

Optimization of Adenylate Energy Charge and NAD⁺/NADH Assays in Resting Cells of Lactic Acid Bacteria

R G Brajesh, Ganesh Nehru, Gopi Gopal Ramakrishnan,
Subramanian Ramalingam*

Centre for Biotechnology, Anna University, Chennai, India.

Abstract In a whole cell mediated redox bio-transformations, flow of electrons and adenylate energy charge (AEC) play a vital role in product formation rate. In *Lactobacillus* resting cells, AEC and NAD⁺/NADH ratio was measured using reverse phase chromatography and microtiter plate assay respectively. The extraction methods used for the estimation of ATP and nicotinamide nucleotides were found to affect the absolute values, but not the derived parameters, AEC and NAD⁺/NADH ratio. It was found that in a redox reaction of *Lactobacillus* coupled to ATP formation, resting cells maintain an AEC of 0.56 and NAD⁺/NADH ratio was found to be 5 and it is maintained throughout the bioconversion process. Initially NADH and NAD⁺ estimation was done in NanoDrop UV-Vis spectrophotometer at 570 nm. Then assay was performed in microtiter plate since estimation in NanoDrop UV-Vis spectrophotometer was time consuming and expensive. The factors affecting NAD⁺/NADH ratio are physiological state of the cell, presence of electron acceptors and product formation. Optimization of these assays in our current work might essentially aid redox and energy engineering of bacterial resting cells for green chemical synthesis like 1,3-propanediol and 3-hydroxypropionic acid.

Keywords: Adenylate Energy Charge, NAD⁺/NADH ratio, Lactic acid bacteria.