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Enzymatic degradation of bisphenol-A with immobilized laccase on TiO_2 sol–gel coated PVDF membrane

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ABSTRACT

Bio-degradation with laccase immobilized on the TiO_2 functionalized membrane offers an attractive option to augment the conventional wastewater treatment processes for the removal of micropollutants such as Bisphenol A (BPA). Immobilization of the enzyme on nanostructured TiO_2 coated membranes addresses the common issues such as the poor activity and stability associated with the free laccase, as well as reducing the loss of laccase. Furthermore, the removal of large molecular weight BPA degradation products presents another benefit with this bio-catalytic membrane system. In this work, the TiO_2 nanoparticles were coated on the membrane surface via a low temperature hydrothermal sol–gel process, and the laccase was subsequently immobilized on these membranes by chemical coupling. The PVDF membranes with different pore sizes (0.1 and 0.45 μm) and coating cycles (up to 4 cycles) were used to examine the effects of the TiO_2 loadings and the nanostructure of the coating layer on the laccase immobilization performance. The optimal apparent activity and activity recovery were achieved on the 0.1 μm membrane with 3 coating cycles based on the 2,2′-azino-bis-(3-ethyl benzothiazoline-6-sulfonic acid) (ABTS) assay, due to the larger BET surface area and BJH pore diameter observed on this membrane. Substantial improvement in BPA removal efficiency and stability under moderate flux conditions were also obtained on the TiO_2 functionalized membranes, in good agreement with the ABTS assay results. In addition, the polymer products derived from the BPA bio-degradation process showed negligible fouling impact on the coated 0.1 μm membrane.

1. Introduction

Bisphenol A (BPA) is heavily used in the plastic industry as the plastic monomer and plasticizer in the production of polycarbonate and epoxy products [1]. Trace amount of BPA has been detected in natural environment as well as in human serum, urine, tissue and blood, due to the BPA leaching from a wide range of plastic products containing BPA [2]. The adverse effect of BPA on human health was evidenced even at low dosage, and its estrogenic activity can lead to cancer in the mammary and the prostate [3]. Therefore it is essential to remove the leached BPA from wastewater to minimize its potential harmful impact propagating through the water cycle. However, the complete removal of BPA via the conventional wastewater treatment processes is difficult to achieve as BPA can potentially absorb into membrane matrix and eventually contaminate the permeate [4]. The search for effective BPA removal from wastewater is therefore of high priority as is with many similar micropollutants.

The use of white rot fungi enzymes (e.g. laccase, lignin peroxidase, and versatile peroxidase) to eliminate BPA has been extensively studied [5–7]. Among them, laccase is the most preferable choice because of its low cost, good stability under moderate operating conditions, high catalytic efficiency, and broad specificity [8]. Furthermore, the oxidation of the phenolic compounds with laccase will form large insoluble polymers (Fig. 1) which can be easily removed from aqueous phase by filtration or sedimentation [9].

Although numerous studies have been dedicated to the use of laccase for BPA removal [10–12], the use of free laccase is undesired due to its high sensitivity to the industrial operating conditions, as well as its poor stability and reusability. The use of immobilized enzyme therefore has been proposed to address these issues. The laccase has been immobilized onto various supports, such as glass, agarose, silica, TiO_2, organic gel, chitosan, and kaolinite [13]. Among them, the TiO_2 nanoparticle is an ideal immobilization candidate due to its tunable surface structure, high surface area, high hydrophilicity, and good stability [14–16]. In addition, the presence of hydroxyl groups on the TiO_2 surface enables further functionalization to couple enzymes via covalent bonding, which is preferable in practice due to the stronger attachment formed between enzyme and support [13]. TiO_2 nanoparticles have been used to immobilize

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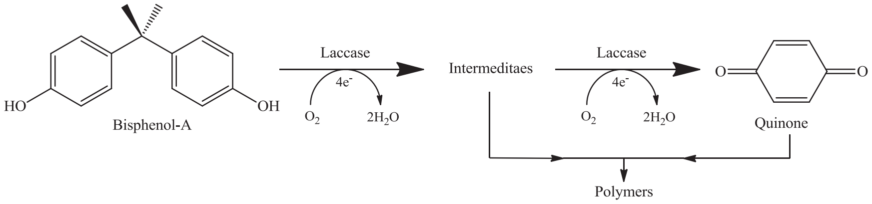

Fig. 1. Schematic degradation pathway of bisphenol-A in the presence of laccase.

enzymes such as glucose oxidase, lipase and urease, and good immobilization performances were achieved [17–19]. In our previous study, laccase was covalently immobilized onto the TiO_2 nanoparticles and nearly 80% of the original laccase activity was preserved [20]. Whilst excellent performance was observed on the laccase immobilized on the TiO_2 nanoparticle, the reuse and recycle of these bio-catalytic nanoparticles still remains a major challenge, especially in the wastewater treatment processes. This technical difficulty can be overcome by using membrane which can be easily integrated with the present wastewater treatment processes instead of a particulate immobilization substrate. In addition, the enhanced mass transfer offered by convective flow through the porous membrane can substantially improve the efficiency of the catalytic reaction. The use of both polymeric and inorganic membranes for laccase immobilization has been explored in the past. The performance of the immobilized laccase on polymeric membrane suffered from low activity recovery and poor reusability mainly due to the weak physical bonding formed between the enzyme and membrane, as well as the low surface area of the membrane [21–24]. While promising immobilization performance was observed on the ceramic membrane due to its fine-tuned, well-ordered surface and pore morphology [9], the difficulty of inorganic membrane preparation and its high cost hindered its wider application.

The nano-composite membrane incorporated with inorganic particles possesses advantages from both organic and inorganic components including low cost, high stability and flexibility, larger surface area, and controllable surface morphology [25,26]. Several studies have been carried out on the use of such nano-composite membrane for enzyme immobilization for biosensors [14,27–29]. In our previous work, TiO_2 particles were blended into the polyethersulfone (PES) membrane to prepare nano-composite membrane for laccase immobilization. Significant improvement in activity, activity recovery and stability was observed in comparison with the pure polymeric membrane [20]. However, due to the inherent limitations of the blending process, the majority of the TiO_2 was embedded within the PES matrix therefore not accessible to the laccase to function as the immobilization support. A potential solution is to strategically locate the TiO_2 particles on the surface of the polymeric membrane readily for laccase immobilization. Recently, a low temperature hydrothermal (LTH) sol–gel coating technique was developed in our group to generate a thin, robust layer of TiO_2 nano-composite coating on the in-house ultrafiltration PES membrane [30]. A uniform coating layer with high TiO_2 content was obtained with good stability and excellent fouling resistance. The membrane with such a unique structure and properties can be potentially used as the laccase immobilization substrate to improve the BPA degradation performance. However, little research has been focused on the laccase immobilization on TiO_2 coated membranes.

In this study, commercial polyvinylidene fluoride (PVDF) microfiltration membranes (0.1 and 0.45 μm pore size) from Millipore were chosen as coating support. Larger microfiltration membrane pores could ensure the formation the TiO_2 coating on both membrane surface and inside the membrane pores, which provided more accessible TiO_2 nanoparticles for laccase immobilization. In addition, larger pores also ensured good filtration performance after laccase immobilization, especially during the BPA degradation process where insoluble polymers were formed as degradation products. The PVDF membranes coated with the TiO_2 nanoparticles via the LTH sol–gel coating technique were explored as novel nanocomposite substrates. The bio-catalytic membranes were prepared by covalently immobilizing laccase onto the coated membrane through 3-aminopropyltriethoxysilane (APTES) and glutaraldehyde (GLU) sequential immobilization process. Comprehensive characterization techniques were applied to investigate the effect of the coating layer properties on the immobilization performance. In addition, the filtration conditions were assessed and optimized based on the BPA degradation performance. The long-term performance of the bio-catalytic membranes as well as the membrane fouling behavior during the BPA degradation process was also evaluated.

2. Experimental

2.1. Materials

Hydrophilic polyvinylidene fluoride (PVDF) membranes with pore sizes of 0.1 and 0.45 μm were purchased from Millipore Pty. Ltd. Laccase from *Trametes versicolour* (EC 1.10.3.2) was supplied by Sigma-Aldrich. 2, 2′-Azino-bis-(3-ethyl benzothiazoline-6-sulfonic acid) (ABTS, Sigma-Aldrich) was used as the assay substrate. Titanium isopropoxide (TTIP) ($\geq$97%, Sigma-Aldrich) was used to prepare the sol solution. 3-Aminopropyltriethoxysilane (APTES, Sigma-Aldrich) and glutaraldehyde (GLU, Ajax Finechem) were used for TiO_2 coating layer functionalization. Bisphenol A (BPA) was also supplied by Sigma-Aldrich. All the chemicals were of analytical grade and used without any purification.

2.2. Preparation of sol–gel coated PVDF membrane

The detailed preparation procedure of TiO_2 sol can be found elsewhere [30,31]. Components were mixed with a molar ratio of TTIP:2, 4-pentanedione:$HClO_4$:H_2O:ethanol = 1:0.5:0.5:0.45:4.76. The sol solution was stirred for 1 h at room temperature, and a light yellow transparent sol solution was formed at pH of 1.2.

Prior to the sol–gel coating, the PVDF membranes were soaked in Milli-Q water and absolute ethanol for 24 h each to remove all the preservatives, followed by drying at room temperature. Each dip-coating cycle was carried out by the following procedures: the membrane was firstly lowered at a speed of 1.0 mm/s into the sol solution. After 8 s soaking time, the membranes were withdrawn from the solution at a speed of 0.2 mm/s. Subsequently, the coated membranes were dried in the oven at 120 °C for 16 h and then placed in a 90 °C water bath for 24 h. Finally, the membranes were rinsed with Milli-Q water 3 times and dried at room temperature. In this work, up to 4 coating cycles were applied on both 0.1 and 0.45 μm PVDF membranes.