Reactive & Functional Polymers 71 (2011) 870–880

Contents lists available at ScienceDirect

Reactive & Functional Polymers

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

A potential enzyme-encapsulating, ultrafine fiber for phenol detection

Dennis A. Oriero [a], Jamie M.F. Jabal [a], Lee Deobald [b], Andrew T. Weakley [a], D. Eric Aston [a,*]

[a] Department of Chemical & Materials Engineering, University of Idaho, Moscow, ID 83844, United States
[b] Department of Microbiology, Molecular Biology & Biochemistry, University of Idaho, Moscow, ID 83844, United States

ARTICLE INFO

Article history:
Received 3 February 2011
Received in revised form 14 April 2011
Accepted 12 May 2011
Available online 18 May 2011

Keywords:
Electrospinning
Sol–gel chemistry
Tyrosinase
Phenol
Ultrafine fibers

ABSTRACT

Ultrafine composite fibers of silicate–polymer matrix structure were synthesized by the combination of sol–gel chemistry and electrospinning to immobilize a tyrosinase enzyme for the purpose of continuous and/or reusable operations. These ultrafine enzyme-carrying fibers with average diameters of less than one micron were studied by their incorporation into a small-scale, flow-through micro-reactor to demonstrate the feasibility of phenol detection with simultaneous remediation through biocatalysis. Biocatalytic activity of the electrospun enzyme (tyrosinase) was confirmed and evaluated using the integrated form of the Michaelis–Menten equation, having the advantage of requiring fewer experimental runs than other approaches. Micro-Raman spectral analysis was also used to demonstrate biocatalysis and the possible biocomposite nature of the enzyme-carrying fibers. Biocatalytic activity of the electrospun enzyme was retained at phenol concentrations of 0.12–5 mM, typical of contaminated waters. This research demonstrates the potential of this cost-effective fibrous material for biocatalysis and detection applicable in environmental and industrial analysis and remediation.

1. Introduction

Phenol ranks 23rd among the top 50 chemicals produced in the world [1,2]. It is an important starting material used in a broad range of chemical manufacturing processes such as for resins, polymers, pharmaceuticals, dyes, pulp and paper, textiles, metal coatings, coal conversion, and petroleum refining [3–5]. Despite its commercial importance, phenol and many of its derivatives are considered to be, not only toxic chemicals, but also highly hazardous materials. This is due to the fact that it can be easily absorbed by animals and humans, through the skin and mucous membrane [6]. In addition, phenol is also known for its persistency in the environment and propensity for bio-concentration and bio-magnification [6]. Phenol concentrations greater than 50 ppb are harmful to some aquatic life, while ingestion of 1 g can be fatal in humans [7,8]. Therefore, the selective and sensitive detection of phenol from industrial aqueous effluents or water contaminated with phenol, followed by remediation, is of practical importance.

In the past three decades, several techniques have been developed for the detection and monitoring of phenol in aqueous environment. These include both non-enzymatic and enzymatic methods. The non-enzymatic methods are mostly analytical techniques such as chromatography, spectrophotometry, flow injection analysis and mass spectrometry [9]. Most of these analytical techniques are expensive, time-consuming, may generate other hazardous by-products, require skilled operators, and sometimes require pre-concentration and extraction steps that increase the risk of sample loss [6,8,10]. Recent advances in biotechnology have promoted the use of enzymes for the detection and monitoring of phenols. Enzymatic methods possess promising potentials in comparison to non-enzymatic techniques due to their specificity, selectivity, speed, simplicity, reliability, predictability and low cost [11,4], in addition to the benefit of eliminating the target species in the process—remediation by default.

Horseradish peroxidase (HRP, EC 1.11.1.7) and tyrosinase (polyphenol oxidase, EC 1.14.18.1) are undoubtedly the most studied enzymes that have been used for phenol detection [4,12]. HRP catalyzes the oxidation of a wide variety of toxic aromatic compounds including phenols, bisphenols, anilines, benzidines, and related hetero-aromatic compounds over broad pH and temperature ranges [4]. The reaction products undergo polymerization via non-enzymatic processes, resulting in the formation of water insoluble precipitates that can be easily removed [4]. HRP requires the presence of hydrogen peroxide that not only acts as a co-reactant but also activates the enzyme, which in turn oxidizes the substrate; however, the drawbacks associated with the use of HRP include the high cost of both the enzyme and hydrogen peroxide in addition to the toxicity of the reaction products [10,12,13].

By comparison, tyrosinase catalyzes the ortho-hydroxylation of phenol in the presence of molecular oxygen, to catechols (diphenols) and their subsequent oxidation to o-quinones [14,15],

* Corresponding author. Address: Department of Chemical & Materials Engineering, University of Idaho, PO Box 441021, Moscow, ID 83844-1021, United States. Tel.: +1 208 885 6953.
E-mail address: aston@uidaho.edu (D.E. Aston).

1381-5148/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.reactfunctpolym.2011.05.004

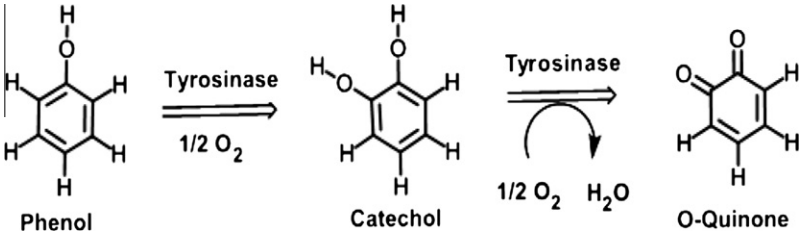

Fig. 1. Enzymatic reaction for phenol detection catalyzed by tyrosinase.

(Fig. 1). The fact that tyrosinase is ubiquitous in nature, abundant in a wide variety of sources such as vegetables, fruits, seafood, etc., and also requires oxygen as a co-reactant presents its use as a less expensive alternative with respect to HRP and other non-enzymatic methods for phenol detection [12] and potential remediation.

Despite the fact that enzymatic methods, in contrast non-enzymatic methods, possess immense potential for hazardous pollutant detection, challenges remain due to their complexity and practical difficulty in implementation, such as successful enzyme immobilization and the related problems of poor stability in terms of suitable reaction conditions, non-reusability, surface area restrictions, enzyme leakage (loss) and complex functionalization chemistries [16]. However, advancements in nanomaterials have facilitated some promising strategies that could overcome some or perhaps all of these challenges.

This research presents the potential of one aspect of the multidisciplinary field of nanotechnology by demonstrating the synthesis of ultrafine composite fibers with a silicate–polymer matrix structure that immobilize enzymes to their relatively large and accessible surface area. The ultrafine fibers, having average diameters of less than one micron, are produced by the combination of sol–gel chemistry and electrospinning [35–41]. The sol–gel process has been demonstrated to be highly successful in the hosting or immobilization of various biomolecules such as active enzymes, cells and proteins [16–18]. In addition to its biocompatibility/biofriendly properties, it is also used for the formation of porous materials. Thus, the combination of both techniques results in a simple, highly versatile and relatively inexpensive method allowing for simultaneous pore formation (for increased surface area and loading) and enzyme encapsulation to reduce leakage during use. To date, only two enzymes—HRP and now tyrosinase—have been successfully immobilized with electrospun fibers in a sol–gel process; the HRP-fibers were used for glucose detection and sensing [16]. The resulting biocomposite fiber mats supporting tyrosinase are further studied by incorporating them into a small-scale, flow-through device to demonstrate phenol detection and biocatalysis feasibility. Biocatalytic activity of the electrospun enzyme (tyrosinase) has been evaluated using the integrated form of the Michaelis–Menten equation, $\left(r_{max} + \frac{km \ln\left[1-\frac{Cp}{Cs0}\right]}{\tau} = \frac{Cp}{\tau}\right)$ having the advantage of requiring fewer experimental runs than by other approaches [19].

2. Materials and methods

2.1. Reagents

Mushroom tyrosinase (polyphenol oxidase C. 1.14. 18.1) with a specific activity of 1460 units/mg was obtained from Worthington Biochemical Corporation (Lakewood, NJ). L-tyrosine, phenol, 4-aminoantipyrine (4-AAP, 98% pure), and poly(vinyl alcohol) (130,000 mol. wt.) were purchased from Sigma Aldrich Chemical Company, Inc. Tetramethyl orthosilicate (TMOS, 97%) was supplied by Spectrum Chemical Manufacturing Corporation (New Brunswick, NJ). D-fructose was obtained from Fisher Scientific (Fairlawn, NJ). Sodium phosphate monobasic monohydrate crystals and sodium phosphate dibasic anhydrous (for preparation of sodium phosphate buffer, pH 6.8) and acetic acid were purchased from Mallinckrodt (Baker, NJ). The buffer solution pH was determined with a Dwyer PH0-1 pH meter (Dwyer Instruments, Inc., Michigan City, IN).

Indium–tin oxide (ITO) glass plates (coated on one surface) were obtained from Delta Technologies (Stillwater, MN). Advance Infusion System series 1200 syringe pump was obtained from Cellpoint Scientific, Inc., Gaithersburg. Syringes and needles (1 mL and 3 mL with 27G 1¼-in. metallic needle) were obtained from Becton, Dickinson (Franklin Lakes, NJ). Aluminum, neoprene rubber and acrylic glass sheets were obtained from MacMaster-Carr (NJ) and cut to design specifications for the small-scale flow-through reactor.

2.2. Preparation of solution for sol–gel electrospinning

The preparation of a silicate–fructose–PVA–tyrosinase sol–gel precursor solution for electrospinning was accomplished by an approach similar to that of Patel et al. [16] with some modifications to make electrospinning of a sol–gel simpler. A mixture of TMOS (0.76 g silica precursor), water (0.18 g for a H_2O:TMOS mole ratio of 2) and HCl (30 μL of 40 mM stock as catalyst to speed up the hydrolysis reaction) were added with continuous stirring to form the hydrolyzed silica sol. Thereafter, the reaction mixture was heated to a temperature of 60 °C for 30 min. The resulting sol was allowed to cool and the pore-forming material (500 μL of 50% w/v aqueous D-fructose) was added with continuous stirring. PVA (700 μL of 15% w/v) was added next to the mixture with continuous stirring. The addition of both PVA and D-fructose made the sol highly viscous, thereby facilitating electrospinning into fibers rather than electrospraying into droplets.

At this stage, the sol had a strong tendency to gel due to the cross-linking of the soluble intermediates formed from the condensation reaction. This was exacerbated by the addition of enzyme solution in sodium phosphate buffer, resulting in rapid gelation inside the syringe, hence electrospinning became impossible. Patel et al. [16] also observed this behavior while preparing their horseradish peroxidase sol–gel electrospinning mixture and their approach towards solving the problem involved cooling the sol to low temperatures (e.g., 0 °C) before electrospinning. This was impractical with the electrospinning system in the present study, hence the introduction of acetic acid (15 μL of 100 mM) was used as the alternative to counteract early gelation and provide a short but feasible window for electrospinning. The actual mechanism for this delayed gel formation effect by acetic acid is unclear however, there is the possibility of inhibitory action by the acid on the cross-linking of the intermediates (the step responsible for formation of the three-dimensional network that builds into the gel) formed from the condensation reaction.

After the addition of acetic acid, 300 μL of the tyrosinase enzyme in buffer solution (6 mg/mL) were introduced into the sol with continuous stirring for about 4–6 min before the mixture