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Immobilisation of cyclodextrin glucanotransferase into polyvinyl alcohol (PVA) nanofibres via electrospinning

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ABSTRACT

Immobilisation of cyclodextrin glucanotransferase (CGTase) on nanofibres was demonstrated. CGTase solution (1% v/v) and PVA (8 wt%) solution were mixed followed by electrospinning (−9 kV, 3 h). CGTase/PVA nanofibres with an average diameter of 176 ± 46 nm were successfully produced. The nanofibres that consist of immobilised CGTase were crosslinked with glutaraldehyde vapour. A CGTase/PVA film made up from the same mixture and treated the same way was used as a control experiment. The immobilised CGTase on nanofibres showed superior performance with nearly a 2.5 fold higher enzyme loading and 31% higher enzyme activity in comparison with the film.

1. Introduction

Cyclodextrin glucanotransferase (CGTase) is an industrially important enzyme that catalyses the transglycosylation reaction of starch and linear oligosaccharides to form ring structured molecules, called cyclodextrins (CDs) [1]. CDs possess the unique property of having a hydrophilic outer surface and a hydrophobic cavity which enables them to form an inclusion complex with guest molecules [2]. Due to this feature, they are widely used in industrial and research applications. The use of CDs has increased by about 20–30% per annum, mainly in food and pharmaceutical products [3].

Generally, free or soluble CGTase are used for the industrial production of CDs [4]. However, the main drawback in the industrial application of free enzymes is their relatively high price and low stability [5]. Immobilising the enzyme by fixing to a solid support will allow the enzyme to be recovered and reused, thus reducing the amount of enzyme required, and simplifying product purification, which makes the enzymatic processes more economically feasible [6–8]. To this end, several CGTase have been immobilised using different supports and immobilisation methods [3,9].

The trend of enzyme immobilisation has now shifted towards the utilisation of nanostructured materials as the enzyme support, which includes nanoporous materials, nanoparticles, and nanofibres [10]. In comparison with the conventional supports, nanostructured materials offer the intrinsic characteristic of an extremely high surface area to volume ratio which is desirable for improvement of the immobilisation efficiency. Immobilised CGTase on nanoporous silica and nanoparticles show a remarkable improvement in terms of enzyme loading and activity recovery [11,12]. However, despite the advantages, some of the problems associated with nanoporous media are such as limited enzyme-substrate interaction due to the enzyme confinement on its inner surface and the complex recovery procedures of the nanoparticles [10]. In this regard, increasing interest has been shown in incorporating enzymes into nanofibrous materials, particularly electrospun nanofibres [6,13–15].

Electrospinning involves electrical atomisation of a viscous liquid that is capable of producing fibres with a diameter ranging

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from the micro to nanometre scale. With high porosity and interconnectivity, the electrospun nanofibres can form a membrane which can be easily recovered, reused and could reduce the mass transfer limitation [16,17]. These unique characteristics enable the application of electrospun nanofibres in diverse fields such as controlled release of compounds in drug delivery, templates or scaffolds in tissue engineering, reinforcement for composite materials and membranes for water filtration systems [6,8]. In biocatalysis, an enzyme immobilised on a nanofibrous membrane allows repeated usage of the enzyme and simultaneous biocatalytic reaction and enzyme-product separation, which are the main requirements for the application of an immobilised enzyme in a membrane-bioreactor [10,13].

In this study, the immobilisation of CGTase into a nanofibrous PVA membrane *via* electrospinning of CGTase and a PVA solution was demonstrated. To the knowledge of the authors, this is the first application of nanofibres as a support for CGTase. The performance of the immobilised enzyme was examined in terms of enzyme loading and catalytic activity with CGTase/PVA nanofibres. In addition, the CGTase/PVA film that was produced from the same solution was used as a control.

2. Materials and methods

2.1. Materials

Polyvinyl alcohol (PVA) with an average molecular weight of 85 000–124 000 Da and a 99% degree of hydrolysis was obtained from Sigma Aldrich, USA. CGTase (EC 2.4.1.19) from *Bacillus macerans* was procured from Amano Enzyme, Inc., Japan. α-CD was provided by Acros Organics, USA. The Bradford reagent, bovine serum albumin (BSA, 67 000 Da) and glutaraldehyde (GA) solution (25% (w/v) in water) were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Soluble starch and hydrochloric acid (32% (w/v)) were provided by Merck, Germany. All the other chemicals used were of analytical reagent grade.

2.2. Methods

2.2.1. Preparation of PVA/CGTase solution

PVA powder was dissolved in a phosphate buffer (100 mM, pH 6) at 90 °C under mild stirring to create a PVA solution of 8% (w/v) concentration. The solution was then allowed to cool at room temperature before mixing with CGTase solution to produce a CGTase/PVA mixture with 1% (v/v) enzyme concentration.

2.2.2. Electrospinning of CGTase/PVA solution

The freshly prepared CGTase/PVA solution was fed into a 10 ml plastic syringe fitted with a 16 G stainless steel capillary (length × OD × ID = 80 × 1.61 × 1.25 mm). The electrospinning was performed by applying a high electric field (−9 kV) to the capillary for a total of 3 h and with a flow rate of 0.5 ml/h. The electrospinning modes were observed using a digital camera equipped with a macro lens. The PVA/CGTase nanofibres were collected using a rotating collector which was covered with aluminium foil (grounded) and placed at 20 cm away from the capillary tip. The nanofibres obtained were then dried overnight in a desiccator before detaching from the aluminium foil and stored at 4 °C before further reactions and analyses. A schematic diagram for the method of CGTase/PVA nanofibre production and the electrospinning system is presented in Fig. 1.

2.2.3. Vapour phase glutaraldehyde (GA) crosslinking

The membrane was subjected to glutaraldehyde vapour phase crosslinking to prevent the dissolution of the hydrophilic PVA as well as to improve the enzyme-PVA interaction. The crosslinking was conducted for 2.5 h at ambient temperature in the presence of hydrochloric acid (HCl) as a catalyst with a 3:1 ratio of GA:HCl, as previously described by Shaikh et al. [18].

2.2.4. Characterisation of PVA/CGTase membrane

The morphology of the PVA/CGTase membrane was observed using a Scanning Electron Microscope (SEM) (JSM 6510, JEOL,

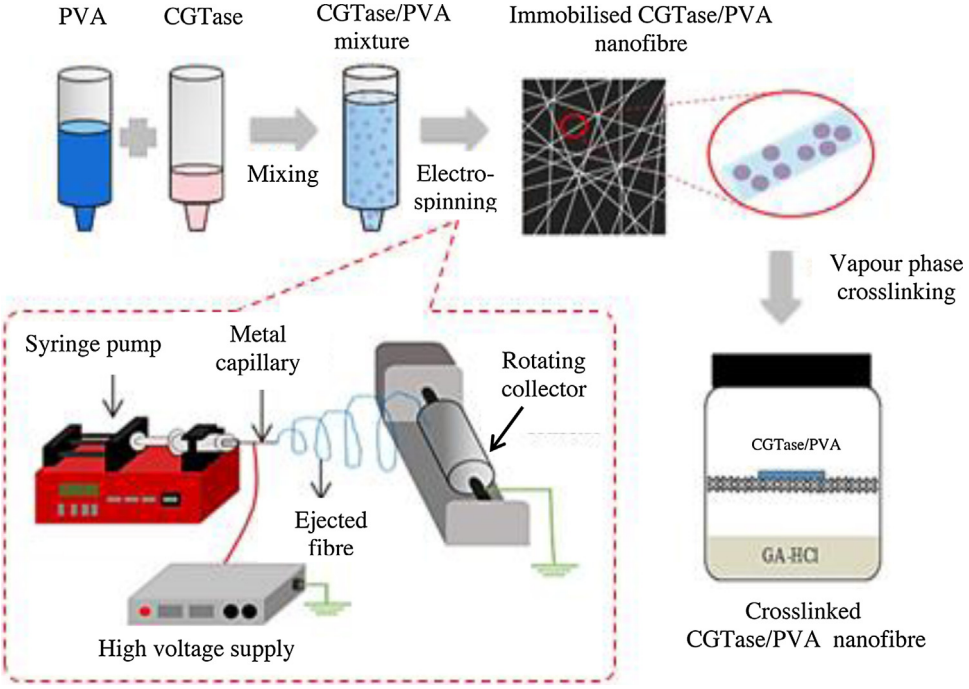

Fig. 1. Schematic of the method used to produce CGTase/PVA nanofibres.