Encapsulation of Baeyer-Villiger monooxygenases as a tool for research of biooxidations

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INTRODUCTION AND OBJECTIVES

Baeyer-Villiger (BV) biooxidations catalysed by BV monooxygenases (BVMOs) enable biotransformations of cyclic ketones to enantiomerically pure lactones as chiral precursors of bioactive compounds (Mihovilovic 2006). Encapsulation using polyelectrolyte complex (PEC) capsules may provide an universal immobilization technique for the whole family of BVMOs overproduced in recombinant cells *E. coli* including cyclohexanone monooxygenase (CHMO) and cyclopentanone monooxygenase (Bučko 2011). The objective of this study was to show the potential of PEC capsules as a tool for research of BVMOs. Therefore, storage and operational stability of encapsulated CHMO was measured in continuous BV biooxidations of rac-bicyclo[3.2.0]hept-2-en-6-one (1) to corresponding lactones (2) and (3) (Fig. 1) as important chiral synthons for the synthesis of prostaglandins and nucleosides. Additionally, monitoring of BV biooxidations by inovative on-line measurements *via* flow calorimetry have been performed (Bučko 2011).

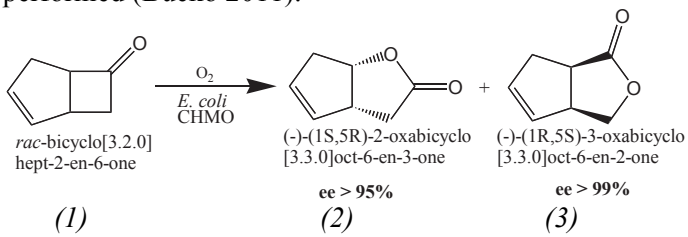

Figure 1. BV biooxidation of ketone substrate (1) to corresponding lactones (2) and (3).

MATERIALS AND METHODS

Materials. Recombinant cells *E. coli* with overexpressed CHMO from *A. calcoaceticus* NCIMB 9871 were kindly donated by prof. M.D. Mihovilovič (VUT Vienna). Substrates, media for cell growth and GC standards for BV biooxidations and other chemicals were purchased from commercial suppliers. (Bučko 2011).

Cultivation conditions. *E. coli* expressing CHMO were cultivated and the biomass was separated as reported previously (Bučko 2011).

Encapsulation of cells. Encapsulation of *E. coli* with CHMO within PEC capsules made of sodium alginate and cellulose sulfate as polyanions, poly(methylene-co-gianidine) as polycation, $CaCl_2$ and NaCl was performed in a custom-made coaxial air-stripping extrusion device fitted with a multiloop reactor reported previously (Bučko 2011).

Flow calorimetry. Detailed procedure have been described elsewhere (Bučko 2011). The thermostatic cell of the flow microcalorimeter (FC) contained the column packed with the CHMO-PEC capsules. The temperature difference between the column input and output is the signal S (in mV) measured by the enzyme thermistor. Phosphate buffer (0.05 M, pH 7.0) was passed through the system at a flow rate 1 ml/min as long as the thermal equilibration (25°C) and oxygen saturation was reached. Afterwards the buffered solutions of substrate (1) (0.92-9.2 mM) saturated with oxygen were continuously pumped through the FC column until the steady-state heat production was obtained. The change of temperature due to the Baeyer-Villiger biooxidation reaction was calculated.

Operational and storage stability. 1.7 g of CHMO-PEC capsules were placed onto column of flow calorimeter (97 mm x 6 mm i.d.). The column was operated as a small packed bed reactor in a continuous mode with recirculation of the reaction mixture in stirred reservoir as described previously (Bučko 2011). After thermal equilibration at 25°C, 100 ml of buffer solution (pH 7.0, $K_2HPO_4.3H_2O$ 16.4 g/l, KH_2PO_4 2.3 g/l, glucose 4g/l, ampicillin 0.2 g/l and substrate (1) rac-bicyclo[3.2.0]hept-2-en-6-one 0.2 g/l was continuously circulated from the reservoir through the column at a flow rate 0.4 ml/l, saturated with oxygen via bubble-free system placed within reservoir. Samples were wihtdrawn every 30 min and analyzed by GC as reported (Bučko 2011). After 12 h, BV biooxidation was terminated, the whole system was flushed with phosphate buffer and next biooxidation cycle was started by addition of substrate to the next batch of buffer solution. 14 biooxidation cycles were performed and operational stability of encapsulated cells was expressed as the percentage of substrate conversion to products after each cycle. Storage stability was expressed as the percentage of substrate conversion to products after each single cycle performed with another column after 14, 26, 35, 48, 60, 70 and 91 days of storage at 4°C (Bučko 2011).

RESULTS AND DISCUSSION

Encapsulation of cells. The CHMO-PEC capsules (Fig. 2a) were uniform in size and membrane thickness. The mean diameter of capsules was 0.80 ±

0.04 mm and the mean membrane thickness was 0.08 ± 0.005 mm. The capsules were slightly deformed at the end of biooxidation tests (Fig. 2b) due to the compression caused by the flow of reaction solution through the column (Bučko 2011).

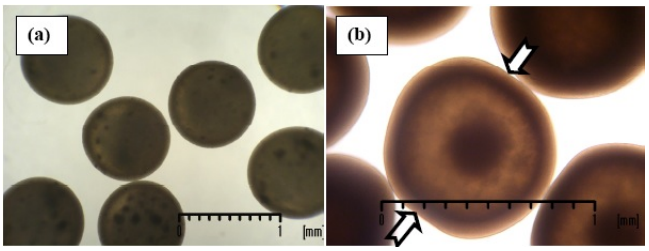

Figure 2. (a) Shape of CHMO-PEC capsules after encapsulation and before placement into the column of the enzyme thermistor; (b) CHMO-PEC capsules after 14 cycles of BV biooxidation.

Flow calorimetry measurements. Recombinant *E. coli* cells overexpressing CHMO encapsulated in the PEC capsules exhibited substrate inhibitory behavior as depicted in Fig. 3. A mild substrate inhibition was apparent when the substrate concentration exceeded 4.0 mM (Bučko 2011).

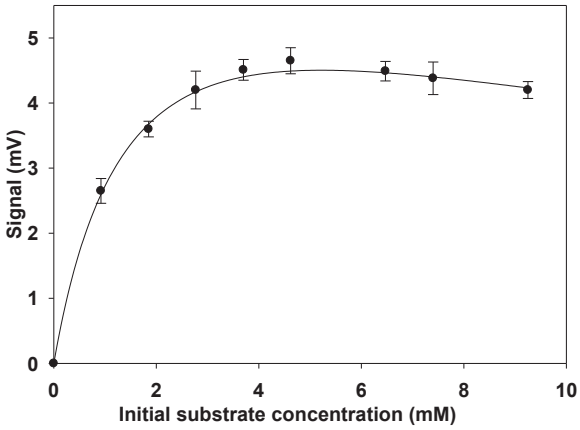

Figure 3. Effect of initial substrate concentration on temperature response by the flow calorimeter during BV biooxidations of rac-bicyclo[3.2.0]hept-2-en-6-one catalyzed by CHMO-PEC capsules.

The operational and storage stability. The CHMO-PEC capsules are able to preserve the catalytic efficiency of encapsulated cells in viable state during 14 repeated continuous BV biooxidations (Bučko 2011) as depicted on Fig. 4.

Evolution of storage stability of CHMO-PEC capsules shown in Fig. 5 proved superior properties of PEC capsules regarding long-term stabilization of encapsulated BVMOs during 91 days of storage (Bučko 2011).

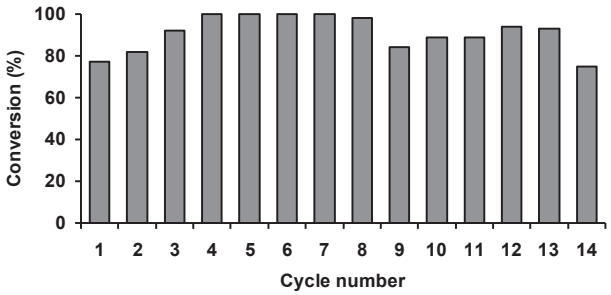

Figure 4. Operational stability of CHMO-PEC capsules during repeated BV.

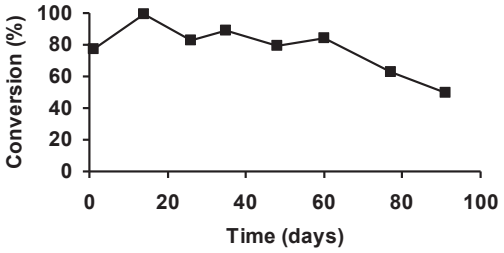

Figure 5. Storage stability of CHMO-PEC capsules during single BV biooxidations.

CONCLUSIONS

The stability of encapsulated cells was indeed improved in a view of long-term stabilization during storage and high operational stability within repeated BV biooxidations. An original approach for determination of kinetics of BV biooxidations by flow calorimetry was introduced. Additionally, stabilization effect of PEC capsules has been currently successfully utilized in production of natural flavours *via* biooxidations (data not shown) using encapsulated cells *Gluconobacter oxydans.*

REFERENCES

- Bučko et al. (2011) *Continuous testing system for Baeyer-Villiger biooxidation using recombinant Escherichia coli expressing cyclohexanone monooxygenase encapsulated in polyelectrolyte complex capsules.* Enzyme Microb Technol 49, 284-288.
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ACKNOWLEDGMENTS

This work was supported by the Slovak Grant Agency for Science VEGA 1/0229/12 and by the Slovak Research and Development Agency under the contract No. APVV-0302-10. We thank to prof. Marko D. Mihovilovic (VUT, Vienna, Austria) for delivery of the expression strain *E. coli* overexpressing CPMO and CHMO.

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