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Highly stable enzymatic membrane for fast treatment of antibiotic-polluted water

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ABSTRACT

The antibiotic water pollution caused by discharge of untreated antibiotic waste water and sewage from animal husbandry is increasingly severe, especially in developing countries. However, current water treatment methods for antibiotic-polluted water are complicated, expensive and time consuming. Here, we have developed a highly effective enzymatic ultrafiltration membrane, which is extremely simple and can degrade antibiotic in a fast manner at a low-cost. Penicillinase, a representative enzyme for antibiotic degradation, was covalently immobilized in bromomethylated poly (2, 6-dimethyl-1, 4-phenylene oxide) (BPPO) ultrafiltration membrane by a simple self-assembling process. The membrane immobilized with penicillinase can thoroughly degrade the antibiotic by a single passing of the polluted water, with a treatment capacity up to 335 $L.m^{-2}.h^{-1}$. A small piece of membrane (ca. 25 cm^2) can provide sufficient clean drinking water for a family (4 people, 10 L per day) overnight. Furthermore, the engineered enzymatic membrane has an attractive stability and reusability for long-term application. We anticipate that our enzymatic membrane will serve as a practical and low-cost solution to antibiotic pollution, in particular for providing antibiotic-free drinking water in developing countries.

1. Introduction

Antibiotic pollution has been increasing globally, especially in developing countries such as China and India [1]. It has been recognized as a profound threat to both ecology and human health due to emerging severe antibiotic resistance [2–4]. Over 90% of antibiotic pollution in developing countries exists in the natural water system, into which untreated antibiotic waste water and sewage from animal husbandry are routinely discharged [5–8]. The direct discharge of antibiotic-related industrial waste water and veterinary and fishery sewage water has been overloading the metabolic capacity of the natural water system [6,9,10]. Due to the unaffordable cost of water treatment processes, such waste water containing high concentrated antibiotics is often discharged without proper treatment [11]. Most of the traditional water treatment methods, such as chlorination and ozonation [12], have shown poor efficiency on removal of antibiotics. Highly concentrated antibiotics in tap water have been reported in a few cities, causing panic of public [6,7,12,13]. Although a few methods, such as light catalysis and mesoporous materials adsorption [11], have shown improved removal efficiency for antibiotics, they have some significant limitations. In addition to the associated high cost, these methods are intrinsically slow, and they may produce a secondary contamination such as concentrated antibiotics in the adsorbent that are even harder to be eliminated [11]. Therefore, a simple and fast method is urgently needed to sustainably treat the antibiotic polluted water at low-cost without secondary contamination.

Combination of enzyme and membrane technology has the potential to meet this need. Enzymes are intensively involved in *in vivo* antibiotic metabolism, and they can degrade antibiotics into relatively harmless chemicals [14]. Free enzyme has been attempted for treatment of sewage water in a batch mode [15], but the low efficiency and non-reusability prevented it from practical application. Membrane technology is well-established as a separation process with advantages including low-cost, easy scale-

Abbreviations: BPPO, bromomethylated poly (2, 6-dimethyl-1, 4-phenylene oxide); EMR, enzymatic membrane reactors; UF, ultrafiltration; SEM, Scanning Electron Microscope; P-BPPO, penicillinase immobilized BPPO membrane; AFM, atomic force microscopy

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up, and fast operation [16], and enzymatic membrane reactor has been explored for degradation of dyes [17,18] and antibiotics in waste water [19]. Despite the promising results, the treatment of antibiotic-polluted water by enzymatic membrane is not yet sufficiently effective. For example, a recent work has reported a degradation yield of 56% for tetracycline treatment over 24 h operation [19].

In this study, we aim to create a highly effective enzymatic membrane that can thoroughly degrade antibiotics by a single pass in a few seconds. Penicillinase, one of the highly efficient enzymes engaging in the human natural antibiotics metabolic system to hydrolyze penicillin [20], has been immobilized onto the surface and within the nano-channels of the BPPO membranes. Selection of penicillinase was based on consideration that penicillin is the most widely applied antibiotic [21]. An asymmetric polymer ultrafiltration (UF) membrane, BPPO membrane, has been selected because of its excellent flux and controllable nano-sized pores on its permselective layer [22]. Also, BPPO membrane has abundant highly reactive bromide groups [23] which can react with amine groups of the enzyme, enabling one step self-assembly and covalently immobilization of penicillinase, to form the stable and reusable enzymatic membrane for antibiotic-polluted water treatment (Scheme 1, Fig. S1). This enzymatic membrane is expected to serve as a highly efficient and yet extremely simple device for treatment of the antibiotic polluted water as shown below.

2. Materials and methods

2.1. Materials

Bromomethylated poly (2, 6-dimethyl-1, 4-phenylene oxide) (BPPO) (Tianwei Membrane Corporation Ltd., Shandong, China); N-Methyl-2-pyrrolidone (NMP) (purity $\geq$99%, Sigma-Aldrich, Australia); phosphate buffer saline (PBS) tablet (Sigma-Aldrich, Australia); Penicillinase from *Bacillus cereus* (lyophilized powder with a molecular weight of 28 kDa, protein content 3.72%, Sigma-Aldrich, Australia); Benzylpenicillin sodium (Sigma-Aldrich, Australia); Alexa fluor-594 (Life technology, Australia).

2.2. Fabrication of BPPO membrane and the penicillinase immobilization

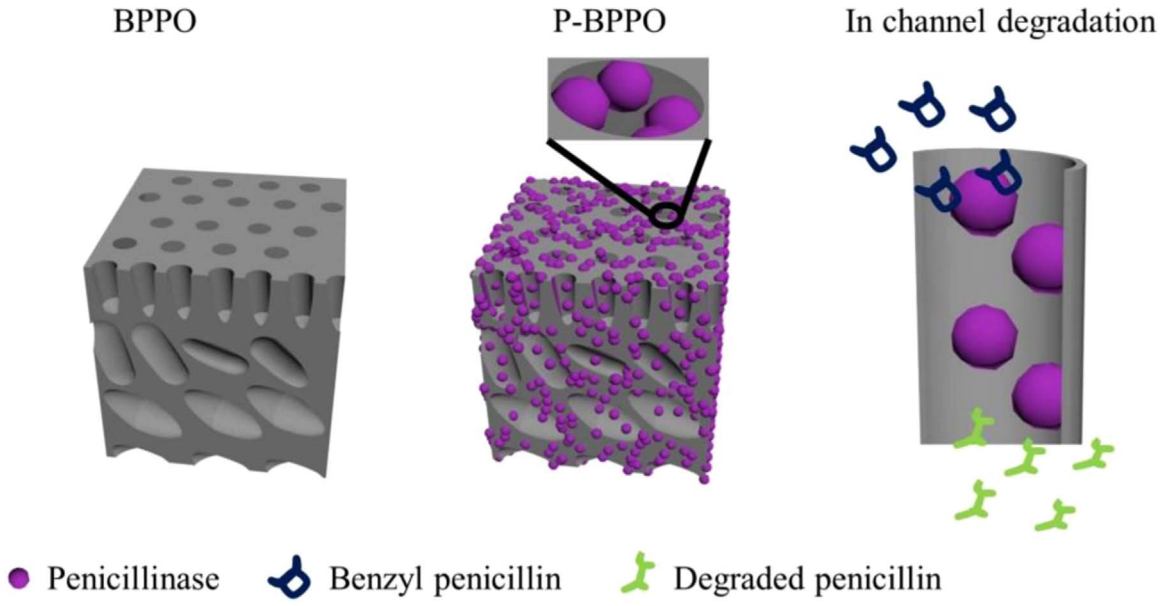
The fabrication of BPPO membrane followed the method reported in previous study [23]. Typically, the 20 wt% BPPO polymer was dissolved in NMP and the mixture was then cast onto a clean glass plate using a casting knife with a 150 μm air gap. After that, the glass was immediately put into deionized (DI) water for phase inversion. After the membrane was prepared, a circle piece of membrane with diameter of 3.5 cm was cut off, and immersed into 2 ml penicillinase solution (5 mg crude powder in 2 ml PBS buffer 0.01 M, pH 7.4) followed by 4 h incubation under room temperature with 60 rpm shaking. Then, this penicillinase immobilized BPPO membrane (P-BPPO) was washed with large amount of NaCl (1 M) and DI water to remove the non-covalent bonded enzyme. The supernatant after immobilization and the original enzyme sample were qualitatively analysed using SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and the protein concentration in the samples were determined by Pierce 660 nm Protein Assay method (Pierce™ 660 nm Protein Assay Reagent, Thermal Fisher Scientific) using bovine serum album (BSA) as standard.

2.3. Characterization of Membranes

The composite surface of the permselective layers was characterized by Fourier transform infrared spectroscopy (FTIR spectrometer, PerkinElmer, Australia), with 20 scans. The morphology of the surface of the permselective layers, bottom surface, and cross-section part was examined by scanning electron microscopy (SEM) (Nova SEM, FEI Company, America). Membrane cross-sections were obtained by fracturing membranes frozen in liquid nitrogen [24].

2.4. Bio-imaging

Bio-imaging was carried out using a Nikon C1 (Inverted) confocal microscope equipped with a 60 × oil immersion objective and running NIS Elements Software (Nikon. Tokyo, Japan). Before imaging, the samples (P-BPPO and BPPO) were soaked in 1 ml 0.01 M PBS. Then, 2 μL of Alexa Fluor® 594 NHS Ester (Succinimidyl Ester) (2.5 mg/ml in water) was added into PBS, and



Scheme 1. Scheme of BPPO, P-BPPO and degrading penicillin using P-BPPO.