DOI: 10.46991/JISEES.2025.SI1.128

The Role of Hydrogenases and F_OF_1-ATPase in Electricity Generation in an H_2-Based Bioelectrochemical System

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ABSTRACT

Hydrogenases (Hyds) are microbial enzymes that reversibly catalyze the oxidation of molecular hydrogen (H_2), playing a critical role in biohydrogen metabolism [1,2,3]. These include oxygen-resistant biological [NiFe]-hydrogenases, which have attracted great interest for their application in hydrogen fuel cell (H_2-FC) technologies. The results provide insight into the potential of bioelectrochemical-based systems for sustainable energy production. The bioelectrocatalytic efficiency of *E. coli* bacteria immobilized on the electrode surface in a volume of 3 µl (1.5 mg cell dry weight) was studied under the conditions of 0.2% glucose fermentation in peptone medium at pH-7.5 [4]. In this study, the electrochemical measurements were performed using a two-electrode system equipped with a computer potentiostat, specifically a hydrogen fuel cell voltammetry (HFCV). The wild-type *E. coli* BW25113, the septuple (BW25113*hyaB hybC hycA fdoG ldhA frdC aceE*) the F_OF_1-ATPase-defective, and the Hyd defective *hyaB*, *hybC*, *hycE*, *hyfG* mutant strains were used in the experiments. Maximal catalytic activity was observed in the *hyaB* and *hyfG* mutants, being stimulated ~2-fold and ~1.6-fold compared to the wild type, reaching values of ~1.26 ± 0.02 V and ~0.98 ± 0.02 V, respectively. The effect of the 10 mM N,N′-dicyclohexylcarbodiimide (DCCD), the F_OF_1-ATPase inhibitor, on the catalytic activity of Hyd enzymes was observed. It was shown that for all strains, the reading of the voltammeter decreased ~1.5 times, reaching the readings recorded by the F_OF_1-ATPase-defective strain. Interestingly, in the case of the the septuple mutant strain, DCCD recorded a stimulating rather than a suppressive effect. The results obtained indicate the great potential of bacteria as anodic biocatalysts and demonstrate the need for further studies.

Keywords: anode biocatalyst, *E. coli* BW 25113, hydrogenase mutants, hydrogenase ferments, voltammeter

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