

Thermal stability and degradation kinetics of polyphenols and polyphenylenediamines enzymatically synthesized by horseradish peroxidase

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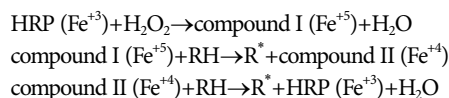
Abstract—Various substituted phenols and phenylenediamines were enzymatically polymerized by horseradish peroxidase in 80% (v/v) organic solvents-aqueous buffer (100 mM sodium acetate, pH 5) mixtures with H₂O₂ as the oxidant. The thermal stability of the polymers was investigated by thermogravimetric analysis (TGA) and represented by the char yield (wt% of the initial polymer mass) after being heated at 800 °C. Poly(*p*-phenylphenol) had the highest thermal stability among the synthesized polymers with a char yield of 47 wt%. The polymers containing amino groups such as poly(*p*-aminophenol) and polyphenylenediamines were also shown to possess high thermal stabilities. The activation energies for the thermal degradation of the polymers determined by derivative thermogravimetric analysis (DTG) using Horowitz-Metzger's pseudo-first-order kinetics were in the range between 23-65 kJ/mol and comparable to those of the chemically synthesized polymers. Dynamic structural changes of the enzymatically synthesized polymers upon heating were studied by differential scanning calorimetry (DSC). The DSC curves of poly(*p*-phenylphenol) showed a broad exothermic peaks between 150-250 °C, indicating that the polymer undergoes complex structural transitions in the temperature range. On the other hand, the DSC curves of the poly(*p*-aminophenol) and the poly(*p*-phenylenediamine) which contain amino groups showed strong sharp endothermic peaks near 150 °C, implying that these polymers possess homogeneous oriented structures which undergo a concerted structural disintegration upon heating.

Keywords: Peroxidase, Polydiamines, Polyphenols, Thermal Property

INTRODUCTION

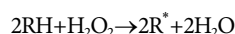
The enzymatic synthesis of polymers has been explored and has drawn interest because the enzymatic processes are environmentally friendly and require mild synthetic conditions [1-6]. A variety of phenols, aminophenols, and anilines have also been polymerized by peroxidases in organic solvents-water mixtures, supercritical solvents, and ionic liquids [5,7-10]. Phenolic polymers synthesized by enzymatic catalysis, especially, have the potential to replace currently used phenol-formaldehyde polymers in many applications for mechanical, electrical and optical uses [11].

Horseradish peroxidase (HRP) is one of the most extensively employed enzymes for the polymerization of aromatic compounds such as anilines and phenols with H₂O₂ as an oxidant [1,2,4,5]. The reaction mechanism of HRP with its two substrates, H₂O₂ and an aromatic compound, is described as shown below [12,13].



The ferric Fe ion (+3 state) at the active site of native HRP is reduced by two electrons to ferryl ion (+5 state) with a bound oxygen atom

from the first substrate, H₂O₂. The native HRP is thus converted to the first enzyme intermediate, compound I. The ferryl Fe ion in compound I is subsequently reduced to ferrous ion (+4 state) in compound II, then returns to the initial ferric ion while two molecules of the second substrate (RH) are oxidized to the corresponding radicals (R[•]). The overall enzymatic reaction of HRP is expressed by the following equation:



HRP catalyzes the formation of the monomer radicals (R[•]) only. The actual polymerization process occurs by non-enzymatic radical-radical reactions between the monomer radicals produced by the catalysis of HRP.

Even though various polymers, including polyphenols and polyanilines, have been synthesized by HRP, few studies have previously reported the thermal properties and heat-caused structural changes of the enzymatically synthesized polymers [2,14]. Such studies are especially scarce for the enzymatically synthesized polyphenylenediamines which are expected to have greater potential than polyaniline due to the presence of additional functional free amino groups on the polymer molecules [15]. For the applications of the enzymatically synthesized polymers in diverse areas, however, investigation on the thermal properties and structural characteristics of the polymers is highly required. This paper presents studies on the enzymatic synthesis of various polymers of substituted phenols and phenylenediamines by HRP and the thermal degradation prop-

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Table 1. Synthetic yields and thermal degradation properties of poly (*p*-phenylphenol) synthesized by HRP in different organic solvents (80% v/v)

Solvent	Synthetic yield	Thermal degradation properties		
		5% Weight loss temperature	10% Weight loss temperature	Char yield at 800 °C
Dioxane	71.0%	290 °C	353 °C	46.5%
DMF	67.9%	140 °C	163 °C	10.8%
Ethanol	82.1%	309 °C	357 °C	32.5%
THF	42.0%	289 °C	355 °C	40.5%

erties of the synthesized polymers.

EXPERIMENTAL

1. Enzymatic Synthesis of Polymers

HRP (Type II, 150-200 units/mg solid), substituted phenols, aminophenols, phenylenediamines, and other chemicals were purchased from Sigma-Aldrich.

The enzymatic synthesis of polymers was as follows. To 16 mL of an organic solvent, typically dioxane, a monomer was dissolved to a final concentration of 100 mM. Then, 4 mL sodium acetate buffer (10 mM, pH 5.0) and 10 mg of HRP were added with gentle magnetic stirring. The reaction was initiated by adding 30% H₂O₂ solution dropwise for 30 min. The total concentration of the added H₂O₂ was 100 mM so that the monomer to H₂O₂ molar ratio became 1. The typical HRP-catalyzed polymerization reactions in aqueous mixtures of organic solvents were reported to be finished within 2 h [2]. In this study, the reaction mixtures were shaken for 5 h at 25 °C to ensure the completion of the polymerization reactions. The final reaction mixtures were dried under vacuum to evaporate the solvents with a rotary evaporator. The remaining polymer residue was washed with 50% methanol for polyphenols and 10% acetone for polyaminophenols and polyphenylenediamines to remove unreacted monomers, salts, and the enzyme [5]. The final polymer samples were obtained by centrifugation, then dried at 60 °C under vacuum for further analysis. The synthetic yields of produced polymers were calculated as the percent of the initially added mass of monomers.

2. Characterization of Thermal Properties of Polymers

Thermal stability of enzymatically synthesized polymers was measured by thermogravimetric analysis (TGA) on a TA instrument (TGA Q50). Dried polymer samples (10 mg) were heated from 50 °C to 850 °C at a heating rate of 10 °C/min under N₂ atmosphere. The thermal stability of the polymers was represented by the % mass residue (char yield) after the heating. Thermal degradation kinetics were analyzed by derivative thermogravimetric analysis (DTG) employing the simplified pseudo-first-order kinetics developed by Horowitz and Metzger [16,17].

Structural properties of the synthesized polymers were analyzed by differential scanning calorimetry (DSC) on a TA instrument (DSC Q20). Dried polymer samples (4-6 mg) were placed in the DSC cells and equilibrated at 30 °C. The temperature of the DSC cell was then increased to 300 °C at a heating rate of 10 °C/min. N₂ gas was used as the purge gas at 50 mL/min. This heating cycle was repeated twice to characterize the thermoplastic or thermosetting

nature of the polymers.

RESULTS AND DISCUSSION

1. Selection of Solvents for the Enzymatic Synthesis of Poly(*p*-phenylphenol)

A variety of organic solvents have been demonstrated to maintain the activity of enzymes [18-20]. The major role of these organic solvents is to increase the solubility of nonpolar substrates of enzymes so as to enhance the productivity of enzymatic processes. To select an effective organic solvent for the enzymatic synthesis of polymers by HRP, aqueous mixtures of several organic solvents (80%

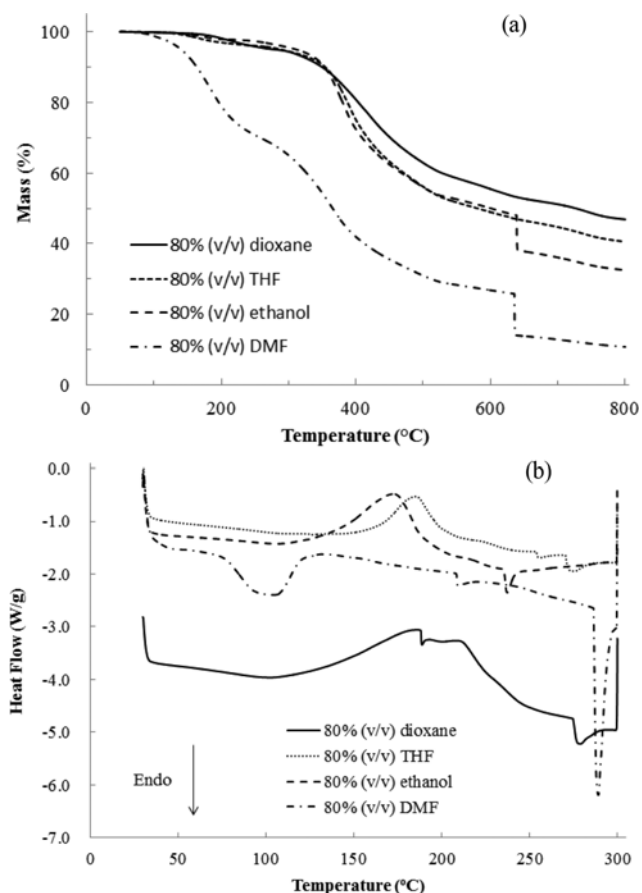


Fig. 1. TGA (a) and DSC (b) thermograms of poly(*p*-phenylphenol) synthesized by HRP in different solvents (80% v/v)-aqueous buffer mixtures (pH 5, 100 mM sodium acetate).

v/v) were tested as the reaction media for the synthesis of poly(*p*-phenylphenol), a representative polyphenol which has been synthesized by horseradish peroxidase [2,21]. The content of the organic solvents was selected to be 80% (v/v) based on the previous results that HRP maintains decent activity while the solubility of the phenolic substrates was enhanced in the solvents [2]. Table 1 lists the synthetic yields and thermal stabilities of the poly(*p*-phenylphenol) synthesized by HRP in the aqueous mixtures of 80% (v/v) different organic solvents. For poly(*p*-phenylphenol), the synthetic yield and thermal stability strongly depend on the type of organic solvents contained in the reaction mixtures. The synthetic yield of poly(*p*-phenylphenol) was the highest in 80% (v/v) ethanol but the lowest in 80% (v/v) tetrahydrofuran (THF). The thermal stability and structural properties of the poly(*p*-phenylphenol) were investigated by TGA and DCS analyses, respectively, and shown in Fig. 1. The char yield of the poly(*p*-phenylphenol) synthesized in 80% (v/v) dioxane was the highest as 46.5%, but it was the lowest as 10.8% when the polymer was synthesized in 80% DMF. DSC thermograms in Fig. 1 show broad exothermic peaks between 150 °C to 200 °C for poly(*p*-phenylphenol) synthesized in different solvents, indicating that the complex bonding nature of the polymer is common regardless of the solvents used as reaction media for the synthesis.

As proposed by Akkara et al., the existence of exothermic reactions for poly(*p*-phenylphenol) on DSC thermograms indicates that the structural changes of the polymer upon heating include crystallization, crosslinking, or blanching [2].

Based on the high synthetic yield and thermal stability of the enzymatically synthesized poly(*p*-phenylphenol), 80% dioxane solution was selected for the subsequent study for the enzymatic synthesis of polymers from various phenolic and nonphenolic functional monomers.

2. Syntheses and Thermal Properties of the Polyphenols and Polyphenylenediamines

Fig. 2 shows the structures of substituted phenols and phenylenediamines used as the monomers to synthesize the corresponding polymers by HRP as the catalyst. For substituted phenolic monomers, synthetic yields strongly depend on the substituted groups on the phenol ring as listed in Table 2. The lowest yield of only 8.1% was obtained for *p*-ethylphenol, while a modest yield of 46.9% was obtained for *p*-methoxyphenol. The synthetic yields of polyphenylenediamines, which lack phenolic hydroxyl groups, were lower than the yields of polyphenols; the yield of poly(*p*-phenylenediamine) was the highest as 57.4%, while that of poly(1,2-phenylenediamine) was the lowest as 13.5%. These results are close to the previously reported results in the literature where the polymerizations were

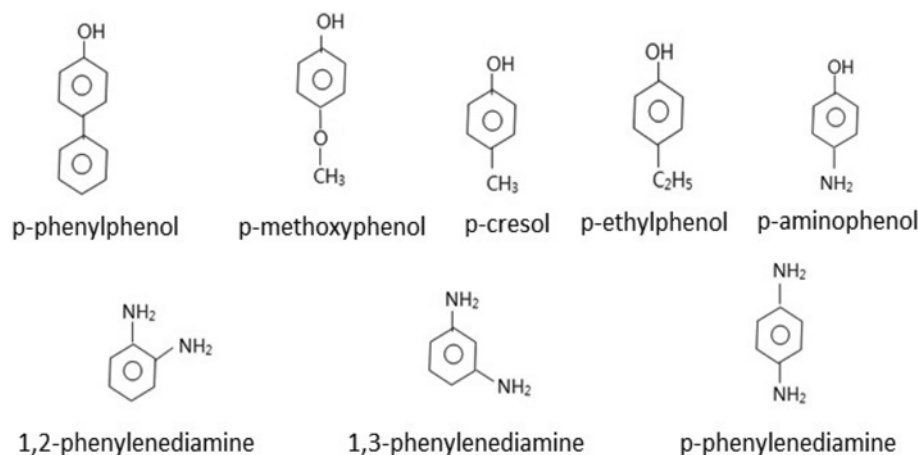


Fig. 2. Chemical structures of various phenols and phenylenediamines to synthesize the polymers catalyzed by HRP.

Table 2. Synthetic yields and thermal degradation properties of various polyphenols and polyphenylenediamines synthesized by HRP in 80% (v/v) dioxane

Class	Monomers	Synthetic yield	Thermal degradation properties		
			T_{max} (°C)	E_a (kJ/mol)	Char yield at 800 °C
Phenols	<i>p</i> -Phenyl phenol	71.0%	417	47.9	46.5%
	<i>p</i> -Methoxy phenol	46.9%	384	42.4	33%
	<i>p</i> -Cresol	19.0%	188	64.7	22%
	<i>p</i> -Ethyl phenol	8.1%	372	22.8	33%
	<i>p</i> -Amino phenol	77.0%	501	28.9	37%
Amines	1,2-Phenylene diamine	13.5%	721	43.6	41%
	1,3-Phenylene diamine	18.5%	723	33.0	38%
	<i>p</i> -Phenylene diamine	57.4%	698	40.8	35%

T_{max} : The temperatures of the maximum rate of weight loss at the peak of DTG thermograms

E_a : The activation energy for thermal degradation of the polymers at T_{max} determined from Horowitz-Metzger's pseudo-first-order kinetics

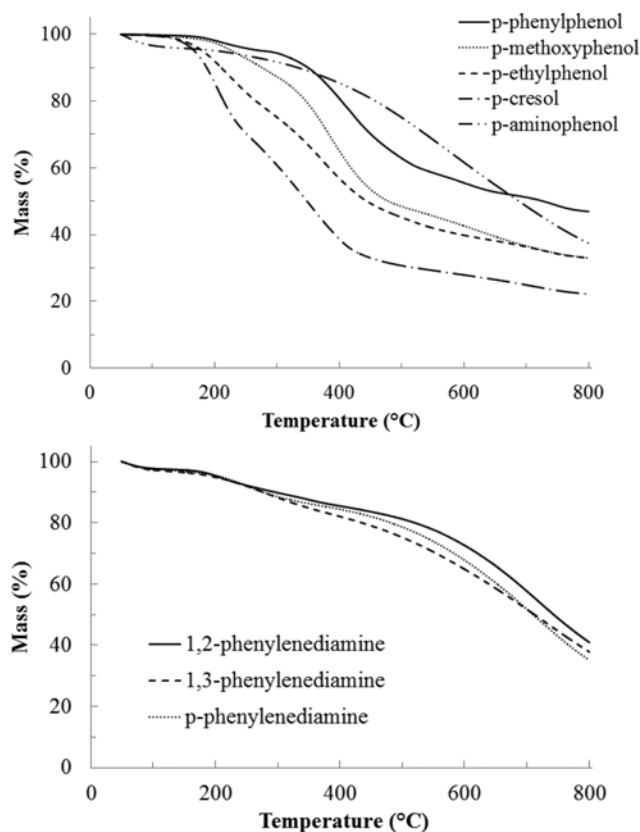


Fig. 3. TGA thermograms of polyphenols and polyphenylenediamines synthesized by HRP in 80% (v/v) dioxane.

performed under the similar reaction conditions with an extended 45 h reaction time [10]. In Fig. 3, TGA thermograms of polyphenols and polyphenylenediamines are shown with the thermogram of poly(*p*-phenylphenol) as the control. The char yields of polymers ranged from 22% to 47% with the highest yield for poly(*p*-phenylphenol). Among the polymers containing amine groups, polymers of 4-aminophenol (77% synthetic yield and 37% char yield) and *p*-phenylenediamine (57% synthetic yield and 35% char yield) were synthesized with high yields and good thermal stability.

To analyze the thermal degradation kinetics and estimate the activation energy, E_a , for the thermal degradation of the enzymatically synthesized polymers, a commonly used simplified pseudo-first-order kinetics derived by Horowitz and Metzger was employed [16,17,22]. The general form of the kinetic equation for the thermal degradation of polymers is expressed as

$$d\alpha/dt = A \exp(-E_a/RT) f(\alpha) \quad (1)$$

where $f(\alpha)$ is a function of the thermal degradation, E_a the activation energy, and α the fractional mass loss defined by Eq. (2).

$$\alpha = (m_0 - m_t)/m_0 \quad (2)$$

where m_t is the mass at any degradation time and m_0 is the initial mass. Therefore, the value of α becomes 0 before the onset of the thermal degradation at time 0 and 1 at the final state of the complete thermal degradation.

Horowitz and Metzger simplified Eq. (1) into Eq. (3) assuming

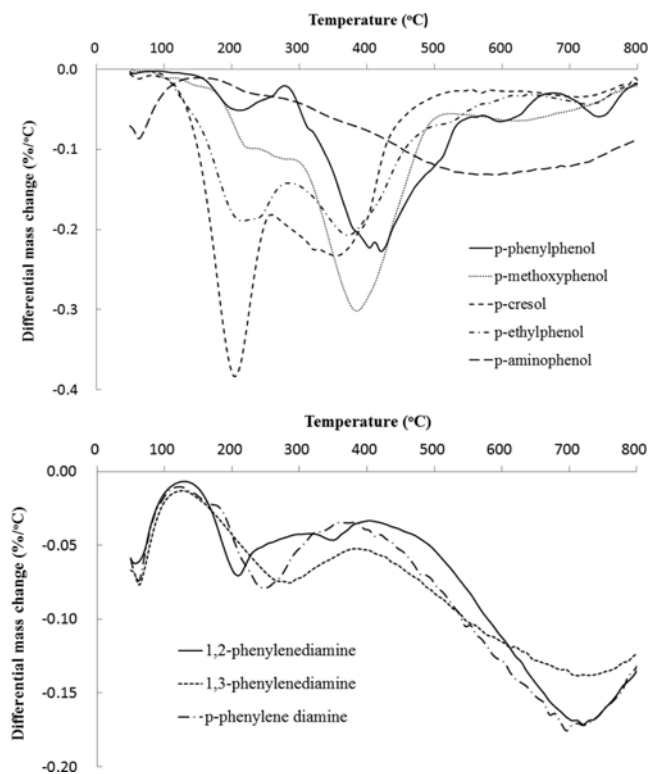


Fig. 4. DTG thermograms of polyphenols and polyphenylenediamines synthesized by HRP in 80% (v/v) dioxane.

a pseudo-first-order kinetics:

$$\ln(\ln(1-\alpha)^{-1}) = E_a/\theta RT_{max}^2 \quad (3)$$

where θ is the difference between T and T_{max} . T_{max} is the temperature at which the rate of weight loss becomes maximal [16,23]. The activation energy for the thermal degradation of the polymers at T_{max} can be calculated from the slope of the straight line between $\ln(\ln(1-\alpha)^{-1})$ and θ . DTG plots for the synthesized polymers are

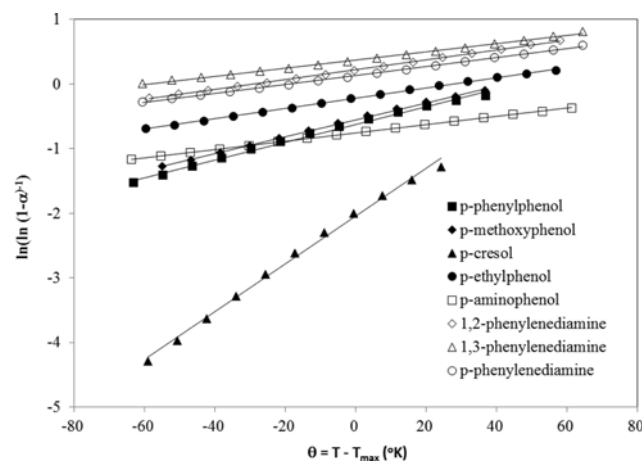


Fig. 5. Horowitz-Metzger plots between $\ln(\ln(1-\alpha)^{-1})$ and θ for the thermal degradation of the polyphenols and polyphenylenediamines synthesized by HRP, where α is the fractional mass loss and θ is the difference between T and T_{max} .

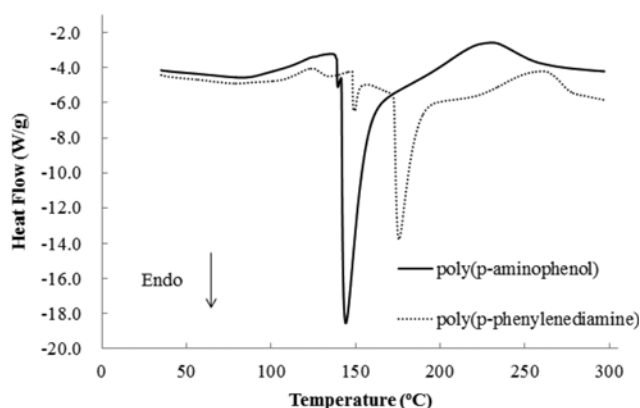


Fig. 6. DSC thermograms of poly(*p*-aminophenol) and poly(*p*-phenylenediamine) synthesized by HRP in 80% v/v dioxane-aqueous buffer mixture (pH 5, 100 mM sodium acetate).

shown in Fig. 4 and the linear Horowitz and Metzger plots are shown in Fig. 5. The activation energy, E_a , for the thermal degradation of the enzymatically synthesized polymers was calculated and listed in Table 2. The E_a values were in the range between 20 to 60 kJ/mol. The T_{max} values of polyphenylenediamines were higher than those of polyphenols. The chemically synthesized polymers such as polyalkyl methacrylates, Novoracs, polypyrroles, polyanilines, and polyphenylenediamines were reported to have similar E_a values to the enzymatically synthesized polymers [22–26].

The structures of poly(*p*-aminophenol) and poly(*p*-phenylenediamine), which were selected as the representative polymers containing amino groups, were investigated by DSC and their thermograms are shown in Fig. 6. The DSC thermograms of the two polymers exhibited strong endothermic peaks at 144 °C and 176 °C, respectively, which were absent in the DSC thermograms of poly(*p*-phenylphenol) shown in Fig. 1(b). The presence of the strong endothermic peaks in the DSC thermograms indicates that these polymers have homogeneous oriented structures which disintegrate in a concerted manner upon heating. In contrast to the polymers containing amino groups, the lack of endothermic peaks and the presence of broad exothermic peaks in the DSC thermograms of the poly(*p*-phenylphenol) indicate that the polymer undergoes complex structural transitions upon heating. The heat flows for all the phenolic polymers synthesized by HRP-catalysis were absent in a second heating of the polymers on DSC, demonstrating that the enzymatically synthesized polymers are thermosetting polymers. As previously reported, the solubility of the enzymatically synthesized phenolic polymers is negligible in most solvents [2]. Therefore, the molecular weights of the enzymatically synthesized polymers were not measured in this study.

CONCLUSIONS

The polymerization of various substituted phenols and phenylenediamines was carried out with high yields by HRP as the catalyst in 80% (v/v) dioxane. The polymers were found to be thermosetting polymers lacking the heat flows in the second heating on DSC. The DSC thermograms of poly(*p*-aminophenol) and poly(*p*-phenylenediamine) show the sharp and strong endothermic peaks,

which implied that these kinds of polymers have homogeneous oriented structures. On the other hand, those of the polyphenols show complex structural transitions. Most importantly, this study showed that thermal stability and the energetics of thermal degradation of the enzymatically synthesized polyphenols and polyphenylenediamines are comparable to those of the conventional polymers which are chemically or electrochemically synthesized [27,28]. Finally, the enzymatic synthesis of the polymers can be used as a mild facile alternative process to replace the conventional non-enzymatic methods to environmentally friendly produce polymers for potential applications requiring high thermal stability such as electrodes for batteries and capacitors, electromagnetic shielding of electronic devices, and flame-retardant organic-inorganic composite materials [29–32].

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