Efficient Regeneration of NADH Cofactor in Laminar Flow Based Microreactors

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The interest in using enzymatic transformation continues to grow very rapidly in many industrial fields such as fine chemistry, pharmacology, and cosmetology. [1] As a result, oxidoreductases that requires nicotinamide cofactors ($NAD(P)^+$-NAD(P)H), which catalyze the asymmetric reduction of carbonyl groups to alcohols and amines or promote the oxygenation of C-H bonds, have become a primary interest But due to the initial expense and physical instability of the nicotinamide cofactor-requiring enzymes themselves, their stoichiometric use is economically not feasible. Hence, various *in situ* regeneration methods, such as chemical, photochemical, enzymatic, biologic and electrochemical methods have been developed with only modest success.[2,3]

From an electrochemical point of view, one can regenerate NADH directly or indirectly (Fig. 1). Direct conversion of NAD^+ into the active NADH at the electrode (Fig. 1A) is difficult because the intermediate radicals dimerize and various inactive isomers are formed.[4] In the indirect regeneration of NADH from NAD^+, a mediator accepts electrons from the electrode and provides these to an enzyme that regenerates NADH from NAD^+ (Fig. 1B).

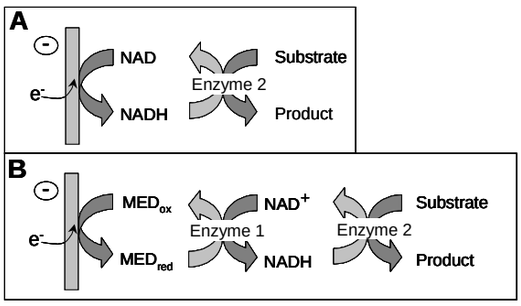

Figure 1. Schematic representation of enzyme-catalyzed electrochemical synthesis via direct (A) or indirect (B) regeneration of NADH. In this work, the mediator (MED) is $FAD/FADH_2$, and enzyme 1 is flavine dehydrogenase (FDH).

In this work, a novel microfluidic electrochemical microreactor that enables indirect regeneration of NADH by use of flavin adenine dinucleotide (FAD) as the mediator has been developed. In general the oxidation of the desired NADH species by the FAD mediator is spontaneous at pH 7.0 without involvement of an enzyme (i.e. the equilibrium is shifted to the right which is undesirable):

$$NADH+FAD+ H^+ \leftrightarrow NAD^+ + FADH_2$$
$$\Delta G'^\circ = -20.3\ kJ.mol^{-1}$$

But due to the ability to focus a stream of reactants closely to the electrode utilizing multistream laminar flow at the microscale (Fig. 2), a normally unfavorable reaction equilibrium essential for enzyme/cofactor regeneration is reversed, in other words, the Gibbs free energy can be forced to change sign.

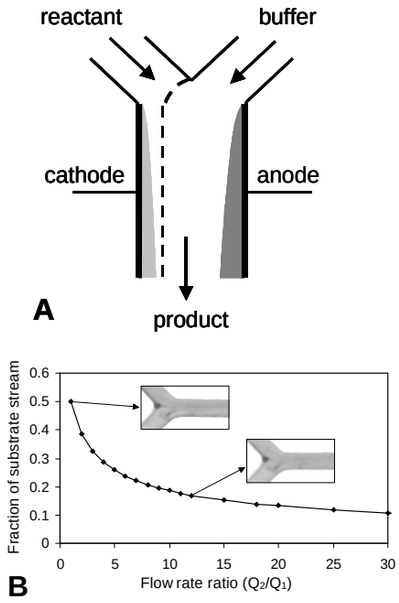

Figure 2. (a) Schematic of a microreactor for regeneration of nicotinamide cofactors. (b) Width of the substrate stream ratio of w_1 / w_1+w_2) as a function of the flow rate ratio (Q_2/Q_1) along with two optical micrographs of two different dyed aqueous streams flowing laminarly in parallel at the flow rate ratios of 1:1 and 1:6.

The biocatalytic microreactor investigated in this work consists of a Y-shaped microfluidic channel in which two liquid streams containing substrates (mediator, enzyme) and buffer merge and continue to flow laminarly in parallel between two electrodes on opposing walls without turbulent mixing. Regeneration of NADH as well as conversion on the substrate pyruvate into the product L-lactate will be shown. Furthermore an approach to increase the regeneration efficiency by integration of a chaotic micromixer[5] will be presented.

References

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