Production, Upscale and Application of Recombinant Whole-cell Styrene Monooxygenase

SLOVAK UNIVERSITY OF TECHNOLOGY IN BRATISLAVA
FACULTY OF CHEMICAL AND FOOD TECHNOLOGY

Dominika Gyuranová[1], Radka Štadániová[2], Zuzana Hegyi[1], Róbert Fischer[2], Martin Rebroš[1]

[1]Institute of Biotechnology, Slovak University of Technology in Bratislava;

[2]Institute of Organic Chemistry, Catalysis and Petrochemistry, Slovak University of Technology in Bratislava

dominika.gyuranova@stuba.sk, martin.rebros@stuba.sk

Introduction

Styrene monooxygenases (SMOs) are highly enantioselective flavoprotein monooxygenases that catalyse the epoxidation of alkenes to chiral epoxides. Chiral compounds containing oxirane ring or products of their hydrolysis comprise a group of important building blocks and precursors in organic synthesis in the pharmaceutical industry. However, industrial production of chiral epoxides usually involves Sharpless and Jacobsen oxidation that demand extreme reaction conditions and suffer from poor enantioselectivity. SMOs, on the other hand, operate under mild conditions, are highly enantioselective, and exhibit an affinity towards a broad substrate spectrum [1-5].

Materials and methods

The genes of StyA and StyB originating from *Marinobacterium litorale* encoding SMO were selected by genome mining and designed for fusion. Upscale SMO production was performed by High Cell Density fermentation (HCD) in a batch mode. Chrial epoxides were analysed by GC-FID and ^{1}H NMR.

Enzyme expression

- expression was optimized by varying media composition, IPTG concentration, and induction temperature
- optimized conditions: M9 medium, 0.25 mM IPTG, 20°C, 22 h, where a specific activity of 12 U/g_{DCW} was achieved

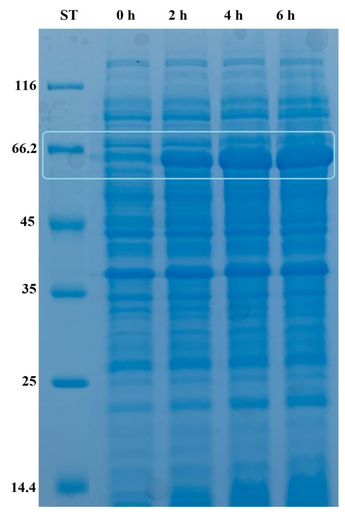


Figure 1. Protein profiles of *E. coli* during fermentation.

Biocatalysis

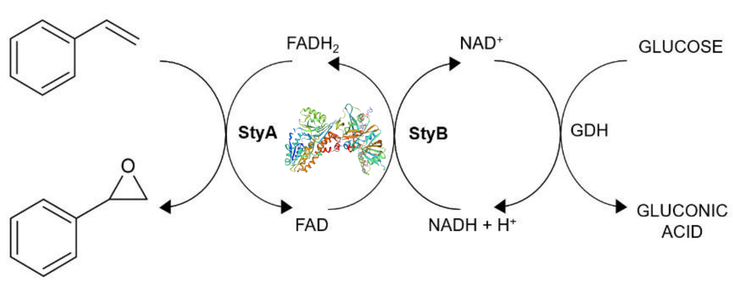


Figure 3. Biocatalytic cascade of styrene epoxidation involving GDH cofactor regeneration system [1].

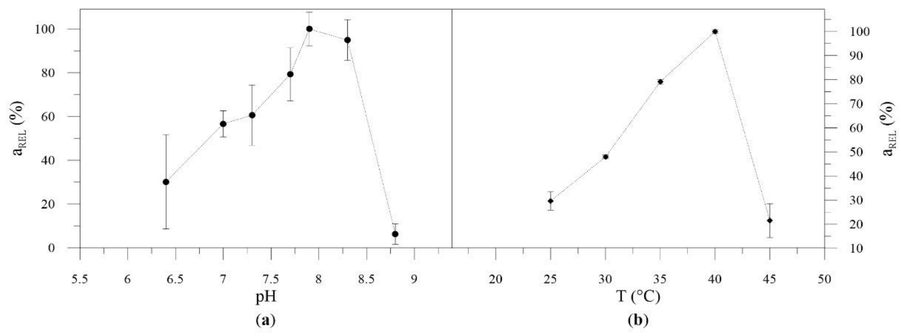


Figure 4. pH (**a**) and temperature (**b**) profile of purified SMO [1].

- SMO exhibited a maximum activity at pH 7.8 and 40°C
- whole-cell SMO exhibited higher activity and stability compare to crude SMO extract and purified SMO
- SMO was tested for epoxidation of 34 structurally different alkenes
- 5 epoxides were produced in excellent enantiopurity

Upscale production

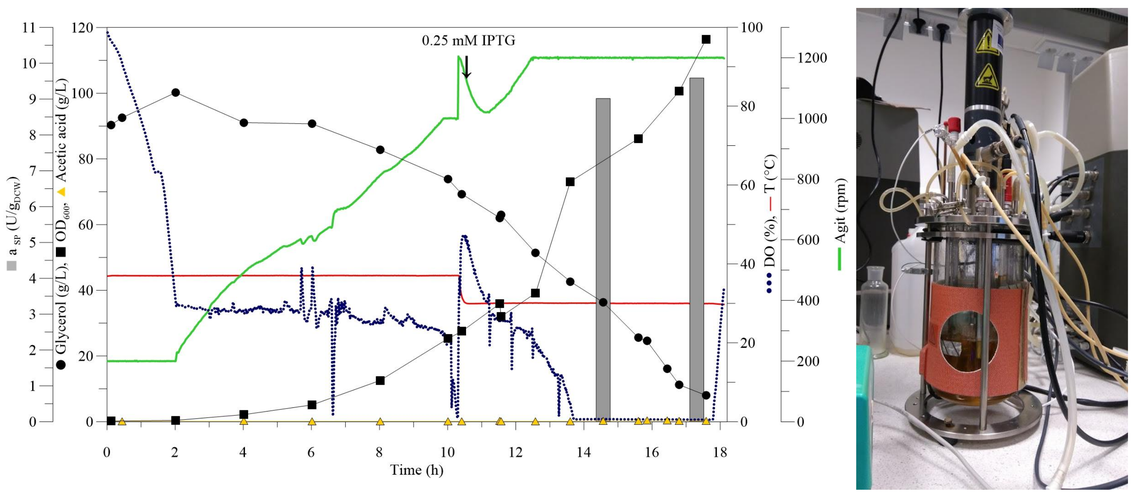


Figure 2. HCD batch fermentation of *E. coli* expressing SMO performed on the 1.5 L scale [1].

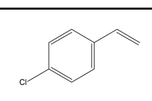
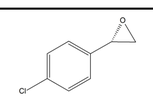
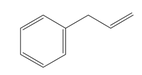
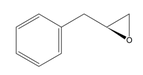
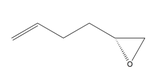
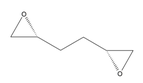
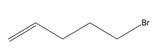
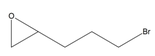
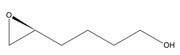
- HCD fermentations were performed to upscale the SMO production
- following the protocol, 1.5 L scale fermentation provided 35 g_{DCW}/L of overexpressed SMO with a specific activity of 9.6 U/g_{DCW}.

Table 1. Results of HCD fermentations [1].

Final volume (L)	Cell concentration (g_{DCW}/L)	Total dry cell weight (g_{DCW})	Enzyme activity (U/g_{DCW})	Total activity (U)
0.5 L	31	15.5	10.5	162.8
1.5 L	35	52.5	9.6	504

Epoxidation of alkenes

Table 2. Summary of upscale biotransformations by whole-cell SMO [1].

Entry	Substrate	Product	Configuration	ee (%)	Conversion (%)	Yield (mg)	Reaction volume/Yield (mL/mg)
1f			*S*	> 99%	99	60	1.27
2a			*S*	> 95%	99	26	2.88
5g			2-*R*,5-*R*	> 97%	99	157	1.28
5j			*ND* [1]	> 99% [2]	93	173	1.73
5d			*S*	> 99% [3]	99	76	1.32

References

[1] D. Gyuranova; *Molecules*, **2021**, 26, 1514.
[2] T. Heine; *Appl Biochem Biotechnol*, **2017**, 181, 1590-1610.
[3] M. Oelschlagel; *Front Microbiol*, **2018**, 9, 490.
[4] M. Breuer; *Angew Chem Int Ed Engl,* **2004**, 43, 788-824.
[5] H. Lin; *Green Biocatalysis,* **2016**, 351-372.

Acknowledgements

This work was supported by the Slovak Research and Development Agency under the Contract no. PP-COVID-20-0056. This article was created with the support of the OP Integrated Infrastructure for the project: Research on COVID-19 progressive diagnostic methods and biomarkers useful in early detection of individuals at increased risk of severe disease, ITMS: 313011ATA2, co-financed by the European Regional Development Fund.