E3B The Elements of Bioremediation, Biomanufacturing & Bioenergy Metals in Biology

Durham University · University of Kent · Manchester 1824 · UKRI Biotechnology and Biological Sciences Research Council

PROJECT PARTNERS: Simone Morra, University of Nottingham and Holly Reeve, HydRegen

POCE3B034 BB/S009787/1

University of Nottingham UK | CHINA | MALAYSIA

HydRegen

Scaling up [FeFe]-hydrogenases to biocatalysis

"This project demonstrated feasibility for scale up of the CbA5H hydrogenase using the bioreactors in Nottingham, which advanced the manufacturing readiness level of promising components in HydRegen's products; we could not have done this on our own within this timescale," Sarah Cleary, HydRegen.

PROJECT AIMS: This project aimed to demonstrate the industrial applicability of the hydrogenase CbA5H by showing that this enzyme is a viable H_2-oxidation biocatalyst for biocatalytic technologies. The project explored factors that enable implementation of CbA5H in industrially applicable processes, such as large-scale enzyme production, process scale-up to grams of product, no requirement for enzyme purification and operation under industry-relevant conditions.

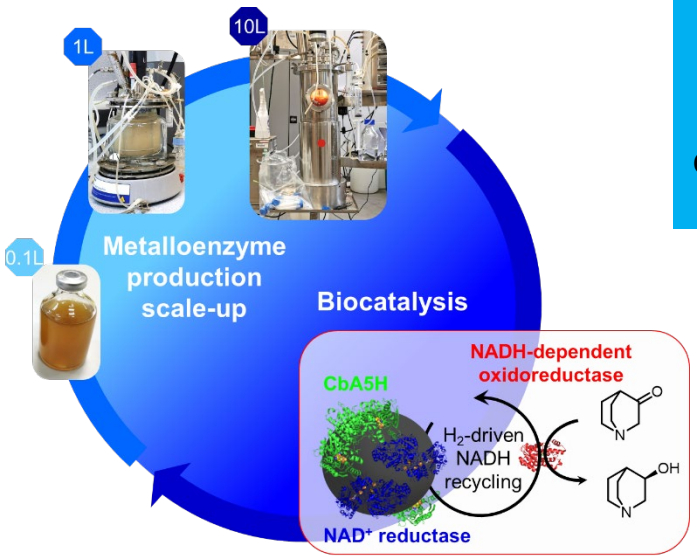
OUTCOMES & NEXT STEPS:

- Funding obtained from Innovate UK for a feasibility study between University of Nottingham and HydRegen to scale up the production of various metalloenzymes.
- PhD scholarship secured from the Nottingham BBSRC DTP CASE program to continue working on aspects of this research.
- Poster presented at the International Conference on Hydrogenases (June 2023, Walla Walla USA).
- HydRegen CSO gave invited talk at Winter Process Chemistry Conference (December 2023, Liverpool UK).

RESULTS:

- We successfully translated CbA5H overproduction from lab-scale to bioreactors at small and medium scale. We initially optimised CbA5H overexpression at 1-L scale, and subsequently assessed the process at 10-L scale. Overall, purification yields significantly improved, up to 24 mg enzyme per litre culture, at high specific activity (665 U/mg).
- We investigated the reaction kinetics of CbA5H, focusing on the reactivation kinetics, the effect of pH and temperature on the enzyme and its stability over time.
- We successfully immobilised CbA5H from five preparations of different purity on carbon particles in an active form; this finding demonstrated that significant cost-savings can be made by omitting enzyme purification or by employing a cheaper, partially purified sample.
- HydRegen Ltd used CbA5H obtained from high-yield bioreactors in their proprietary technology, coupling H_2 oxidation to cofactor recycling. They demonstrated 100% conversion in a process for the production of the API 3-quinuclidinol with a total turnover number of 136,600 on a 25 mg scale. Subsequently, HydRegen has scaled up the reaction conditions demonstrating ≥80% conversion at 0.5 gram scale, demonstrating the feasibility of our approach.

Change in technology readiness level: 2 to 4



[FeFe]-hydrogenases can be produced in high yields for exploitation in biocatalytic cofactor recycling applications.