catalysts

Supplementary Material: Co-Immobilization of Ketoreductase and Glucose Dehydrogenase

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1. Analysis of expression of cloned enzymes KRED and GDH in *E. coli.*

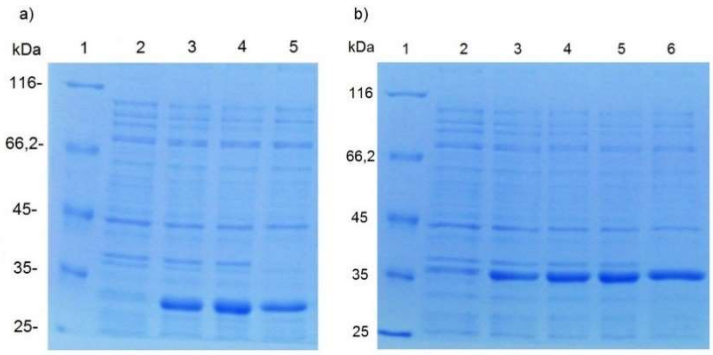

Figure S1 (**a**) expression of KRED (32 kDa); column 1- molecular standard, 2- start of induction, 3- after 2h, 4- after 3h, 5- cell extract (**b**) expression of GDH (35 kDa); 1- molecular standard, 2- start of induction, 3- after 1h, 4- after 2h, 5- after 3h, 6- cell extract. Conditions of induction for both KRED and GDH: 30°C, 0.5 mM IPTG.

2. Purification of KRED and GDH.

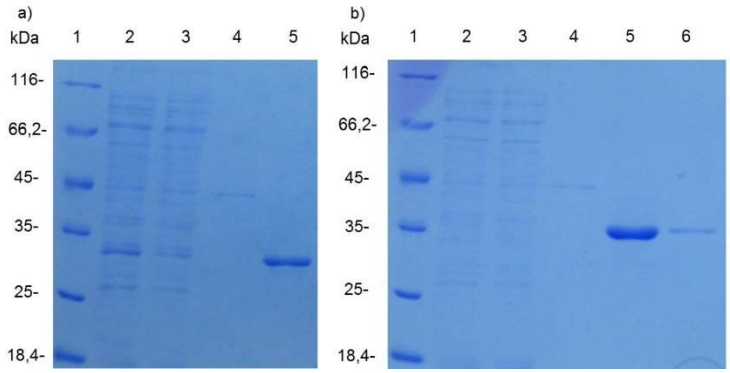

Figure S2 Purification of (**a**) KRED (1- molecular standard, 2-3- flow through, 4- unspecific proteins eluted with 25% elution buffer, 5- KRED eluted with 80% elution buffer) and (**b**) GDH (1- molecular standard, 2-3- flow through, 4- unspecific proteins eluted with 20% elution buffer, 5-6- GDH eluted with 75% elution buffer).

3. Biotransformations with free enzymes.

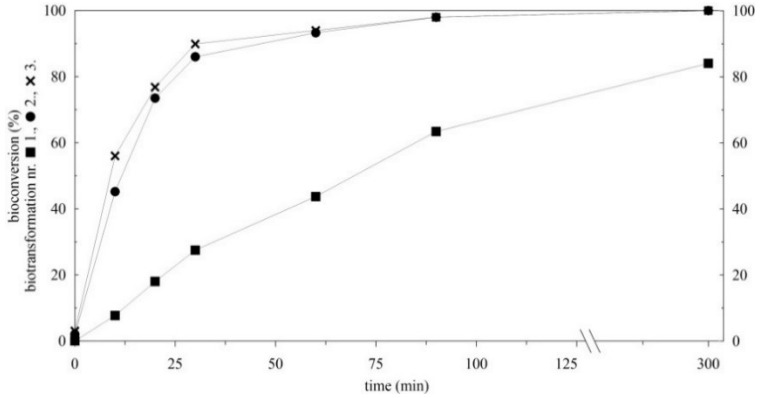

Figure S3 Product conversion in biotransformations with free ketoreductase (KRED) and glucosedehydrogenase (GDH) with three different ratios (KRED:GDH) of enzymes: nr. 1– 1:1.7; nr. 2– 5:1.7; nr. 3– 5:6.9.

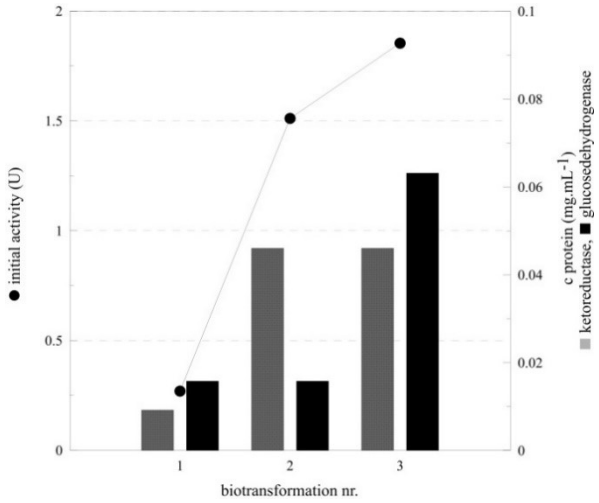

Figure S3 Influence of KRED:GDH ratio and enzyme concentration on initial activity in biotransformation with free enzymes.

4. Optimization of co-immobilization of KRED and GDH in PVA particles.

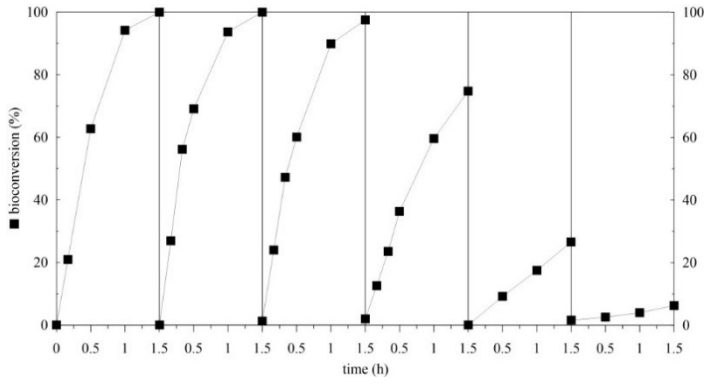

Figure S4 Product conversion in biotransformations with co- immobilized enzymes nr. 1 during six biotransformations with 12-hours storage at 4 °C in potassium phosphate buffer (0.1 M; pH=6.5) after 3rd, 4th and 5th batch.

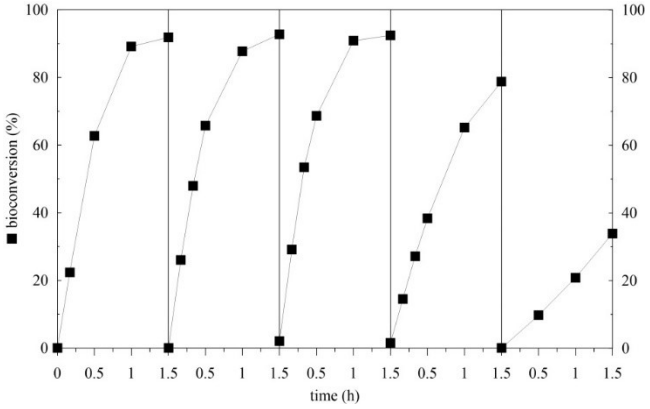

Figure S5 Product conversion in biotransformations with co-immobilized enzymes nr. 2 during five biotransformations with 12-hours storage at 4 °C in potassium phosphate buffer (0.1 M; pH=6.5) after 3rd and 4th batch.

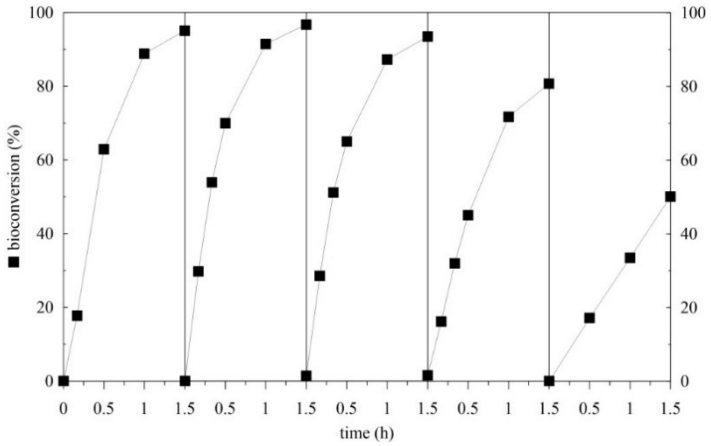

Figure S6 Product conversion in biotransformations with co-immobilized enzymes nr. 5 during five biotransformations with 12-hours storage at 4 °C in potassium phosphate buffer (0.1 M; pH=6.5) after 3rd and 4th batch.

5. Biotransformations in nonstop mode using co-immobilized enzymes nr. 1.

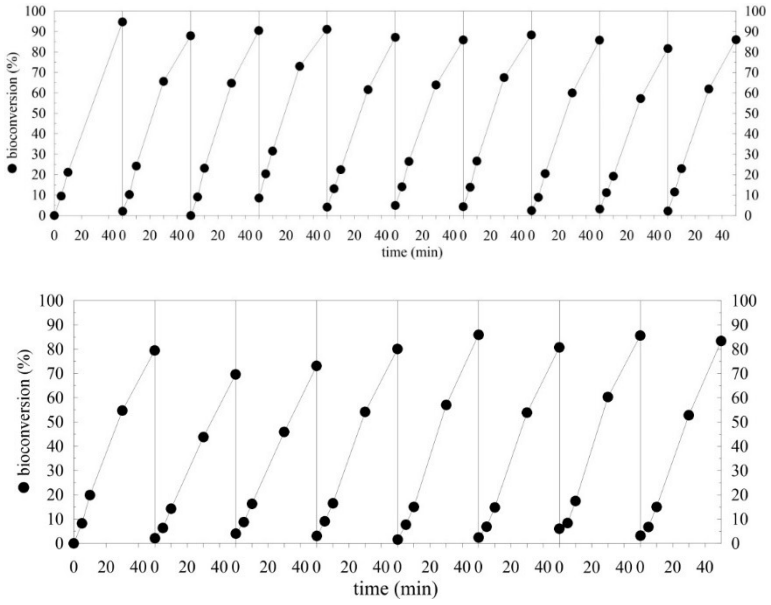

Figure S7 Product conversion in 18 batches of 50-minutes reactions.

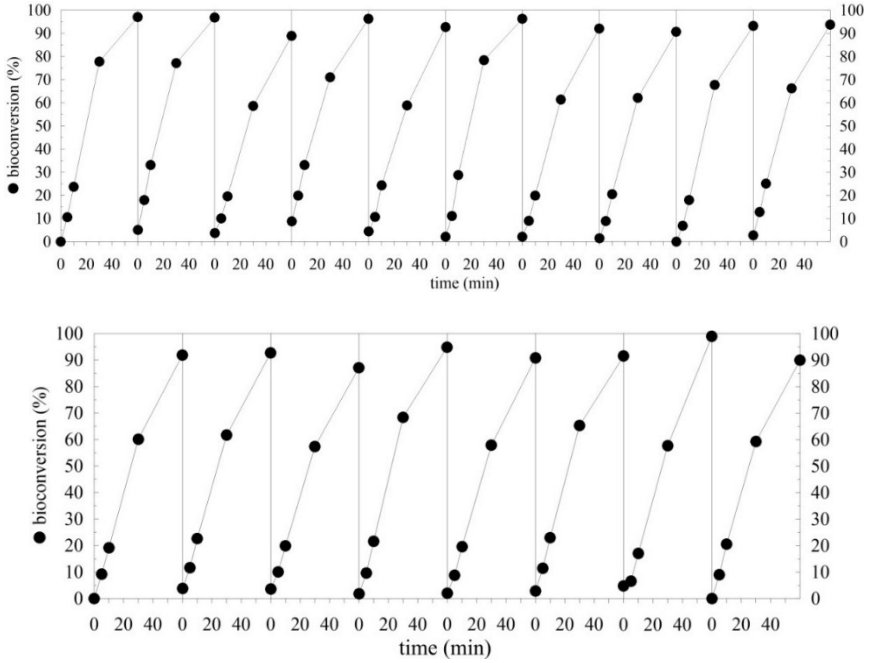

Figure S8 Product conversion in 18 batches of 60-minutes reactions.

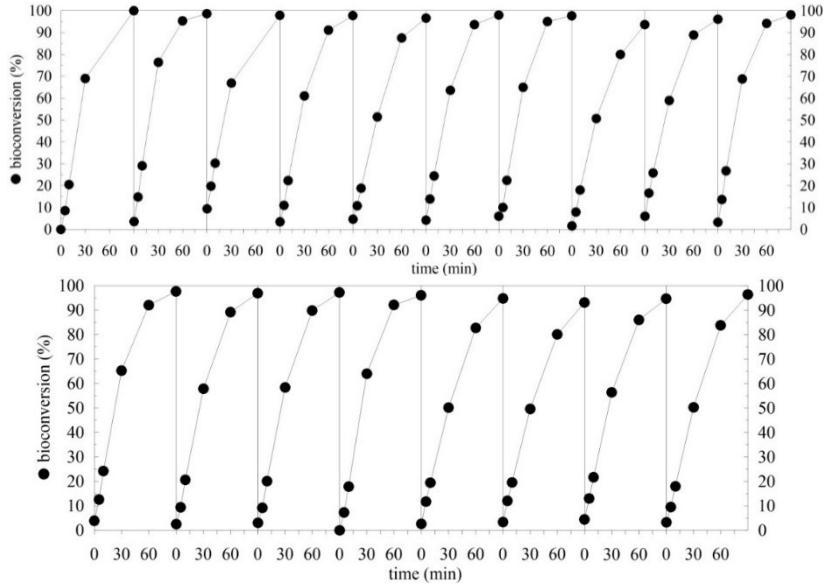

Figure S9 Product conversion in 18 batches of 90-minutes reactions.

6. Chromatographic analysis of biotransformation's hydroxy ester product by GC-FID with chiral column.

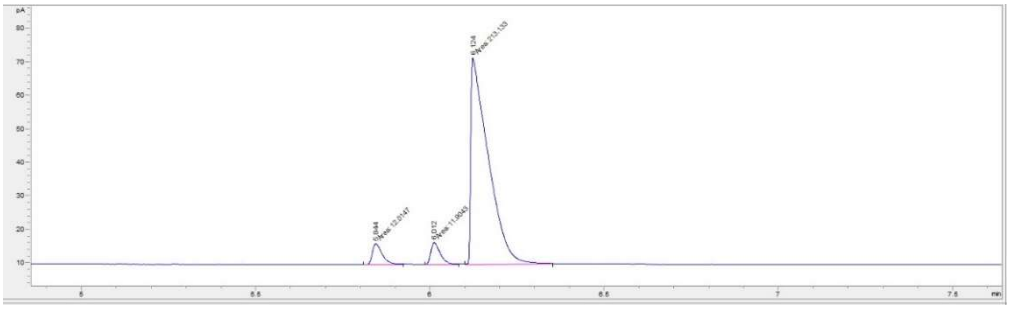

Figure S10 Chromatogram of hydroxy ester product after biotransformation with free enzymes.

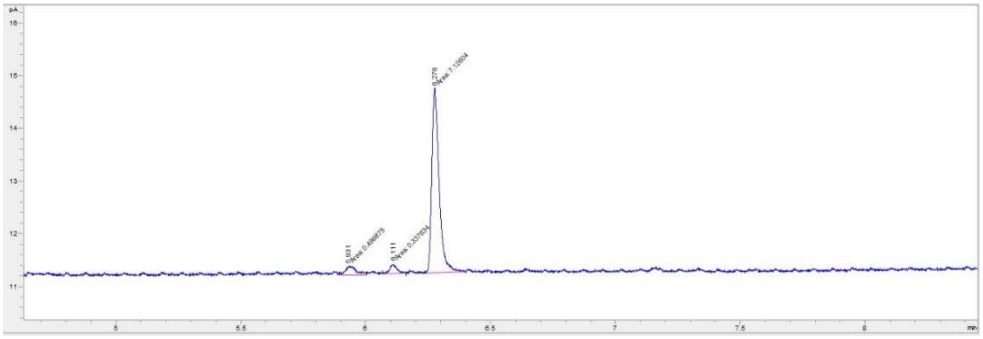

Figure S11 Chromatogram of hydroxy ester product after biotransformation with co-immobilized enzymes.

7. Biotransformations with differnet concentrations of β-ketoester substrate.

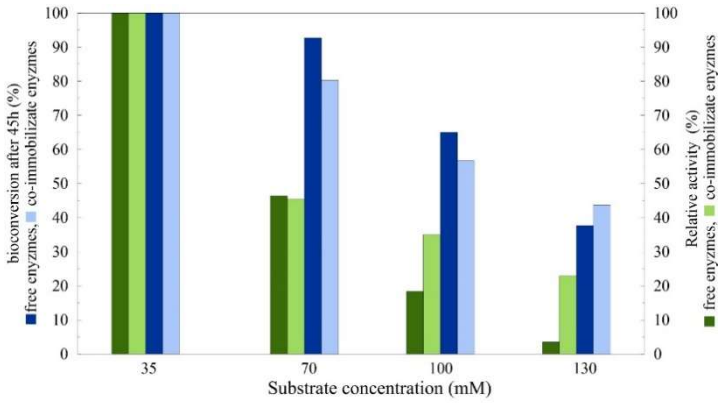

Figure S12 Bioconversions after 45 hours and relative activity of free and co-immobilizedenzymes with different substrate concentrations.

It is obvious that at the concentration 35 mM of substrate (used in experiments in whole manuscript) the free and immobilized enzymes perform equally regarding the reached conversion and relative initial activity (Figure S.13). However, with the increase of the substrate concentration, the final conversion and initial free enzyme activity drops more than immobilized one. This confirms that immobilized enzymes compared to free ones are being protected by PVA gel.