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Enzymatic CO_2 reduction to formate by formate dehydrogenase from *Candida boidinii* coupling with direct electrochemical regeneration of NADH

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ABSTRACT

Enzymatic conversion of CO_2 to formate was carried out in the cathodic cell of a two-chamber electrochemical apparatus where NAD^+ was reduced on the surface of a Copper foam electrode. Formate dehydrogenase (FDH) was used as the biocatalyst in both free form and immobilized on the modified electrospun polystyrene nanofibers (EPSNF). The fabricated EPSNF were modified by a multistage procedure including acid treatment, silanization followed by activation with glutaraldehyde. The effects of regenerated NADH concentration and time of enzymatic reaction on the formate production in the both systems were studied. The results indicated that the EPSNF immobilized FDH had a desirable activity, long-term storage stability (41% after 20 days) and reusability after eight cycles of successive reactions (53% of the initial activity). Moreover, it was revealed that the increase of cofactor concentration at the early times of reaction was favorable to the formate production. However, an inhibitory effect was observed at higher concentrations of NADH, and the optimum values of 0.45 mM and 0.51 mM were obtained for the maximum enzyme activity by the free and immobilized enzymes respectively. The produced formate at the optimum cofactor concentration after 300 min was 0.61 mM and 0.31 mM for the free and immobilized enzyme systems. Finally, it can be concluded that the presented process is a promising approach to the enzymatic conversion of CO_2.

1. Introduction

Global warming is one of the major environmental issues worldwide which is strongly correlated with the production and accumulation of greenhouse gases especially CO_2 in the atmosphere [1,2]. There are several approaches to mitigate CO_2 emission such as promoting the energy efficiency, CO_2 capturing and storage [3–6]. However, to achieve sustainable development, it would be necessary to convert the captured CO_2 to useful products through green processes. Several CO_2 recycling methods have been suggested based on various chemical, electrochemical, photochemical and biological principles [7–9]. Despite the diversity in mechanisms and details, these processes have a catalyst based conversion reaction in common. Advances in developing and improvement of CO_2 reducing chemical catalysts have frequently been reviewed [9,10]. However, chemical catalysts such as ruthenium, rhodium, and iridium are precious metals that catalyze the CO_2 conversion reaction at high temperatures and pressures [10–12]. In contrast, enzymes exhibit the ability of catalysis for CO_2 reduction at mild conditions with high specificity [2,3,10]. For example, carbonic anhydrase, pyruvate decarboxylase and formate dehydrogenase (FDH) have been used for biological CO_2 conversion [10,13]. Recently, FDH has received much attention due to its ability to reduce CO_2 directly to formate without any other co-products. There are two types of FDH based on their cofactor requirements namely, the NADH dependent and metal dependent FDHs. The metal-dependent group includes molybdenum (Mo)-based or tungsten (W)-based FDHs. Although metal containing FDHs have a higher catalytic activity for CO_2 reduction reaction, these NADH-independent FDHs contain extremely oxygen-labile components such as metal ions (tungsten or molybdenum), iron-sulfur clusters, and selenocysteine which limit their industrial applications. However, the more stable type of FDH activity depends on the presence of NADH which is an expensive cofactor. Moreover, the aqueous enzyme solution has low reusability [10,14,15]. The cofactor problem has been addressed either by feeding the reaction with excess amounts of NADH or by introducing a cofactor regeneration system. Various regeneration systems and their influence on the enzymatic conversion of CO_2 have been reported [5,14,16–19]. Among them, the electrochemical and coupled redox enzyme systems are more popular. In fact,

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electrochemical based methods are especially interesting because of the convenient monitoring of the reaction progress, lower process costs and elimination of reducing agents which leads to a simple downstream product isolation [20–24]. For example, Kim et al. reported the electroenzymatic conversion of CO_2 to formate by FDH from *Candida boidinii*. They regenerated NADH electrochemically at the Cu electrode using an Rh complex as the mediator [14]. Jung et al. presented electroenzymatic reduction of CO_2 to formate by FDH with electrochemical regeneration of NADH. They used Cu nanorods electrodeposited on glassy carbon electrode as the electron mediator for the electrochemical NADH regeneration [5]. Aresta et al. represented a hybrid enzymatic/photocatalytic system for the reduction of CO_2 to methanol. They used TiO_2-based photocatalysts and Rh complex for regeneration of NADH [16]. Chung et al. reported the electrochemical reduction of CO_2 to formate at an integrated bioelectrode. They immobilized both the FDH and cofactor (NADH) on polydopamine thin films as cathode and cobalt phosphate/bismuth vanadate ($CoPi/BiVO_4$) as photoanode [18]. In the majority of the reported results, the electrochemical regeneration has been carried out indirectly on a mediator coated electrode which increases the complexity and cost of operation. Obviously, a direct system can be more advantageous if it could produce high regeneration efficiency.

The low reusability problem of the enzyme can be addressed by employing a proper immobilization method [25,26]. Accordingly, the successful immobilization of FDH on a variety of supports and carriers (e.g., polyacrylic matrix, coated iron oxide nanoparticles, magnetic nanoparticles, alginate–silica hybrid gel) has been reported [27–30]. The merits and disadvantages of the enzyme immobilization on various nano-structured supports have been reviewed as well [31,32]. Aside from the important parameters such as the high enzyme activity, stability, and loading capacity, the immobilized enzyme should be separated from the aqueous medium conveniently. As a result, magnetic nanoparticles have been vastly investigated as the proper immobilization supports. However, when the electrochemical cofactor regeneration and the enzymatic reaction have been integrated into one chamber, the magnetic nanoparticles would be useless. Alternatively, polymeric nanofibers seems to be promising supports and offer several advantages such as high enzyme loading capability, high porosity, highly homogenous dispersion in liquid phase, low hindrance for mass transfer, low cost, simple separation and easy manipulation of surface modification [33–35]. Several studies on using electrospun nanofibers for enzyme immobilization have been published. For example, Herricks et al. used polystyrene and polystyrene-co-maleic anhydride electrospun nanofibers for α-chymotrypsin immobilization. They showed that modification of nanofibers with glutaraldehyde stabilized the enzyme activity [36]. Wu et al. studied immobilization of cellulose in electrospun polyvinyl alcohol nanofibers [37]. Misson et al. immobilized β-galactosidase onto polystyrene nanofibers to increase enzyme stability and its practical usability [38].

In this work, the CO_2 conversion to formate reaction catalyzed by the NADH-dependent FDH combined with an electrochemical regenerating system has been investigated. Firstly, the application of an electrospun polystyrene nanofibrous membrane as a support for the FDH immobilization has been studied. Several immobilization parameters such as immobilization time, storage stability and reusability have been investigated. Then, the functionality of the immobilized enzyme along with the NADH direct electrochemical regeneration system has been investigated.

2. Materials and methods

2.1. Chemicals and reagents

In this work, the commercially available materials were used without further purification. Polystyrene 1540 was purchased from Tabriz Petrochemical Company (Iran). High-purity copper sheet (thickness 1 mm), glutaraldehyde and absolute ethanol were obtained from Merck. β-NAD^+ (sodium salt), formate dehydrogenase (F8649 from *Candida boidinii*), bovine serum albumin (BSA), Coomassie Brilliant Blue (G-250), 3-aminopropyltriethoxysilane (APTES), *N,N*-dimethylformamide, Nafion 117 and sodium formate were purchased from Sigma Chemicals Co. $CuSO_4$, H_2SO_4, NaH_2PO_4, Na_2HPO_4, HNO_3 and H_3PO_4 were purchased from Daejung chemicals (S. Korea). Carbon felt (3.18 mm thick, 99.0%) was from Alfa Aesar (US).

2.2. Preparation, modification, and characterization of polystyrene nanofibers

There are several parameters which affect the morphology and size of the electrospun nanofibers (i.e., polymer average molecular weight and its concentration, viscosity, conductivity and surface tension of the solution, the applied voltage and flow rate, the distance between the needle tip and collector, temperature and humidity). Polystyrene (PS) has several appropriate characteristics such as the non-toxicity, good mechanical properties, chemical stability, and low cost which make it a suitable candidate for electrospinning. In addition, it has no interactions with the electrical current in the cofactor regeneration system, its nano-fibers can be easily separated from the solution and their hydrophobic nature can increase the adsorption of CO_2 gaseous bubbles in the system. Moreover, PS can be chemically modified to introduce functional groups for covalent enzyme immobilization. It has been reported that fine fibers can be obtained using PS with large MW and low concentration. The critical concentration at which bead free PS fibers were produced was decreased with increasing MW. Also, bead formation and fiber diameter were increased with increasing polymer concentration [33,39,40]. *N,N*-dimethylformamide (DMF) was used as the solvent for electrospinning of the PS fibers because of its low viscosity and high conductivity [41,42]. The optimum conditions for electrospinning PS nanofibers were PS concentration of 10% in DMF and the operating voltage of 20 kV (data not shown). The electrospinning of PS to nanofibers was performed through the described procedure by Jin et al. [33] with minor modifications. PS was dissolved in DMF at 25 °C and 60 rpm overnight to prepare PS stock solution. 0.5 mL of PS stock solution was placed into a plastic syringe which was connected to a high voltage power supply. The syringe was horizontally fixed on a syringe pump (model SP-500). Electrospinning process was conducted with a flow rate of 1 mL/h at operating voltages of 20 kV at room temperature. A grounded aluminum foil collector was located 12.5 cm away from the needle tip. After electrospinning, the fibers were dried overnight in a vacuum oven at 60 °C.

The fibers were modified as described by Raman Suri et al. [43]. Briefly, EPSNFs were treated with a mixture of nitric acid (63%) and sulfuric acid (98%) with a ratio of 10:1 (v/v) for 30 min at 25 °C and 50 rpm to add nitro functional groups to the benzene ring of styrene moieties. The modified fibers were washed several times with deionized water to remove all the remaining acids and then treated with 5% (v/v) APTES aqueous solution at 50 °C and 50 rpm for 2 h. The amine-functionalized EPSNFs were submerged in phosphate buffer saline (PBS, 200 mM, pH 7) containing glutaraldehyde (2% v/v) for 2 h at 25 °C and 50 rpm. Finally, the EPSNFs were washed with PBS and dried at 60 °C in a vacuum oven.

The presence of amino groups on the surface of the modified EPSNFs was investigated qualitatively by the ninhydrin assay [44–46]. A small piece of fibrous membrane was immersed in a 1 M ninhydrin/ethanol solution and heated in boiling water for 15 min. The NH_2 groups on the surface of EPSNF reacted with ninhydrin and sufficient amounts of amino groups on the surface would change the color of the EPSNF to purple.

The morphology of the obtained electrospun nanofibrous membrane and their elemental composition were studied using Mira3-XM (TESCAN) scanning electron microscopy (SEM) coupled with an energy dispersive X-ray spectroscopy (EDX). The presence of surface functional