Enzyme and Microbial Technology 54 (2014) 38–44

Contents lists available at ScienceDirect

Enzyme and Microbial Technology

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

Bioremediation of pesticide contaminated water using an organophosphate degrading enzyme immobilized on nonwoven polyester textiles

Yuan Gao [a,*], Yen Bach Truong [a], Paul Cacioli [b], Phil Butler [b], Ilias Louis Kyratzis [a]

[a] Materials Science and Engineering, Commonwealth Scientific and Industrial Research Organization (CSIRO), Bayview Ave, Clayton 3168, Australia
[b] Textor Technologies, Melbourne, Australia

ARTICLE INFO

Article history:
Received 30 July 2013
Received in revised form 2 October 2013
Accepted 4 October 2013

Keywords:
Enzyme immobilization
Bioremediation
Organophosphate
Organophosphate hydrolase
Polyester
Nonwoven textiles

ABSTRACT

Bioremediation using enzymes has become an attractive approach for removing hazardous chemicals such as organophosphate pesticides from the environment. Enzymes immobilized on solid carriers are particularly suited for such applications. In this study, the organophosphate degrading enzyme A (OpdA) was covalently immobilized on highly porous nonwoven polyester fabrics for organophosphate pesticide degradation. The fabrics were first activated with ethylenediamine to introduce free amine groups, and the enzyme was then attached using the bifunctional crosslinker glutaraldehyde. The immobilization only slightly increased the K_m (for methyl parathion, MP), broadened the pH profile such that the enzyme had significant activity at acidic pH, and enhanced the stability of the enzyme. The OpdA-functionalized fabrics could be stored in a phosphate buffer or in the dry state at 4 °C for at least 4 weeks without a large loss of activity. When used in batch mode, the functionalized textiles could degrade 20 μM MP in un-buffered water at liquor to fabric ratios as high as 5000:1 within 2 h, and could be used repeatedly. The fabrics could also be made into columns for continuous pesticide degradation. The columns were able to degrade 50 μM MP at high flow rates, and could be used repeatedly over 2 months. These results demonstrate that OpdA immobilized on nonwoven polyester fabrics is useful in environmental remediation of organophosphate compounds.

1. Introduction

Organophosphates are highly toxic to animals due to their irreversible inhibition of acetylcholinesterase which regulates the neurotransmitter acetylcholine [1]. Organophosphate compounds constitute the bulk of pesticides in agricultural use worldwide and while improving the quality and quantity of produce, their widespread use has led to the contamination of environmental waters, posing a threat to aquatic life and human health. The hazardous nature of organophosphates has necessitated the development of methods for the decontamination of polluted environments. Conventional methods for the disposal of organophosphates, such as incineration, chemical hydrolysis/oxidation, are difficult to be applied to large bodies of contaminated water.

Advances in biotechnology have enabled the development of novel applications for enzymes in many fields. Enzymes have a high degree of specificity and can therefore be exploited to degrade particular groups of toxic chemical into harmless or less toxic products. Of particular interest for detoxification of organophosphates is the enzyme OpdA (Organophosphate degrading enzyme A, also known as phosphotriesterase, organophosphorus hydrolase (OPH), or parathion hydrolase) [2]. This enzyme has a broad substrate specificity, being able to cleave P—O, P—CN and P—F bonds in organophosphate compounds, and a very high catalytic activity (*e.g.* k_{cat} of ~3000 s^{-1}) [3]. Such features make OpdA an ideal tool for bioremediation of organophosphate compounds [4].

The CSIRO in Australia has developed a scalable process to enable manufacture of commercial quantities of OpdA for bioremediation of purposes, under the trade name of Landguard™ (http://www.csiro.au/Outcomes/Environment/Australian-Landscapes/Pesticide-Bioremediation.aspx). The product is a lyophilized crude cell homogenate containing approximately 1% OpdA protein. In a recent study, OpdA has been successfully used to treat agricultural runoff in California [5] and is also being tested in other applications. In these pilot trials, Landguard™ is directly added to the contaminated water. While effective and simple, such practice means that the enzyme is lost after its application.

Enzyme immobilization offers several advantages and has attracted considerable interest for industrial applications [6,7].

* Corresponding author. Tel.: +61 3 9545 2104; fax: +61 3 9545 2363.
E-mail address: yuan.gao@csiro.au (Y. Gao).

0141-0229/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.enzmictec.2013.10.001

When immobilized on solid carriers, enzymes can be conveniently recovered from the process solution enabling repeated use and reduced operating costs. In addition, immobilized enzymes generally exhibit higher thermostability and tolerance to organic solvents [6,7]. OpdA (or OPH) has been immobilized on several types of carriers for organophosphate degradation, including adsorption onto trityl agarose [8] and cellulosic materials [9], entrapment in polyurethane foams [10,11], silica-silicone composites [12], PVA and alginate [13], agrose [14] and hydrogels [15], or covalent attachment to nylon membranes [16] and amyloid fibrils [17]. More recently, a novel approach was reported to immobilize OpdA on the surface of polyester inclusions inside bacterial cells [18]. The work exploits the ability of the polyester synthase (PhaC) to mediate polyester granule formation and covalently attach to their surface inside bacteria. Therefore a fusion protein of OpdA-PhaC could be overexpressed and immobilized on the granules at the same time to facilitate purification [18]. These immobilized enzymes demonstrated catalytic activities and were able to degrade various organophosphate compounds.

We realize that nonwoven polyester textiles are an excellent choice of carrier for enzyme immobilization in bioremediation applications. These textiles are highly porous with high surface areas, which not only facilitate enzyme immobilization but also contribute to good fluid dynamical properties during their use. These textiles are also inexpensive and easy to handle. In this study, the enzyme OpdA was covalently immobilized on nonwoven polyester textiles and used for organophosphate pesticide degradation in un-buffered water in both batch and continuous operating modes.

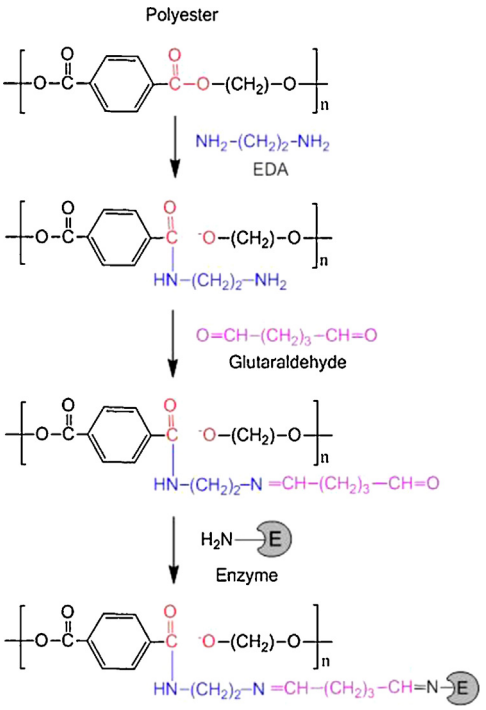

Fig. 1. Overview of the covalent immobilization procedure. Polyester fabrics were activated with EDA to introduce free amine groups and the enzyme was then covalently attached using the crosslinker glutaraldehyde.

2. Materials and methods

2.1. Materials

Ethylenediamine (EDA, 99%), glutaraldehyde (50% solution) and methyl parathion (MP) were purchased from Sigma–Aldrich. Nonwoven polyester (polyethylene terephthalate) fabrics (30 g/m^2) were supplied by the nonwoven textile company Textor Technologies in Melbourne, Australia. Recombinant OpdA was expressed in *E. coli* as previously described [19]. The crude OpdA preparation (Landguard™) was a lyophilized crude cell extract produced at pilot-scale fermentation at CSIRO, Australia. It contained approx 1% (by mass) OpdA protein and had a specificity of 0.017 mmol/min/mg at 20 °C under assay conditions described below. The highly purified enzyme had a specific activity of 2.15 mmol/min/mg at 20 °C, using Landguard™ as the starting material.

2.2. Immobilization of OpdA on polyester

The immobilization procedure comprised three steps (Fig. 1). First, polyester fabrics were treated with EDA (99%) at room temperature at a liquor:fabric ratio of 50:1 for 2 h with agitation, and thoroughly rinsed in water. During this treatment one amine group in these compounds reacted with the polymer in the fibre while other amine group remained free on the fibre surface [20]. Second, the amine-treated textiles were reacted with the bifunctional crosslinker glutaraldehyde (1% in 100 mM phosphate buffer, pH 7.4) for 2 h at room temperature, and thoroughly rinsed again in water. Finally, the activated fabrics were reacted with the highly purified OpdA (10 μg/mL) in phosphate buffer (100 mM phosphate buffer, pH 7.4) overnight at 4 °C (liquor to fabric ratio of 125:1) and washed four times over 2 h in copious amounts of the phosphate buffer containing 0.1% Triton X-100 to remove unbound enzyme. The treated fabrics were air dried at room temperature and stored at 4 °C if necessary.

2.3. OpdA activity assay

Unless specified otherwise, OpdA activity was assayed in 100 mM Tris, pH 8.0, using 0.2 mM MP as the substrate at 20 °C. OpdA activity immobilized on polyester fabrics was assayed using fabric discs of 10 mm in diameter (2.5 mg). The discs were wetted in 0.1% Triton X-100, rinsed with water and placed individually in 6-well plates. 3 mL of the assay buffer was added to each well. After shaking for 3 min at 100 rpm on an orbital shaker, 1 mL of the solution was withdrawn and combined with 0.1 mL of 1 M Na_2CO_3 to raise the pH. The absorbance was read at 405 nm immediately. The absorption coefficient of the MP hydrolysis product, *para*-nitrophenol (*p*NP) (1.74 × 10^4/M/cm), was used to calculate enzyme activity [21]. Activity assays on fabrics had four replicates and results were presented as average ± standard error.

To study the effect of pH on enzyme activity, citric acid/Na_2HPO_4 buffers were used for pH 4.0–6.0, sodium phosphate buffer (100 mM, Na_2HPO_4/NaH_2PO_4) for pH 6.5–8.0, and sodium carbonate/bicarbonate buffer (100 mM) for pH 8.5–10.0. All solutions were made according to standard recipes and the final pH was adjusted as required.

For storage stability, OpdA treated fabrics were either stored in the dry state at 20 °C or at 4 °C, or stored in phosphate buffer containing 0.02% NaN_3 at 4 °C before activity assays. For thermostability studies, soluble and immobilized OpdA were incubated in the phosphate buffer containing 0.1 mg/mL BSA at 55 °C in a water bath for the designated time.

2.4. Batch-wise degradation of methyl parathion

OpdA-functionalized polyester fabrics (specific activity ~0.1 μmol/min/g) were cut into squares of 10 mm and immersed in MP solutions (20 μM in un-buffered water) at liquor to fabric ratios of 500:1, 1000:1, 2000:1 or 5000:1. The mixture was shaken continuously at room temperature. At designated times, 1.0 mL of the solution was withdrawn and combined with 0.1 mL of 1 M Na_2CO_3 solution and the absorbance read at 405 nm. Degradation was calculated from the accumulation of *p*NP in the solutions. To demonstrate reusability of the fabrics, the same fabrics were used for MP (20 μM in un-buffered water) degradation at a liquor to fabric ratio of 1000:1 on five consecutive days. The fabrics were rinsed in water and stored in the phosphate buffer containing 0.02% NaN_3 at 4 °C between uses.

2.5. Continuous degradation of methyl parathion

The crude OpdA preparation (Landguard™) was used for textile functionalization. Ten grams of Landguard™ was resuspended in 400 mL of the phosphate buffer pre-warmed at 50 °C. The solution was incubated at 50 °C for 5 min in a water bath to precipitate contaminating proteins and then clarified by centrifugation for 10 min at 15,000 × *g*. This treatment enriched the specific activity of the enzyme by three fold.

Nonwoven polyester fabrics (~2 mm thickness) were punched into discs of 30 mm in diameter and 25 g was tightly packed into a glass column of 240 mm in length. To activate the fabrics *in situ*, the column was connected to a peristaltic pump (set at 10 mL/min) and circulated with EDA (99%, 300 mL) for 2 h at room temperature, washed with 2 L of water, then circulated with a glutaradehyde solution (400 mL of 1% in phosphate buffer) for 2 h at room temperature and again flushed with 2 L of water. The partially purified OpdA solution from above was then circulated through the activated column overnight at 4 °C. The column was washed with 2 L of the phosphate buffer containing 0.1% Triton X-100 to remove any unbound enzyme. The column was stored in the phosphate buffer containing 0.02% NaN_3 at 4 °C when not in use. The void volume of the column was 170 mL.