Immobilization of Catalase Using PAES-C Polymer for Wastewater Biological Treatment Research

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Abstract. PAES-C polymer modified by glutaraldehyde (GA) was selected to immobilize the catalase. Infrared spectrum analysis and $^1$H NMR test the presence of -COOH bond in PAES-C, which favored the cross-linking of PAES-C with catalase. The SEM image showed that its surface had a porous structure, which increased its surface area and provided more active sites for the immobilization of catalase. Indeed, the influence of various factors including GA mass concentration, modification time of GA and carrier, cross-linking time of modified carrier and catalase on the immobilization capacity were deeply investigated. In particular, the achieved optimal conditions for immobilization of catalase were as following: GA mass concentration was set at 1.0 wt%, GA modification time was 1.0 h, and the binding time between the modified carrier and the enzyme was 1.0 h. Herein, the findings in this work revealed that PAES-C polymer might be one of the promising candidates for wastewater biological treatment and immobilization of other enzyme.

1. Introduction

Polyarylethersulfone (PAES-C), as a thermoplastic engineering polymer, possessed excellent heat resistance, corrosion resistance, electrical insulation stability, film-forming processing, good mechanical strength and other comprehensive properties\textsuperscript{[1,2]}. Moreover, PAES-C also exhibited high glass transition temperature, good resistance to hydrolysis, good industrial solvent resistance, excellent oxidation stability\textsuperscript{[3]}. It was accepted that its thermal deformation temperature was much higher in comparison with other polymers such as polyamide, polycarbonate, pom, ABS resin\textsuperscript{[4]}. Therefore, it could be used for a long time under 150°C\textsuperscript{[5]}. Another remarkable merit for PAES-C was that it could maintain its good performance at a lower temperature, even in the arctic area, which might enlarge its application. In addition, the typical morphology and pore structure of polyarylethersulfone could keep its relatively large specific surface area, fast adsorption speed, good stability and biocompatibility, strong corrosion resistance for acid and alkali and good recyclability. Hence, this type of polymer have been applied in many fields such as electronics and electrical, aerospace, machinery, military equipment and facilities etc\textsuperscript{[6,7]}

Catalase (CAT)\textsuperscript{[8]} is a kind of biological macromolecule with the attractive activity in the catalytic decomposition of hydrogen peroxide. Therefore, it is used in the consumption of hydrogen peroxide contained in oxygen bleaching wastewater in textile industry. However, it is difficult to recycle catalase which greatly prevent its pilot application. Immobilized catalase was more loosely used than free enzymes, and it was more widely used. Moreover, the immobilization process might also enhance the activity of enzyme and its operating conditions to a certain extent, which could expand its application. There were also many materials for immobilization, including glyoxal, silicate, cotton
Catalase was widely used, and the research on catalase was getting more thorough. In recent years, the types of materials that immobilize catalase were also becoming more extensive, such as tomato straw and collagen. In 2019, Kowalski et al. explored the peroxidase, oxidase and reductase enzymes immobilized in protein crystals to maintain activity in the form of single crystals and volume measurements. Several binding strategies were used, including metal affinity and physical capture, to promote the adsorption of the enzyme into the protein crystals, and to retain the enzyme for multiple cycles. Comparing with the free enzyme in solution, the activity of the immobilized enzyme is lower, partly because the diffusion of the substrate in the crystal pores is limited. However, the immobilized enzyme is stable for long time using and get higher heat resistance. The potential applications of enzyme-carrying crystals as sensing devices, transfer capsules and microreactors promote the future development of this technology. Considering the rapid development of polymer synthesis, the immobilization of catalase using polymer materials might an attractive progress to increase its recyclability.

2. Experimental

2.1. Chemicals
Main chemicals: Catalase purchased from Novozymes (China) Biotechnology Co., LTD. Glutaraldehyde, purchased from Aladdin; Toluene; Anhydrous ethanol; 4,4'-dichlorodiphenylsulfone (DCDPS); Bisphenol acid (DPA); N, N-dimethyl formamide (DMF); N, N-dimethyl acetamide (DMAC); Dimethyl sulfoxide (DMSO); Tetrahydrofuran (THF); The above organic reagents were purchased from Sinopharm Group Chemical Reagents Co. LTD. Main instruments: Infrared spectrometers, Perkin Elmer; Jsm-6460lv scanning electron microscope, Japan Electronics Corporation; Uv-visible spectrophotometer, Shanghai Mountain Scientific Instrument Co., LTD.

2.2. PAES-C preparation
2.2.1. Pretreatment of chemicals. DMAC and DMSO were distilled under pressure, respectively. After the distillation, 4a zeolite was added for further drying. DPA and DCDPS were dried under vacuum at 100 °C for 24 h. K2CO3 was dried at 150 °C for 24 h, and KOH was dried at 100 °C for 24 h.
2.2.2. Synthesis of PAES-C. A mixture containing 0.03 mol K2CO3, 0.02 mol DPA, 0.02 mol DCDPS and 0.01 mol KOH were added into the three-mouth flask, and then a solution (40 mL) of toluene, DMAC and DMSO with a volume ratio of 3:2:2 was further added. Under the protection of continuous nitrogen flow, the achieved compound was mechanically stirred at 25°C for 2 hours. Subsequently, it was slowly heated to 150°C, and further refluxed for 3 h. After cooling down to room temperature, 0.02 mol DCDPS was added into the flask and the solution was slowly heated to 150°C for 3 h. After that, the mixture of toluene and water were removed from the separator. The residue in the flask was then heated to 175°C for 24 h. After complete reaction, it was cooled down to room temperature. The above achieved product was poured into a 500 mL conical flask, and the mixed solution of THF and HCl with a volume ratio of 4:5 was added before the product was acidified for 12 h. Then the acidifying liquid was discarded, and the white precipitate was washed for 4 times alternately with deionized water at 100°C and hot anhydrous ethanol. Finally, the product was put into a vacuum drying oven and dried at 100°C for 24 h to obtain PAES-C.

2.3. Characterization of PAES-C
2.3.1. Infrared spectrum analysis of PAES-C. 3 mg PAES-C was ground into powder and the sample was prepared by means of potassium bromide tablet. The infrared spectrum was recorded in the light region of 4 000 ~ 400 cm⁻¹ on the Infrared spectrometers (Perkin Elmer Instrument Co., LTD.).
2.3.2. SEM analysis of PAES-C. A small amount of PAES-C samples were fixed onto the sample table. After gold spraying, the morphology structure of PAES-C was checked by jSM-7800F ultra high resolution thermal field emission scanning electron microscope (SEM).
2.4. Immobilization of Catalase

1 g PAES-C was put into 20 mL 0.5% glutaraldehyde solution and the mixture reacted at 20°C for 1 h. After complete reaction, the remaining solid substances were washed with deionized water for 4 times to remove the GA solution on the solid surface. The free enzymes were dissolved in 50 mmol phosphate solution buffer at pH=7. The free enzyme was set at a 1 mg/mL free enzyme solution. The solid material after glutaraldehyde treatment was put into the solution of free enzyme for cross-linking reaction at 5°C for 1 h. After reaction, the product was filtered and thoroughly washed with the phosphoric acid buffer for 4 times. After drying, catalase was successfully immobilized.

2.4.1. Optimization of glutaraldehyde modification concentration. Under the above conditions, the contents of glutaraldehyde were set as 0.5%, 1.0%, 1.5% and 2.0% respectively for the preparation of immobilized CAT.

2.4.2. Optimization of modification time of glutaraldehyde. Under the above conditions, the GA reaction time was set to be 20 min, 40 min, 60 min and 80 min respectively for the immobilization of CAT.

2.4.3. Optimization of CAT fixation time. Under the above conditions, the reaction time between the enzyme and the carrier was 20 min, 60 min, 100 min and 140 min to prepare the immobilized CAT.

2.4.4. Orthogonal analysis. In order to precisely determine the optimal conditions, orthogonal experiments with three factors and four levels were carried out. The three factors were A (GA mass concentration), B (reaction time between GA and PAES-C) and C (enzyme reaction time), respectively. The four levels were shown in Table 1 respectively.

| Table 1. Levels of orthogonal factors. |
|---|---|---|---|
|   | 1   | 2   | 3   | 4   |
| A  | 0.5% | 1.0% | 1.5% | 2.0% |
| B  | 20 min | 40 min | 60 min | 80 min |
| C  | 20 min | 60 min | 100 min | 140 min |

2.5. Analysis Method.

2.5.1. Condition optimization of immobilized CAT. By UV-Vis spectrophotometry, hydrogen peroxide solutions with different concentrations were accurately configured, and their absorbance A was determined at 240 nm. With solution concentration C (PPM) as the horizontal coordinate and absorbance A as the vertical coordinate, linear regression analysis was conducted to obtain the standard curve related to solution concentration and absorbance, as shown in Equation (1).

\[ Y = 0.0003X - 0.0013 \quad R^2 = 0.9998 \quad (1) \]

2.5.2. Determination of activity of immobilized catalase. At 30 °C, 0.05 g immobilized enzyme was added into 10 mL 3000 ppm H₂O₂ phosphate buffer solution. After reaction (10 min), the immobilized enzyme was recycled. The absorbance value A of the solution before and after the reaction was collected at 240 nm respectively. The concentration of hydrogen peroxide was quantified based on the standard curve of hydrogen peroxide, and the activity of immobilized enzyme was calculated according to Equation (2).

Enzyme activity was defined as the amount of immobilized enzyme required to decompose 1 ppm hydrogen peroxide per minute (based on the total mass of immobilized enzyme) under specified conditions at 30°C, pH=7.0. The unit of enzyme activity was expressed as U/g.

\[ W = V \times (C_0 - C_e) \times 10^3 / (T - m) \quad (2) \]
V, volume of hydrogen peroxide solution (mL); C₀, initial concentration of hydrogen peroxide (PPM); Cₑ, residual hydrogen peroxide concentration (PPM). T, reaction time (min). M, total mass after immobilized enzyme (g).

3. Results and Discussion

3.1. Infrared Spectrum Analysis of PAES-C.
Figure 1 shows the FT-IR spectrum of the as-prepared PAES-C material. It was clearly observed that the vibration band at 343.38 cm⁻¹ corresponded to O-H stretching vibration[18]. The peak at 1737 cm⁻¹ was assigned to the stretching vibration of -C=O- groups[19]. Moreover, the bands centered at 1293 cm⁻¹, 1145 cm⁻¹ were attributed to the -SO² symmetric and antisymmetric stretching vibration -C-S- stretching vibration band was observed at 687 cm⁻¹[20,21,22]. On the other hand, the peaks centered at 1493 cm⁻¹, 819 cm⁻¹ and 733 cm⁻¹ respectively might be due to the stretching vibration of -C-C-bond on the benzene ring[23]. The symmetric and anti-symmetric stretching vibration of -Ph-O- bond were related with the bands at 1242 cm⁻¹ and 1012 cm⁻¹[24]. The findings indirectly evidenced the presence of -COOH bond in PAES-C, which favored the cross-linking of PAES-C with catalase.

![Figure 1. FTIR spectrum of PAES-C.](image)

3.2. SEM Analysis of PAES-C
As depicted in Fig 2, the surface morphology of PAES-C-NA was reticulated, with relatively uniform and dense pores distributed on the surface. The pore structure of the molecular chain, which was closely connected to the toes, might enhance its specific surface area and provide more active sites for the fixed cross-linking of catalase.
3.3. The $^1$H NMR Test and Chromatographic Analysis of the Polymer PAES-C-Na
PAES-C-Na was purified for several times to achieve the purity of the NMR test, and then PAES-C-Na was dissolved in the deuterium dimethyl sulfoxide reagent. The LIQUID NMR test was conducted with TMS as the internal standard using INOVA 400-mhz NMR analysis equipment of Valium, as shown in figure 3.

![Figure 3. $^1$H NMR spectra of polymer PAES-C-Na.](image)

According to $^1$H-NMR NMR spectrum, the peaks (g, e, f) in the range of 1.5 PPM to 2.5 PPM are attributed to the chemical shift of proton hydrogen on the long chain in aliphatic polymer molecules. The peaks (a, b, c, d) in the range of 6.5 PPM to 8.5 PPM are attributed to the chemical shift of protic hydrogen on the aromatic ring.

3.4. Determination of Molecular Weight of Polymer PAES-C
The relative molecular weight of PAES-C was determined by gel permeation chromatography (GPC) equipped with a differential refractive index detector. During the measurement, the molecular weight
of 0.5mL/min chromatogram pure tetrahydrofuran was used as eluent, and the polystyrene standard substance was used to calibrate the molecular weight, which ranged from 103 to 106. The sample with concentration of 2mg/mL was first dissolved in chromatogram pure tetrahydrofuran, then filtered by 0.45 μm oil phase filter membrane and injected into GPC for detection. The test results are shown in Table 2. The weight-average molecular weight of PAES-C is 125762 g/mol.

### Table 2. GPC data of PAES-C, as calculated towards polystyrene standards.

| Dist name | $M_n$ | $M_w$ | $M_p$ | $M_z$ | $M_{z+1}$ | Polydispersity |
|-----------|-------|-------|-------|-------|-----------|---------------|
| PAES-C    | 52841 | 125762| 82341 | 225154| 326243    | 2.38          |

3.5. **Optimal Conditions for Immobilization of CAT**  
The results of the orthogonal tests are presented in Fig 4,5,6. It was revealed that the optimal conditions for immobilization of CAT were as following: 1.0wt% GA, 1.0 h GA modification, and 1.0 h carrier and enzyme binding.
4. Conclusion
In conclusion, the carboxyl polyethersulfone polymer (PAES-C) modified by glutaraldehyde was selected as the catalase immobilized agent, and the schiff base reaction between glutaraldehyde and the carboxyl group had a certain influence on the immobilized enzyme experiment. Infrared spectrum analysis and $^1$H NMR test showed that it could be cross-linked with GA. The SEM image showed that its surface had a porous structure, which increased its surface area and provided more active sites for the immobilization of catalase. Therefore, the change of GA mass concentration and the length of GA cross-linking time will affect the final effect of immobilized enzyme. The optimal conditions for catalase immobilization (GA mass concentration 1.0%, GA modification time 1.0 h, and carrier-enzyme binding time 1.0 h) were obtained in detail by an immobilized optimization experiment. In addition, the ability of hydrogen peroxide decomposition to immobilize catalase has also been investigated.

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