Novel industrial frontiers for alcohol dehydrogenases: hemiacetal oxidation is a self-sufficient biocatalytic production of esters

Sofia Ceccarossi[a], Mirko Zago[b], Immacolata Serra[c], Paola Branduardi[c]
lead presenter: s.ceccarossi@campus.unimib.it

[a]Department of Earth and Marine Sciences, University of Palermo, Palermo, Italy
[b]Soft Chemicals S.r.l., ASTROBIO™ Division, Via Sandro Pertini 14, Arsago Seprio, Varese, Italy
[c]Department of Biotechnology and Biosciences, University of Milano-Bicocca, Piazza della Scienza 2, Milano, Italy

Esters are versatile organic molecules essential to numerous industrial sectors, yet their conventional chemical synthesis often relies on energy-intensive processes and corrosive catalysts. While biocatalytic routes using esterases or lipases offer greener alternatives, they are frequently limited by thermodynamic equilibrium and the need for continuous water removal. This study presents a novel, self-sufficient biocatalytic production route leveraging the enzymatic oxidation of hemiacetals by alcohol dehydrogenases (ADHs). We demonstrate that multiple ADHs, including Adh1 from *Saccharomyces cerevisiae* (*Sc*Adh), *Homo sapiens*, *Neurospora crassa*, and *Clostridium beijerinckii*, possess hemiacetal dehydrogenase (HADH) activity. Crucially, we discovered an intrinsic $NAD(P)^+$ regeneration pathway operated by the enzymes themselves, which utilizes the initial aldehyde substrate to re-oxidize the cofactor. This mechanism allows ester production to significantly exceed traditional stoichiometric limits, without external cofactor regeneration systems or sacrificial substrates. In batch experiments, *Sc*Adh achieved methyl formate titers 4.5 times higher than the stoichiometric threshold. Through process optimization and iterative aldehyde feeding, methyl formate titers were further increased to 11787 mg L^{-1}, approximately 65 times the expected maximum. Furthermore, the versatility of this route was confirmed by the successful synthesis of ethyl formate, methyl acetate, and ethyl acetate using various ADHs. These findings establish hemiacetal oxidation as a robust and industrially viable platform for the sustainable synthesis of high-value esters.