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Aquatic micro-pollutants removal with a biocatalytic membrane prepared by metal chelating affinity membrane chromatography

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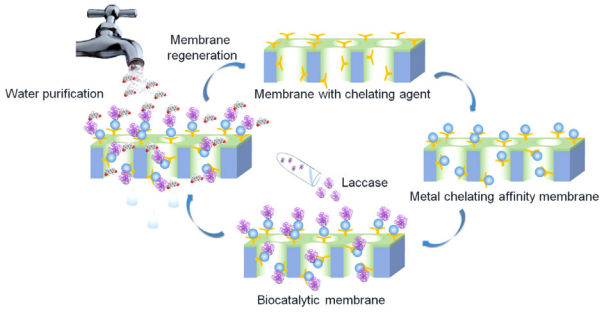
HIGHLIGHTS

- Metal chelating affinity membrane chromatography is used to prepare biocatalytic membrane.
- Metal ions have a significant effect on laccase activity recovery and its specific activity.
- Cascade catalysis with stacked biocatalytic membranes improves BPA degradation.
- Membrane fouling by BPA polymerization products causes BPA removal decline.
- Biocatalytic membrane regeneration is achieved by simple elution-cleaning-reloading.

GRAPHICAL ABSTRACT



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ABSTRACT

Biocatalytic membranes are promising to remove micro-pollutants in aqueous environment due to their mild and green operation condition. However, more efforts need to be devoted to improving their removal efficiency and stability. In this study, metal chelating affinity membrane chromatography (MCAMC) was used to construct a biocatalytic membrane by selectively capturing laccase from a crude fermentation broth. Metal ions had a significant effect on the activity of the immobilized laccase and copper ion was the best choice. A pH of 4.5 was selected for laccase adsorption and its loading seemed the same under flow rates from 0.5 to 10 mL min^{-1} thanks to the inherent convective transport of membrane chromatography. The pH value and salt concentration in the storage buffer had an obvious effect on the stability of the immobilized laccase, and the prepared biocatalytic membrane retained 87% of initial activity after 20 days storage. When applying such membrane to micro-pollutant removal (taking bisphenol A (BPA) as an example), a high BPA removal efficiency (99.3%) could be obtained. The biocatalytic membranes could be operated at a high flux of 50 L m^{-2} h^{-1} without recycling the permeate into the feed, and its throughput and BPA removal rate were superior to the most results in the literature. However, BPA removal decline (from 99.6% to 56.6% after five cycles) occurred during the successive water treatment due to the membrane fouling caused by BPA polymerization products. Membrane regeneration could be achieved by simple elution-cleaning-reloading, and the laccase activity and BPA removal were fully recovered.

1. Introduction

Metal chelating affinity chromatography was first introduced by Porath et al. to fractionate and purify proteins in 1975 [1]. Since

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then, thanks to high resolution and high reliability, it has become an important tool to isolate various proteins such as recombinant proteins, biomarkers and enzymes [2] for disease treatment and industrial production. The affinity coordinate bond is triggered by the metal-binding residues (e.g. histidine, lysine, glutamic acid, etc.) on proteins and the metal ions (e.g. Mn^{2+}, Zn^{2+}, Ni^{2+}, and Cu^{2+}, etc.) on ligands [3,4]. Nowadays, due to the advantages of strong multipoint attachment, mild adsorption as well as easy regeneration [5], various metal chelating adsorbents based on magnetic nanoparticles [6], microspheres [7], beads [8] and membranes [4], have been widely applied for enzyme immobilization, which could increase the stability, recyclability and reusability of enzymes during the reactions.

Among them, metal chelating affinity membranes (MCAMs) harbored the biocatalyst in their unique interconnecting pores, endowing the carrier with more catalytic sites, and the prepared biocatalytic membranes could be carried out under flow-through mode, overcoming the mass transfer limitation. Meanwhile, the attached enzymes could also be easily recycled, thus making it suitable for continuous operation [9,10]. In recent years, several studies on enzyme immobilization with MCAMs have been reported. For instance, laccase was immobilized on a Cu^{2+} chelated chitosan membrane via dipping incubation, and since the enzymes were anchored on the membrane surface with polymer chains, the diffusion limitation of substrate and products was negligible [4]. Wang et al. claimed that the performance of the immobilized enzymes on nanofibrous membranes depended on the quantity of the chelated metal ions [11]. Liu et al. immobilized penicillin G acylase on a Cu^{2+} chelated membrane and established a regeneration strategy [12,13]. However, the above-mentioned immobilization and enzymatic catalysis processes were lengthy and most of them were conducted by dipping incubation, which could not fully exploit the immobilization and catalytic sites inside the membrane. Actually, operating MCAMs under flow-through mode (dynamic filtration) was able to solve such problem. However, there are few studies regarding enzyme immobilization and subsequent catalysis with membrane under dynamic filtration.

On the other hand, metal chelating affinity membrane chromatography (MCAMC) with stacked MCAMs has been widely used for protein purification [14]. Due to the inherent convective transport, a high recovery and a high resolution could be well maintained under high flow rates [15]. With such outstanding features, it could be used to purify and immobilize enzymes by selectively capturing them from fermentation broth with a high volumetric throughput. Therefore, this can potentially provide an efficient, rapid and cost-effective approach for one-step enzyme purification and immobilization. However, the relevant study on the flow-through preparation of the biocatalytic membrane based on MCAMC has not been reported yet.

Employing biocatalytic membranes to remove some harmful and recalcitrant micro-pollutants for water purification has surged in recent years [16–18]. Bisphenol A (BPA) as an endocrine disruptor has an adverse impact on human health, which commonly exists in water system by leaching from a wide range of plastic products [19]. This micro-pollutant can be effectively degraded by laccase (polyphenoloxidase, E.C.1.10.3.2) that exhibits a high catalytic efficiency in oxidation of phenolic compounds [20,21]. Thereby, the biocatalytic membrane with immobilized laccase has been applied for the removal of BPA [17,19,22,23]. Cao et al. developed a multifunctional membrane for BPA removal by laccase catalysis, membrane rejection and adsorption, while there lacked an effective regeneration strategy to prolong the membrane working life [24]. Ji et al. fabricated a biocatalytic membrane based on a carbon nanotube (CNTs) coated membrane for BPA removal [17], while a full recycling mode was required to obtain a high level of micro-pollutants removal. Therefore, in order to further improve BPA removal efficiency, more efforts need to be devoted to increasing enzyme activity and reloading enzyme in the membrane reactor.

In this study, a MCAM was first developed by modification of a polyethyleneimine (PEI) coupled membrane [25]. As illustrated in Fig. 1, iminodiacetic acid (IDA) as a chelating agent was grafted onto the PEI chains to adsorb metal ions. The fabrication of biocatalytic membrane was then carried out by direct purification and immobilization of laccase from a crude fermentation broth with dynamic loading. The membrane structure and surface property during preparation were characterized and the influence of purification/immobilization conditions including different divalent metal ions, pH and flow rate were systematically investigated. Additionally, the effect of storage time, pH and salt concentration on laccase stability was examined. Finally, the prepared biocatalytic membrane was employed to remove BPA in aqueous solution under flow through mode. The influence of membrane configuration, operation flux, membrane regeneration and reuse on BPA removal was evaluated. To the best of our knowledge, there has no previous study regarding the fabrication of biocatalytic membrane with MCAMC for micro-pollutant removal. The outcome of this work would not only offer a novel strategy to prepare biocatalytic membrane, but also provide new insights into the catalytic behavior of such membranes for micro-pollutant removal.

2. Experimental

2.1. Materials

Polyvinylidene Fluoride (PVDF) microfiltration membranes with a pore size of 0.45 μm were bought from Jiuding High-Tech Filtration, China. A Mustang coin units (0.35 mL, an effective area of 1.5 cm^2) was purchased from Pall Corporation, USA. Dopamine hydrochloride, PEI (average Mw 70 kDa) from Sigma-Aldrich. 2.6-Dimethoxy phenol (DMP), BPA, sodium chloroacetate were bought from Aladdin. Cupric sulphate ($CuSO_4{\cdot}5H_2O$), zinc sulphate ($ZnSO_4{\cdot}7H_2O$), cobaltous sulphate ($CoSO_4{\cdot}7H_2O$), manganese sulphate ($MnSO_4{\cdot}H_2O$), nickel sulphate ($NiSO_4{\cdot}6H_2O$), isopropanol (IPA), ethanol, acetonitrile, sodium chloride, sodium hydroxide (NaOH), tris-base, hydrochloric acid (HCl), acetic acid (HAC), sodium acetate (NaAC), sodium citrate, sodium phosphate, citric acid, ethylenediamine tetraacetic acid disodium salt (EDTA), potassium thiocyanate (KSCN) and other chemicals for buffer preparation, sample analysis and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) were purchased from Beijing Chemicals Reagent Company, Beijing, China. Laccase was produced by *Trametes versicolor* (CICC14001) with a 7-day fermentation (25 °C, 150 rpm min^{-1}, pH 4.0). The crude fermentation broth was kindly provided by Prof. C.Z. Liu's group (Institute of Process Engineering, Chinese Academy of Sciences), which was obtained by a centrifugation (8000 rpm, 30 min, 4 °C) to remove the biomass.

2.2. Biocatalytic membrane preparation and characterization

A MCAM was prepared by the modification of a PEI grafted membrane as shown in Fig. 1. First, dopamine coating on a PVDF membrane (12 h, 90 rpm min^{-1}, room temperature, 20 mL 2.0 mg mL^{-1} dopamine solution with 10 mM pH 8.5 Tris-HCl buffer) was carried out to activate membrane by forming a polydopamine (PDA) layer [26], and then PEI was coupled onto the PDA layer (12 h, 120 rpm min^{-1}, 60 °C, 20 mL 2.0 mg mL^{-1} PEI in pure water) [25]. The modified membrane was then soaked in a sodium chloroacetate solution (12 h, 120 rpm min^{-1}, 60 °C, 20 mL 40 mg mL^{-1} sodium chloroacetate in pure water, pH = 12) to form IDA groups. After that, Cu^{2+} coupling was carried out by soaking the membrane in a $CuSO_4$ solution (8 h, 120 rpm min^{-1}, 25 °C,