Colloids and Surfaces B: Biointerfaces 170 (2018) 588–595

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Reusable anionic sulfonate functionalized nanofibrous membranes for cellulase enzyme adsorption and separation

Noha Amaly[a,b], Yang Si[a], Yike Chen[c], Ahmed Y. El-Moghazy[a,b], Cunyi Zhao[a], Ruihong Zhang[c], Gang Sun[a,*]

[a] Fiber and Polymer Science, University of California, Davis, CA 95616 USA
[b] Polymeric Materials Research Department, Advanced Technology and New Materials Research Institute, City of Scientific Research and Technological Applications (SRTA-City), New Borg El-Arab City, 21934, Alexandria, Egypt
[c] Biological and Agricultural Engineering, University of California, Davis, CA 95616, USA

ARTICLE INFO

Keywords:
PVA-co-PE
Nanofiber
Sulfonation
Adsorption
Cellulase

ABSTRACT

Poly (vinyl alcohol-co-ethylene) nanofibrous membranes (PVA-co-PE NFM) were successfully modified by sodium-3-sulfobenzoate to become negatively charged with sulfonate groups, and the sulfonated (PVA-co-PE) nanofiber membrane SS (PVA-co-PE NFM) was used in non-covalent adsorption of cellulases via electrostatic attraction. The modified NFM showed excellent adsorption to the enzyme molecules due to the incorporated static charge interaction with the fibers, high open-porosity and ultrahigh surface areas of the nanofibers. Such unique morphology and chemical structures lead to the adsorption capacity of 130 mg g^{-1} and reusability for 5 cycles without significant change in catalytic functions. The morphology changes of the nanofibrous membranes were observed by using a scanning electron microscopy, and chemical structures of the membranes were characterized by using FTIR and water contact angle measurements. SS (PVA-co-PE NFM) is a promising solid support media for enzyme immobilization, and the immobilized enzymes can be applied in industrial applications.

1. Introduction

Bioethanol, biomethanol, biodiesel, etc. known as biofuels are an alternative and sustainable energy source that will likely become more prevalent in the future [1] due to the fact that fossil fuels are fast depleting by current consumption rates. The search for an alternative energy source that is renewable, economical, and environmentally friendly is urgent. Cellulose is the most abundant biopolymer in nature and renewable in agricultural production. Thus, bioethanol made from cellulose is therefore a very promising resource of the biofuels. Production of ethanol from cellulose usually involves two steps: First, hydrolysis of cellulose to glucose; and second, fermentation of glucose to ethanol. Although the first process can be performed by either chemical or enzymatic process [2]. Using enzymes as green catalysts of the hydrolysis is preferred because the process is environmentally friendly and operated under mild conditions. The enzyme molecules, specifically cellulases in most cases, are relatively expensive, chemically or thermally unstable, and difficult for handling, purification, and reuse, which have limited the large-scale operations in industrial applications [3,4]. Cellulases are a mixture of enzymes that act synergistically to convert cellulose into glucose, [5], usually composed of three enzymes: endoglucanase (EC 3.2.1.4), cellobiohydrolase (EC 3.2.1.91) and cellobiase (EC 3.2.1.21) [6]. Endoglucanase and cellobiohydrolase are responsible for decreasing degree of polymerization of cellulose to produce cellobiose, which are further hydrolyzed to glucose by cellobiase [7].

Recovery of these free cellulases from the solution after the hydrolysis process is quite difficult, which significantly limits the reusability of the enzymes and increases the costs of the process [8]. Many approaches to recover the enzymes have been attempted, including ultrafiltration (UF) [9]. However, many challenges exist, such as fouling and concentration polarization on the membranes, cleaning costs, and membrane damages during applications [10,11]. Column separation technique is only employed in systems processing small quantity of hydrolysis because of the instrumentation complicacy, lower yield, diffusion limitation and high production cost, though it revealed high resolution capacity [12,13]. So far, enzyme isolation, separation, and purification by adsorption onto solid materials are still considered as the best practical techniques to recycle the enzymes and consequently reduce the cost of the bioethanol production. Here, the

* Corresponding author.
E-mail address: gysun@ucdavis.edu (G. Sun).

https://doi.org/10.1016/j.colsurfb.2018.06.019
Received 19 January 2018; Received in revised form 21 May 2018; Accepted 14 June 2018
Available online 25 June 2018
0927-7765/

adsorption mechanism and solid media are two important factors that can significantly influence recycling and reuse of the biocatalysts. Generally speaking, the adsorption of proteins onto solid adsorbents is due to physical adsorption caused by electrostatic interactions, hydrogen bonding, and hydrophobic interactions between solid surfaces and protein molecules, without affecting conformational structure and active center of enzyme molecules [14]. Thus, such an adsorption is affected by surface area of the solid materials and can retain their biological activities. Materials possess high surface areas is a determining factor in recycling of cellulase enzymes. Inspired by the development of nano materials, which possess ultra-high surface area, many efforts have been made to immobilize enzymes onto surfaces of nanoparticles [2], mesoporous materials [15] and nanofibrous membranes [16]. Overall, nanofibrous membrane materials should be a better solid carrier versus nanoparticles and nanowires [17–19].

Various nanofibrous membranes have been prepared for adsorption of BSA and have shown good efficiency [20]. However, nonspecific interactions between enzymes and hydrophobic nanofiber surfaces could induce nonselective adsorption onto the membranes, leading to reduced separation of the targeted enzyme molecules from the reaction systems. Thus, it is recommended to modify hydrophilic nanofibrous membranes with function groups and promote interactions with specific enzymes and assist enzyme adsorption [21].

In this research, we selected poly (vinyl alcohol-co-ethylene) nanofibrous membranes (PVA-co-PE NFM) for further chemical modifications because of the hydrophilicity and existence of reactivity of hydroxyl groups in the polymer. Sodium-3- sulfobenzoate (SS) can bring strong anionic groups to PVA-co-PE by reacting the benzoate group in SS with hydroxyl group on PVA-co-PE. To promote the reaction between hydroxyl groups on PVA-co-PE with carboxylic groups on SS, N-Ethyl-N′-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) was first mixed with SS to form an activated intermediate [22], according to Scheme 1, which then reacts with PVA-co-PE to form ester bond. Thus modified PVA-co-PE nanofibrous membranes (PVA-co-PE-NFM) was prepared and employed in separation of cellulase enzymes. The highly open-porous structure and the numerous sulfonate groups introduced on the membranes, make sulfonated (PVA-co-PE NFM) exhibit excellent adsorption of the enzymes due to the increase electrostatic charges, high open-porosity, and robust mechanical strength. Such unique morphology and chemical structure lead to high efficient protein adsorption capacity, short adsorption time and improved reusability.

2. Experimental section

2.1. Materials

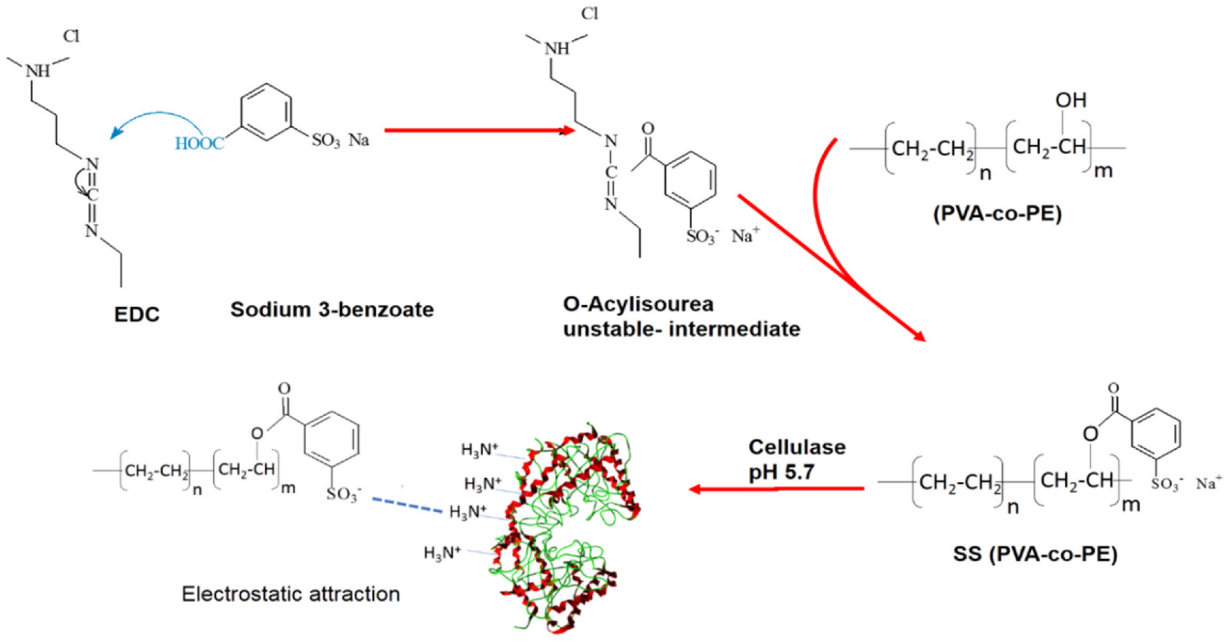
PVA-co-PE (ethylene content 27, 32 and 44 mol%), sodium 3-sulfobenzoate, sodium chloride (NaCl), calcium chloride ($CaCl_2$), potassium chloride (KCl) and magnesium chloride ($MgCl_2$), were purchased from Sigma-Aldrich (Milwaukee, WI, USA), N-Ethyl-N′-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), disodium hydrogen phosphate (Na_2HPO_4), monosodium orthophosphate (NaH_2PO_4), Bradford solution (Biorad), sodium hydroxide (NaOH), and hydrochloric acid (HCl) were supplied by Acros Chemical (Pittsburgh, PA,USA). Cellulase enzyme was supplied by Novozymes (Davis, CA, USA) with total protein soluble, 70 g L^{-1}, 267–323 g L^{-1} glucose and 50% total solids. All water used was purified via a Millipore Milli-Q plus water purification system. All chemicals were used as received.

2.2. Fabrication of PVA-co-PE nanofibrous membranes

The PVA-co-PE solution (8 wt %) was prepared by dissolving a certain weight of copolymer in the mixture solvent of isopropanol and ultra-pure water (7/3, v/v) with vigorous stirring in a water bath at 60 °C for 8 h [23]. Afterward, the as-prepared solution was transferred to 10-mL plastic syringes with tubular metal needles. The electrospinning process was implemented by utilizing a DXES-1 spinning equipment supplied with an applied voltage of 30 kV, a controllable propulsion velocity of 4 mL h^{-1}, and a distance of 23 cm from spinneret to membrane collector. The spinning process was at room temperature and varied humidity. The resultant nanofibrous membranes (PVA-co-PE NFM) were collected on the grounded roller covered with paper and rotated at speed of 100 rpm, and then dried by using a vacuum oven at 40 °C for 12 h.

2.3. Modification of (PVA-co-PE NFM)

PVA-co-PE NFM was chemically modified following this procedure: Different sodium-3-sulfobenzoate (SS) concentrations (0, 2.5, 4.5, 5, 6,



Scheme 1. Scheme of (PVA-co-PE NFM) sulfonation and cellulase adsorption.