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# Solving NAD(P) Recycling Challenges in Organic Solvent Biocatalysis with Enzyme Immobilization

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#### PURPOSE OF THE ABSTRACT

Ene-reductases (EREDs) are enzymes that have gained significant attention as valuable catalysts for the (stereoselective) reduction of activated alkenes.1,2 The poor solubility of target compounds in aqueous systems usually hamper the implementation of biocatalytic process on industrial scale,3 and in fact, there is currently limited knowledge available on ERED enzymes performance in organic solvents.4,5 One of the major challenges of using redox enzymes in organic solvents is the supply of the required NAD(P)(H) cofactor for transformations. The recycling of NAD(P) in these media is particularly challenging due to the insolubility of the nicotinamide cofactor, severely hindering the hydride equivalent transfer between the reductase and the recycling enzyme in a coupled-enzyme system.

To address these challenges, the use of an ERED from Zymomonas mobilis has been fully investigated using cyclohex-2-en-1-one as model substrate. Special attention has been given to searching for efficient alcohol dehydrogenases (ADHs) for NAD(P) cofactor recycling purposes, as well as optimizing the (co)immobilized catalytic system in organic solvents. With this aim, ADH from Thermoanaerobacter ethanolicus was found to be a suitable enzyme, and both ERED and ADH were immobilized on different types of EziG polymer-coated controlled porosity glass carrier materials. The results of this study demonstrate the potential of using redox enzymes in organic solvents and provide insights into their optimization for future industrial applications.

FIGURE 1

#### FIGURE 2

#### **KEYWORDS**

enzyme immobilization | organic solvent | cofactor recycling | ERED, ADH

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