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Designing membrane electrochemical reactors for oxidoreductase-catalysed synthesis

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Abstract

The purpose of this work was to design an electrochemical reactor to enhance the high selectivity of enzyme-catalysed processes. In order to develop economically efficient syntheses, the enzymes must be confined in the strict vicinity of the electrode surface. Here the confinement was achieved with a dialysis membrane in a so-called Dialysis-Membrane Electrochemical Reactor (D-MER). Oxidation of glucose into gluconic acid catalysed by glucose oxidase was a first example. The ADH-catalysed reduction of cyclohexanone into cyclohexanol was also tested in a new type of MER. NADH was electrochemically regenerated thanks to mediator (methyl viologen or rhodium complex). The key point in developing electro-enzymatic process is to ensure perfect suiting of the reactor design to the reactions that are to be processed.

Keywords: Reactor; Electrochemical; Enzyme; Synthesis; Membrane

1. Introduction

The association of oxidoreductase-catalysed reactions with the electrochemical regeneration of some intermediate species should be a powerful basis for new highly selective synthesis [1–4]. In order to develop economically efficient synthesis, the enzymes must be confined in the strict vicinity of the electrode surface. Here we chose to retain the enzyme(s) near the electrode with a semipermeable membrane. To our knowledge only one example of compact reactor has been proposed until now [5]. This reactor was only a first attempt, the working electrode is simply inserted in a dialysis tubing. This process should surely take great benefit of a better design.

2. Experimental

The MER shown in Fig. 1 was a filter-press reactor which contained a platinum or carbon felt working electrode, a platinum auxiliary electrode and a saturated calomel electrode. The working electrode was covered by a dialysis membrane which confines the enzyme for the GOD-catalysed synthesis. The inner design of the reactor was modified to improve its performance. This new MER (N-MER) was used for cyclohexanol production. The solution circulated tangentially with respect to the dialysis membrane. When necessary, Nafion® membrane was inserted to separate the working and auxiliary compartments. When anaerobic conditions were required, a flux of inert gas such as helium or nitrogen was set through the storage tank.

The chemicals were purchased from Sigma, Boehringer-Mannheim and Fluka. The rhodium complex, pentamethylcyclopentadienyl-2,2′ -bipyridinechloro-rhodium(III), was synthesised by the group of Pr. Steckhan (University of Bonn, Germany). The regenerated cellulose dialysis membranes were supplied by Bioblock. Electrolysis at constant potential was performed with a 1286 Solartron Schlumberger interface. The transformation of glucose into gluconic acid and of cyclohexanone into cyclohexanol was followed by enzymatic test and gas chromatography, respectively.

3. Results and discussion

The transformation of glucose into gluconic acid catalysed by a glucose oxidase (GOD) also produces hydrogen

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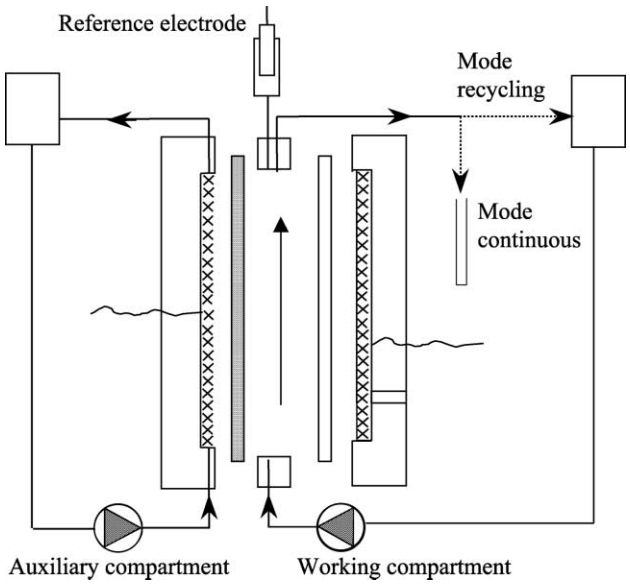

Fig. 1. Schematic representation of the Dialysis-Membrane Electrochemical Reactor (D-MER).

peroxide, which strongly inhibits glucose oxidase. An activity loss of 80% was observed in the presence of 50 mM hydrogen peroxide [6]. Experiments were first carried out in a beaker containing 250 mM glucose and 100 U GOD to quantify the transformation ratio obtained without elimination of the hydrogen peroxide. For all the tests carried out in these conditions, the transformation rate stayed below 4% as reported in Fig. 2. The addition of 4900 U catalase, which catalyses the dismutation of hydrogen peroxide into water and oxygen, increased slightly the transformation ratios, which remained lower than 10% (Fig. 2). The same reaction was finally conducted in the D-MER where hydrogen peroxide is oxidized into oxygen, 30% transformation was obtained in 3 h. The electrochemical step revealed to be very efficient. However, the transformation of high concentration of glucose is limited by the

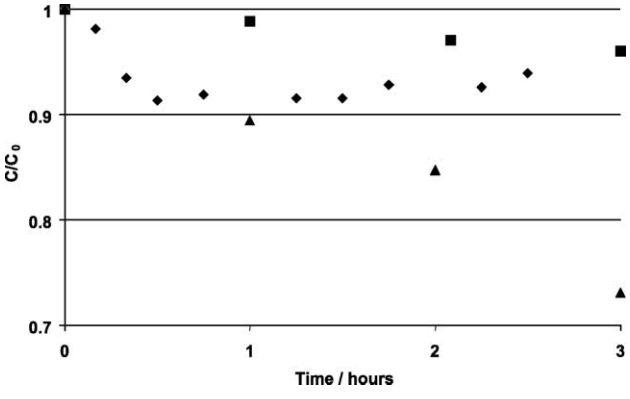

Fig. 2. Dimensionless evolution of glucose concentration as a function of time in a beaker without ■ and with ♦ catalase and in the MER ▲ for a membrane of 12–14 kDa cut-off. Phosphate buffer 0.1 M pH 7.0, 250 mM glucose, 100 U GOD, volume without catalase 43 ml, with 4900 U catalase 10 ml, in the D-MER 44 ml.

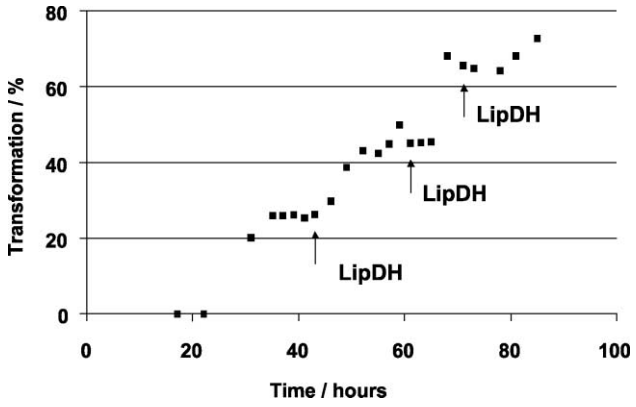

Fig. 3. Influence of LipDH on the transformation of cyclohexanone into cyclohexanol in the N-MER in continuous mode. Regeneration of NADH with methyl viologen as mediator. Phosphate buffer 0.1 M pH 8.0, Cyclohexanone 100 mM, MV^{2+} 5 mM, NAD^+ 1 mM, HLADH 60 U, LipDH 500 U, Flow 0.13 μl/s.

autoinactivation of enzyme which was proved to be clearly distinct from inhibition by hydrogen peroxide [7].

The synthesis of cyclohexanol from cyclohexanone catalysed by an alcohol dehydrogenase requires the cofactor NADH. Methyl viologen and a rhodium complex were successively used to electrochemically regenerate NADH. In the first case, lipoamide dehydrogenase is required to catalyse the reaction between MV^{o+} and NAD^+. On the contrary, regeneration with the rhodium complex does not require any enzyme. With methyl viologen as mediator, the results obtained with the N-MER in continuous mode (only one passage through the reactor) are given in Fig. 3. The potential was imposed after 19 h to check that there was no transformation without electrochemical assistance. After a few hours of electrolysis at -0.70 V/SCE, 26% transformation was obtained with 500 U lipoamide dehydrogenase. Adding of 250 U and then 350 U lipoamide dehydrogenase yielded 45% and 65% transformation, respectively. This demonstrates that the enzymatic reaction between NAD^+

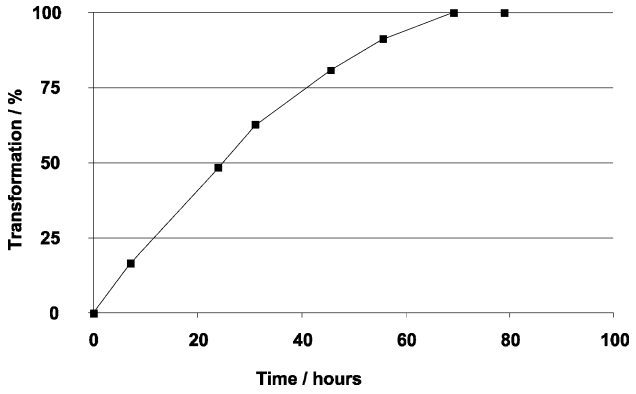

Fig. 4. Transformation of cyclohexanone into cyclohexanol in the N-MER in recycling mode. Regeneration of NADH with rhodium complex as mediator. Tris–HCl buffer 0.1 M pH 7.5; Cyclohexanone 100 mM; Rh complex 1 mM; NAD^+ 1 mM; HLADH 73 U.

and viologen methyl is the limiting step. Moreover, it was important to work without oxygen because oxygen reacts very quickly with MV^{o+} [8] and oxygen reacts also with NADH in the presence of LipDH. The regeneration of NADH with the rhodium complex as mediator was also carried out in the N-MER in continuous mode with a flow rate of 0.075 μl/s. The transformation ratio increased up to 55%. The flow rate was then increased, and the transformation ratio decreased and reached 45% for 0.3 μl/s and 33% for 0.45 μl/s. The transformation ratio was directly controlled by the residence time in the reactor. For a constant flow of 0.075 μl/s, the transformation ratio kept a constant value of 75% over 36 h. When the N-MER was used in recycling mode, the transformation of cyclohexanone into cyclohexanol was complete after 70 h of electrolysis (Fig. 4).

4. Conclusion

The two examples chosen allowed to show the potentialities of the MER. For the glucose transformation, the electrochemical step really increased the conversion ratio for high concentration. For the cyclohexanol production, the N-MER allowed to reach a complete transformation of cyclohexanone. The results demonstrated that it is important to choose well the reactor adapted to the reaction synthesis.

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