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Enzymatic bioreactor for simultaneous electrosynthesis and energy production

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ABSTRACT

The proof-of-concept for enzymatic electrosynthesis with simultaneous energy production has been demonstrated by using the regioselective conversion of D-sorbitol to D-fructose by D-sorbitol dehydrogenase as a model reaction. The bioreactor was composed of two electrodes, a carbon felt bioanode modified by multi-walled carbon nanotubes (MWCNT) containing D-sorbitol dehydrogenase immobilized in a silica matrix and an oxygen gas-flow cathode. The electrochemical regeneration of the NAD^+ cofactor at the bioanode was achieved by using a poly(methylene green) mediator electrodeposited on the carbon felt/MWCNT electrode. The conversion rate was 1.18 mg day^{-1} cm^{-3} and an energy power output up to 14.6 μW cm^{-3} at 0.1 V was reached.

1. Introduction

Enzyme biocatalysis has become well-recognized in organic synthesis and biotechnology because enzymes are proficient biocatalysts likely to offer much more competitive processes compared to chemical catalysts [1,2]. In view to replacing chemical processes with cleaner, safer, and more eco-friendly biocatalytic processes [3], enzyme biocatalysis has emerged in substituting traditional chemical processes in some areas, owing especially to new technologies in enzyme engineering [4]. For large-scale applications, however, enzymes should be immobilized to ensure long-term operational stability and easy recovery and re-use [3]. A generic method to prepare efficient biocatalysts which are easy to recycle is their encapsulation inside inorganic sol-gel matrices [5], thanks to the soft synthesis conditions and low temperature processing associated to sol-gel chemistry (i.e., maintaining the biological activity of enzymes immobilized in a biocompatible surrounding) [6,7].

Actually, most developments in enzyme electrochemistry are based on oxido-reductases [8] and targeted to enzymatic biosensors and biofuel cells [9–12]. Thereby, most bioelectrocatalytic devices are either systems that need electrical power input from the outside (i.e., electrochemical biosensors, except some cases of self-powered biosensors including a biofuel cell component [13,14]) or systems that are able to deliver electric energy but produce molecules that are not useful (e.g. glucose oxidase-based biofuel cells in which the glucose fuel is transformed into D-gluconolactone that can be considered as waste) [12,15].

Here, we plan to combine these two approaches in a single one in order to design a bioelectrocatalytic reactor that produces valuable molecules and, at the same time, delivers electrical energy. It should be mentioned that the simultaneous production of energy and chemicals has been already envisaged for microbial bioelectrochemical systems [16], but the energy generation was mostly accompanied by wastewater treatment [17,18] and dye bleaching [19,20], i.e., substance decomposition, and not generation of selected compounds, and these systems suffered from poor yields and no chemo-selectivity [21].

A bioelectrocatalytic reactor for simultaneous electrosynthesis of a fine chemical and electric energy production has to fulfill some prerequisites. It first implies the use of oxido-reductases gathering in a single biomolecule the requirements of bioenergy generation and effective bioconversion of substrates into added-value products (thanks to remarkable stereo-, enantio-, and regio-selectivity [22–24]). Second, to have high voltage output, the electro-enzymatic oxidation should operate at low overpotentials, which is usually achieved by an appropriate choice of mediator

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and/or electrode material [25]. Third, most functional components have to be immobilized at the electrode surface to help at reducing waste production to a minimum and therefore decreasing purification and separation steps of the added value products. The immobilization method should provide stable biocompatible media for the preservation of enzymatic activity for a reasonable period of time, in a way enabling long-term operational stability and effective enzyme recycling [26,27]. Finally, large amounts of enzyme must be immobilized in the bioreactor for the sake of high bioconversion yields and large output power density, which could be achieved by using porous electrodes [28], for instance. Association of an enzymatic bioelectrode with a gas diffusion electrode (GDE) [29–31] appears to be an attractive strategy to design bioreactors, in the frame of the recent review on combination of bioelectrochemical syntheses and energy conversion [32].

Here, we report an enzymatic reactor combined with GDE used for simultaneous bioconversion and electricity production. A model reaction was chosen for this proof-of-concept, i.e., the electroenzymatic oxidation of D-sorbitol to D-fructose by NAD-dependent D-sorbitol dehydrogenase (DSDH) immobilized in a sol-gel matrix. The electrochemical regeneration of NAD^+ was achieved by using poly(methylene green) electrodeposited on carbon felt modified by multi-walled carbon nanotubes (MWCNT). Effective bioconversion has been characterized by chromatographic analysis of both substrate and products of the electroenzymatic reaction. Bioconversion experiments have been first performed with applying a constant potential to the biocathode (energy consumption), before evaluating the bioconversion reaction together with the energy production. Continuous production of D-fructose by continuous feeding of the bioreactor with D-sorbitol has been finally tested.

2. Material and methods

2.1. Chemicals and Reagents

D-sorbitol dehydrogenase solution (DSDH, 1000 units mL^{-1}) was prepared by overproduction of the His(6) tagged protein in Escherichia coli BL21GOLD (DE3) and purification of the enzyme was achieved with Histrap columns (GE Healthcare). Tetraethoxysilane (TEOS, 98%) was from Alfa Aesar. Poly(ethylene imine) (PEI, 50% w/v in water, $M_n = 60000$) was supplied by Fluka. Methylene green (MG), 3-glycidoxypropyltrimethoxysilane (GPS, 98%), tris (hydroxymethyl) amino-methane (Tris, 99%) and β-nicotinamide adenine dinucleotide (NAD^+) were from Sigma–Aldrich. All other reagents were of analytical grade. All solutions were prepared with high purity water (18 MΩ cm) from a Purelab Option water purification system (Elga). Multi-walled carbon nanotubes functionalized by carboxylic groups (MWCNT, 95%, ∅ 15 ± 5 nm, L 1–5 μm) were provided by Nanolab and dispersed in water by sonication for 12 hours. Carbon felt (CF) was from Sigratherm® (GFD 4.6 EA, density 0.09 g cm^{-3}, electrical resistivity 3–6 Ω mm).

2.2. Preparation of the bioelectrode

A piece of CF (4 × 4 × 0.46 cm; 16 cm^2 geometrical area) was treated by electrochemical cycling (10 cycles) between −0.7 and 1.9 V in 0.1 M H_2SO_4 in order to activate and to increase the hydrophilicity of the surface. After drying at 200 °C, CF was dipped into suspension of MWCNT (1 mg mL^{-1}) and dried at 130 °C for 30 min. This process was repeated several times to get the desired number of MWCNT-layers (usually 10). Finally, methylene green (MG) was electropolymerized on the surface of CF-MWCNT by cycling it in a 0.5 mM MG solution between −0.5 and 1.2 V. A sol was prepared according to a protocol that was previously optimized [33]. Briefly, 0.18 g TEOS was pre-hydrolyzed by mixing with 0.13 g GPS, 0.5 mL water and 0.625 mL 0.01 M HCl for 8 h. Then, this sol was diluted 3 times and a 400 μL aliquot was mixed with 200 μL PEI (20%), 200 μL of water and 300 μL of DSDH. This mixture was spread over the whole surface of CF-CNT-MG. The modified bioelectrode was left until complete drying at 4 °C before use. The concentration of the enzyme in the starting sol was 2.73 mg cm^{-3}, after immobilization on the bioelectrode this corresponds to 0.375 mg cm^{-3} in the electrode bulk. This protocol has been flat electrodes [33], however no optimization has been done for carbon felt.

2.3. Procedures

In the bioreactor, the bioelectrode was used as anode while a gas diffusion electrode (GDE, purchased from Paxitech, France, and installed over a graphite bipolar plate, with flow pattern derived from fuel cell technology) was used as cathode (Fig. 1). 100 mL of reaction mixture containing 0.1 M Tris-HCl pH 9 buffer, 0.1 mM NAD^+ and 1 mM D-sorbitol was flowed continuously through the bioelectrode at a rate of 6 mL min^{-1}. At the same time, compressed air was introduced through the GDE at a rate of 10 mL min^{-1}. In the energy-consuming mode, the potential of the bioelectrode was fixed to +0.1 V *versus* a silver wire covered by AgCl (Ag/AgCl pseudo-reference electrode) placed into the bioreactor. The

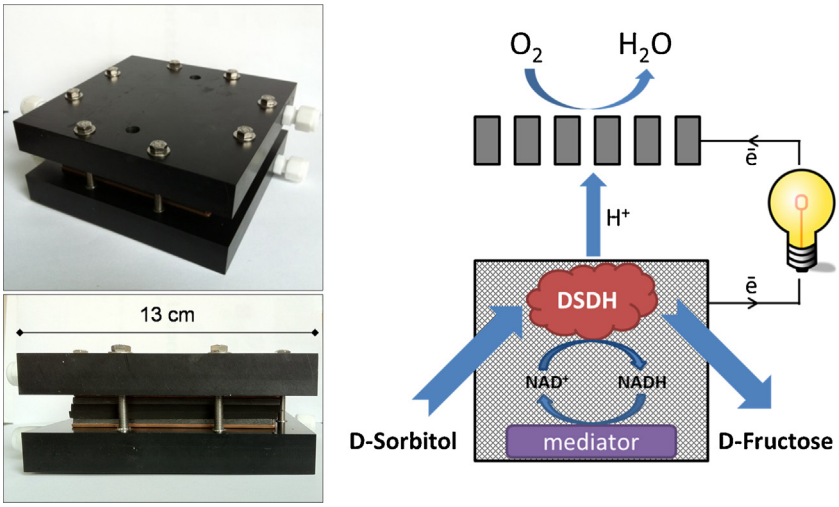

Fig. 1. Photographs and functional scheme of the flow bioreactor.