKST11). Moreover, in contrast with the requirement of isopropyl β-D-thiogalactopyranoside induction for *E. coli* JM109 (pKST11), isopropyl β-D-thiogalactopyranoside was not needed to realize biotransformation by these three strains. To make a further improvement, Ouyang et al. (2007b) integrated the *vgb* gene, which encoded the *Vitreoscilla* hemoglobin protein that enhanced the oxygen microbial utilization rate under low dissolved oxygen concentration, into the *P. putida* KT2442 genome. The mutant strain *P. putida* KTOY02 (pSPM01) showed higher oxidation ability and higher production of *cis*-diols was achieved. *P. putida* KT2442 was also genetically modified to transform benzoic acid (benzoate) to benzoate *cis*-diol, also named 1,2-dihydroxycyclohexa-3,5-diene-1-carboxylic acid (Sun et al. 2008). These results indicate that *P. putida* KT2442 could be used as a cell factory to biotransform aromatic compounds.

There are three principal industrial modes of fermenting microorganisms: batch, fed-batch, and continuous fermentation, among which the continuous fermentation is the most demanding process, with nutrients continuously fed to the fermentor while an equal amount of spent growth medium is withdrawn to achieve a highly consistent product. The batch and fed-batch processes are simpler, the first one comprising inoculation of a sterile fermentor containing the growth medium with a live bacterial culture, and the latter one comprising continuous addition of a controlled amount of an essential growth element (Ballard et al. 1994). For a laboratory-scale fermentation for research, batch and fed-batch processes using shake-flasks and 3-6 L fermentors are mostly used for convenience.

Batch operation to synthesis of DHCD requires the supply of a biocatalyst, that is, a strain as the enzyme source, oxygen or air, and an aromatic reactant. Attention should be paid to the supply of the aromatic reactant since the aromatic reactant has damaging effects on microbial cells, and thus cannot be added to the reactor in too great an amount at one time. A detailed study of toluene hydroxylation kinetics (Woodley et al. 1991) showed that the aqueous toluene concentration should be

maintained between 0.05 and 0.20 g L^{-1} for optimization of biocatalytic activity. A concentration of toluene higher than 0.20 g L^{-1} would be toxic to the cells, whereas at concentration lower than 0.05 g L^{-1} poor use is made of the available dioxygenase activity. As the aromatic reactant is poorly water soluble and volatile, it may reach the bacteria via three routes: aqueous phase, vapor phase, or organic phase (Ballard et al. 1994). Bacteria may either catalyze the reaction with the aromatic reactant in the aqueous phase via mass transfer from the vapor or organic phase, or via direct contact with the nonaqueous phase, or both. However, vapor-phase addition has resulted in low reaction rates and product concentration (de Bont et al. 1986; van den Tweel et al. 1986). In contrast, organic-phase addition has been done successfully on the laboratory scale by dissolution of the reactant in a selected organic solvent (Harrop et al. 1992; Collins and Woodley 1993; van den Tweel et al. 1987; Yarmoff et al. 1988).

A two-phage cultivation system has been developed in the biotransformation of benzene (Quintana and Dalton 1999; Qu et al. 2003). To reduce benzene toxicity, a 3–10 times volume of water-insoluble organic solvent, such as tetradecane or liquid paraffin, was used for dissolution of benzene before the benzene was added into the culture broth. However, this large volume of organic solvent would reduce the effective working volume of the bioreactor, leading to increasing DHCD production cost and complicating the downstream extraction procedure. These disadvantages may hinder the commercial-scale production of *cis*-diols and the productivity of DHCD using the recombinant *E. coli* system was limited.

With use of *P.putida* KT2442 as the host, which has high resistance to benzene or its derivatives and high dioxygenase enzyme activity, the highest yield of benzene *cis*-diol ever reported, near 60 g L^{-1} on the 5-L fermentor scale, was achieved (Ouyang et al. 2007a).

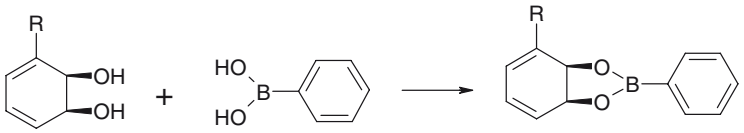
For a ton scale operation, a simpler approach has been successfully used to supply the reactant to cells via the aqueous phase. The reactant is added at a controlled rate so that that the supply is matched by the reactant dihydroxylation to DHCD. A control system is needed to maintain the correct aqueous-phase reactant concentration as the activity of the cells varies during the stages of the biotransformation (Ballard et al. 1994).

3.4 Recovery of cis-DHCD

The DHCD product is more water-soluble than the starting material aromatic compounds; thus, it is a problem to isolate the compound. Other water-soluble components in the cells, such as proteins and nucleic acids, may create difficulties in extracting the DHCD into an organic solvent. It is also important to keep the pH above 7 to avoid DHCD dehydrating into phenol (Ballard et al. 1994).

There are several methods for isolating the DHCD product in a short sequence in high yield and with high purity. An elegant method is the formation of an insoluble phenylboronate complex (Herbert et al. 1990; Scheme 6). This reaction occurs

when the phenylborate is added stoichiometrically to the DHCD. The precipitated product can be filtered and recovered. Subsequently, when the phenylboronate adduct is broken, the phenylborate can be recovered and reused, and the DHCD can be recrystallized.



(6)

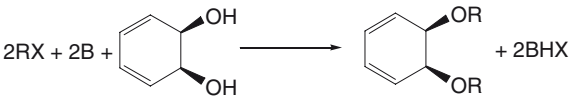
Another method of recovery of DHCD is to absorb it onto an insoluble and hydrophobic solid such as diatomaceous earth (Güuzel et al. 1990) or charcoal, by passing the aqueous solution down a column. The column binds DHCD and DHCD may be eluted by washing the column with a polar organic solvent such as methanol.

The most obvious way of isolating DHCD is to extract it from the aqueous medium into an organic solvent and then recrystallize it. This method needs careful selection of solvent and operation owing to the high water solubility of DHCD.

Some DHCD products have been found to be unstable as solids left at room temperature over time; exothermic decomposition results in the formation of phenol. DHCDs produced by ICI are formulated as solutions in ethyl acetate containing a small amount of basic triethylamine stabilizer, and are fully stable at room temperature (Ballard et al. 1994).

4 Polymerization: From *cis*-DHCD to PPP

The process of synthesis of PPP from *cis*-DHCD is as follows: first, derivatization of *cis*-DHCD (Scheme 7); second, polymerization of *cis*-DHCD derivatives (Scheme 8); finally, the aromatization to obtain PPP (Scheme 9).



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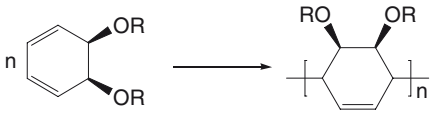

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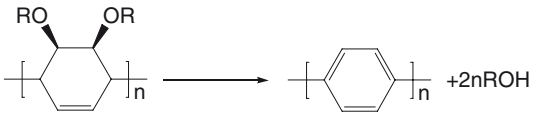

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4.1 Derivatives of cis-DHCD

For synthesis of PPP from *cis*-DHCD, the first step is the derivatization of DHCD. The reason not to directly polymerize DHCD is that after its polymerization, which is in fact difficult, the subsequent aromatization step gives rise not to PPP but to a polymer of undefined structure containing nonaromatic and phenolic groups (Ballard et al. 1994).

Derivatization of DHCD can be carried out at and above pH 7.4 without the formation of phenol, by the reaction shown in Scheme 7, where RX can be an acid chloride, anhydride, or iodide, and B can be an organic tertiary base. The thermal properties of derivatives of DHCD have to be identified after the polymerization and aromatization processes. The aromatization process can be carried out smoothly at a temperature greater than 100°C. Acetic anhydride is often used to produce cis-3,5-cyclohexadien-1,2-diol diacetate (DHCD-DA). The dimethylcarbonate (DMC) derivative has also been used for the majority of polymerization work covered by a range of ICI patents (Ballard et al. 1983a, b, 1984; Chenshire 1984; Nevin and Shirley 1985).

4.2 Polymerization of cis-DHCD Derivatives

In early experiments, contamination by small amounts of impurities such as phenol made experiments attempting the polymerization of DHCD and its derivatives unsuccessful. When pure DHCD was used, the initiating radicals facilitated the formation of phenol, also inhibiting the polymerization. However, most of the acyl derivatives could be polymerized by using radical initiators either as the pure compound or dispersed in an organic solvent in which they were insoluble. The details of the polymerization, including conversion rate, molecular weight, temperature, and the relationships among these parameters, were described by Ballard et al. (1994).

Most effective polymerizations were achieved in the absence of solvent and would proceed almost to completion without difficulty. For example, with use of benzoyl peroxide as an initiator, the conversion rate of DHCD-DA would reach 90% in 40 h. Moreover, the variation in molecular weight, which was represented by the number-average degree of polymerization and the dispersity, showed that the bulk polymerization of these monomers was similar to that of acrylic esters (Ballard et al. 1994).

The relationship between polymerization rate and concentration of monomer and catalyst is shown in Eq. 1:

$$R_p = \frac{-d[M]}{dt} = K[M]_0^{3/2}[I]_0^{1/2} \tag{10}$$

where $[M]_0$ and $[I]_0$ are the initial monomer and initiator concentrations, respectively (Ballard et al. 1994). This is a general feature of the polymerization of

vinyl monomers such as styrene and methyl methacrylate. It was also found that the polymerization had a pressure coefficient and the rate of polymerization at 3,000 atm was 5–7 times greater than at atmospheric pressure. But the molecular weight was not significantly higher (Ballard et al. 1988, 1994).

The molecular weight of the polymer is sensitive to the concentration of the monomer, and would be reduced markedly by the presence of an aromatic solvent. Thus, the polymerization should be performed in the absence of solvent to obtain a high molecular weight. The reaction temperature is also a sensitive parameter. For example, the molecular weight increases by a factor of 5–10 times when the polymerization is carried out at 60°C as compared with 90°C. Through a consideration of the temperature effect on the polymerization rate, the energy of activation is calculated to be 16.9 kcal mol^{-1} using the Arrhenius equation (Ballard et al. 1994). The molecular weight of the polymer can be markedly increased by using deuterated analogs of DHCD. This effect of deuteration is well known in radical polymerization of vinyl monomers, and is due to the retardation of the bimolecular termination reaction and possibly to differences in the proton or deuteron abstraction from the monomer, leading to degradative chain transfer (Ballard et al. 1988).

Polymerization in organic diluents, in which the polymer is insoluble, has been achieved by using dispersing agents consisting of a poly(methyl methacrylate) backbone with a side chain derived from 12-hydroxystearic acid. Polymer powders from acetate, benzoate, and methylcarbonate derivatives of DHCD have been obtained. Particularly, the benzoate and methylcarbonate derivatives are polymerized at high rates and give polymers with molecular weights up to one million (Ballard et al. 1994).

4.3 Aromatization Process to PPP

The polymers of DHCD derivatives can be aromatized by heating the polymers as fibers or films in the solid state and in solution (Ballard et al. 1994). The process is done with reaction shown in Scheme 9, accompanied by elimination of two molecules of the acid for each phenylene group formed. In the case of DHCD-DA aromatization, the ROH is acetic acid.

The DMC derivative is another preferred one for aromatization for practical reasons, as the eliminated acid is methylcarbonic acid, which decomposes to methanol and carbon dioxide. This DHCD-DMC aromatization process is catalyzed by alkali metal salts and a tertiary organic nitrogen compound. Tertiary bases such as *n*-octylamine, the oligo bases, and other bases of low volatility are the preferred catalysts for the process at 240°C using 0.5 mol%. Moreover, the base can be removed by volatilization at 350°C after the conversion is complete. This is definitely an advantage over using metal salts (Ballard et al. 1994).

N-Methylpyrrolidone is not only a catalyst for the conversion to PPP, but is also a good solvent for the partially aromatized poly(DHCD-DMC) with up to 35 mol% of phenylene groups. Thus, it is a good base for the study of the initial stages of the

aromatization process. It has been demonstrated that the process is autocatalytic, that is, as the relative number of phenylene groups in the chain increases, the adjacent DHCD-DMC residues are more readily aromatized. And the aromatization does not produce chain scission (Ballard et al. 1994).

There is a fundamental difference between aromatization in the solid state and in solution. The conversion in solution up to 26% does not involve chain scission as the conformational changes occurring are accommodated. On the other hand, in the solid state, indirect evidence shows that chain fracture takes place, accompanied by crystallization of polyphenylene (Ballard et al. 1988).

Alternative methods of polymerization to obtain a polymer chain with no 1,2 phenylene units have been developed at the California Institute of Technology (Gin et al. 1992a). In this process, *cis*-5,6-bis(trimethylsiloxy)-1,3-cyclohexadiene (DHCD-TMS) is prepared by the reaction in Scheme 7. It is then polymerized by an organometallic catalyst, bis(allyltrifluroacetonickel(Ni^{2+})), to give an exclusively 1,4 polymer in 93% yield. Poly(DHCD-TMS) cannot be aromatized, so it is converted to poly(DHCD-DA) by removal of the trimethylsilane groups with a fluoride ion and methanol and is reacetylated using acetic anhydride. This is aromatized thermally, and the PPP is shown to be of longer chain length than polymers from radical-initiated polymerization (Ballard et al. 1994).

5 Properties and Applications of PPP

Some properties of PPP are related to the aromatization process (Ballard et al. 1994). The level of the crystallinity of PPP is affected by the aromatization temperature. When the process is done below the glass-transition temperature (T_g) of the precursor molecule, which is 185°C, a predominantly amorphous PPP powder or coating is produced. The subsequent annealing of this powder at temperatures below or above 290°C has quite distinct effects on the crystallinity. When the temperature is below 290°C, there is no recognizable increase in crystallinity. In contrast, once the temperature goes up above 290°C, a high level crystallinity polymer is produced. On the other hand, if the precursor molecule is aromatized above its T_g, small crystals would be formed during the aromatization process and impede the further reorganization of the macromolecules, making the maximum possible crystallinity unachievable. But these small crystals are absent from the polymer produced by aromatization below T_g. The distinct change of crystallinity at 290°C also shows that it is associated with the increase in chain mobility and thus this temperature is the T_g for amorphous PPP.

The T_g of the precursor molecule rises with the increase in aromatization degree, as more phenyl groups are formed and the flexible precursor becomes more rigid. The phenyl groups are formed initially in blocks and are not randomly distributed along the chain, so the T_g increases smoothly. After the aromatization degree reaches about 30%, the T_g increases more rapidly (Ballard et al. 1994).

Another property of PPP related to the aromatization process is the thermal stability. One of the optimal options of the process is to carry it out in an inert atmosphere, complete it by heating at 260°C, and finally at 320°C, to remove the amine catalyst and oligomers. The PPP coatings obtained in such a way can be used at temperatures near 400°C, and in the absence of oxygen, even at 500°C. In these conditions, no recognizable decomposition of PPP occurs. At temperatures above 400°C in air, only a small amount of weight is lost in the form of hydrogen and methane. Breakdown of phenyl groups only occurs at a significant rate at 600–800°C. In an inert atmosphere such as N_2, at a temperature of 900°C, only 7% of weight is lost with production of oligophenyls containing three to 11 phenyl units, and no benzene or diphenyl is produced. In contrast, other aromatic polymers and coatings can only withstand temperature of 350°C for short periods without significant breakdown (Ballard et al. 1994). This thermal stability of PPP is among the best in all polymer materials.

Other properties of PPP are also the among best of all artificial and natural polymer materials, such as (1) it is the most resistant to acid corrosion, it is only dissolved slowly by 98% sulfuric acid, much superior to other polymer materials; (2) it is the most resistant to radiation, without any changes under radiation of 8.95×10^8 rad from cobalt; (3) it is the hardest to burn; (4) it has the highest rigidity; and (5) its refractive index of 1.833 is higher than that any other organic polymer.

Oligophenyl absorbs in the infrared, at about 800 cm^{-1}. It has been shown that when the unit number of PPP increases, the absorption wavenumber decreases, and this decrease is in direct ratio to $1/\alpha^2$, in which α is the unit number. This is due to the decline of the vibrational energy of Π*** conjugation by polymerization. As a result, vibration would happen even from irradiation by a low-energy wave, and thus the absorption takes place. If the unit number was 20, the theoretical absorption wavenumber would be 2 cm^{-1}, and the frequency would be 60 Hz. This would meet the need for materials with absorption of long electromagnetic waves. Moreover, since the structure of PPP is symmetrical, its dielectric constant is very low, and it would not be activated by a general electric or magnetic field.

As described above, PPP has novel rigidity and thermal stability, and its density is only 1.228 $g\,ml^{-1}$, one sixth that of iron. This indicates that PPP can be suitably applied in special environments where materials of high rigidity, thermal stability, and corrosion resistance are needed, for example, aerospace.

PPP coatings are very good electrical insulators, with electrical resistivity of 10^{13}–$10^{16}\,\Omega$cm. However, the treatment with n- and p-type dopants such as sodium naphthalide, ferric chloride, and AsF_5, the electrical resistivity decreases markedly to that of a semiconductor. The electrically insulating coatings have been studied for use in the design of advanced liquid crystal displays for computers. By deposition PPP on glass plates coated with an electrically conducting layer of indium tin oxide (ITO), the plates encapsulate a solution of liquid-crystalline molecules. The supertwisted birefringent effect enhances the visualization of the display. It has also been shown that a light-emitting diode (LED) can be constructed with PPP (Grem et al. 1992). A glass plate is coated with a layer of ITO and aluminum with PPP sandwiched between them. The application of 12 V at a frequency of 60 Hz

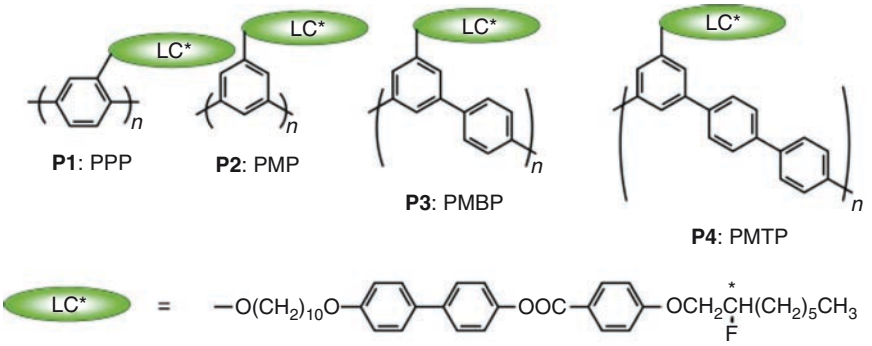

Fig. 5 Chemical structures of chiral liquid-crystalline conjugated polymers*PMP* poly (*meta*-phenylene), *PMBP* poly (*meta*-biphenylene), *PMTP* poly(*meta*-terphenylene) (Suda and Akagi 2008)

between the electrodes produced blue light. This type of LED differs from that of poly(phenylene vinylene) (Burroughes et al. 1990), which emits radiation of yellowish light. From these studies PPP may be suggested for development of computer screens. In addition, the blue emitted radiation is photochemically active and may be used for electroluminescent devices.

Recently, liquid-crystalline polyphenylene derivatives have been synthesized through substitution of a fluorine-containing chiral liquid-crystalline group into side chains, with an aim to develop ferroelectric liquid-crystalline conjugated polymers (Fig. 5; Suda and Akagi 2008). These are attracting interest because they can afford anisotropies in electrical and optical properties when they are macroscopically aligned. This study also elucidated that PPP can be used to prepare new types of polymer materials.

6 Summary and Future Developments

In this chapter we have described the production of DHCDs from aromatic compounds, their polymerization into PPP, and the properties and applications of the polymer. Large-scale synthesis of DHCD has been demonstrated, and DHCD is widely used in the pharmaceutical industry, as well as in chemical industries for polymer productions. A review of these points was given by Ballard and others in 1994. We have added recent study results obtained since then, such as research on different types of dioxygenases, strain development by recombination, and genetical modification. More research needs to be done, on one hand, to develop the process technology for commercialization of this new polymer and chemical intermediates. On the other hand, research on a series of DHCDs and their derivatives and new applications of the polymers would create many new opportunities for industry.

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