Catalysts 2018, 8, 603; doi:10.3390/catal8120603

Supplementary Materials: Immobilization of Arylmalonate Decarboxylase

Kristína Markošová [1], Jana Husarčíková [1], Monika Halásová [1], Robert Kourist [2], Michal Rosenberg [1], Radek Stloukal [3], Ludmila Zajoncová [4] and Martin Rebroš [1,*]

[1] Institute of Biotechnology, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, 812 37, Bratislava, Slovakia; kristina.markosova@stuba.sk (K.M.); j.husarcikova@gmail.com (J.H.); halasova.monika@gmail.com (M.H.); michal.rosenberg@stuba.sk (M.R.)
[2] Institute of Molecular Biotechnology, Graz University of Technology, Petersgasse 14, A8010 Graz, Austria; r.kourist@googlemail.com
[3] LentiKat´s, a.s., Evropská 846/176a, 160 00, Prague 6, Czech Republic; radek.stloukal@seznam.cz
[4] Department of Biochemistry, Palacký University Olomouc, Šlechtitelů 27783 71, Olomouc, Czech Republic; ludmila.zajoncova@upol.cz
* Correspondence: martin.rebros@stuba.sk, Tel.: +421-259-325-480

Received: 25 September 2018; Accepted: 27 November 2018; Published: date

Activity calculations:

The initial rate of biotransformation was represented by the slope b of the linear part of a graph of α-phenylacetic acid concentration against time measured in minutes and was obtained by linear regression analysis. Calculations of the activity was according to formula:

$$a = (b.\ V_R)/\ (M_w)$$

a activity [U]

b slope [$g.L^{-1}.min^{-1}$]

V_R middle volume of the reaction mixture [L]

M_w molar weight of α-phenylacetic acid [g/mol]

Specific activity was calculated per mg of crude enzyme extract (for free enzyme) or g of LentiKats particles (for immobilized enzyme).

Methods for modified PVA gel:

The prepared CEE was immobilized by entrapment in PVA gel using the LentiKats® system (www.lentikats.eu). 10 g of PVA and 3 g (1 g respectively) of polyethylene glycol (PEG) were mixed together in 82 mL (84 mL respectively) of distilled water. The mixture was heated to 94 °C for approx. 30 min at constant mixing, after which it was cooled to 40 °C and 5 mL (6.25 mg) of prepared CEE was added. The gel drops were printed on plastic carriers using laboratory equipment for immobilization (LentiPrinter®). The immobilized enzyme particles were dried at 40 °C for approx. 45 min and then hardened in 0.1 M Na_2SO_4 solution for an additional 45 min. The immobilized enzyme was used for biocatalytic reactions or stored in Tris HCl (100 mM, pH = 8.5) buffer at 4 °C.

Methods for CLEA immobilization:

The prepared CEE (20 mg/mL) was mixed with 0.527 g of ammonium sulphate and Tris HCl (100 mM, pH = 8.5) buffer to a final volume 1 mL. The solution was incubated at 4 °C for 15 minutes. After, 0.025 mg of glutaraldehyde were added, the pH was adjusted to 8.5 using hydroxide sodium solution and the solution was vortexed and stored at 4 °C for 2 h. Then the solution was centrifuged for 15 minutes at 7197 g and 4 °C. The formed CLEA were 3 times washed in 10 mL Tris HCl (100 mM, pH = 8.5) buffer using the same centrifugation conditions. To 0.1 g CLEA 900 μL of Tris HCl (100 mM, pH = 8.5) buffer were added and immediately entrapped in PVA gel according previously described method.

Figures:

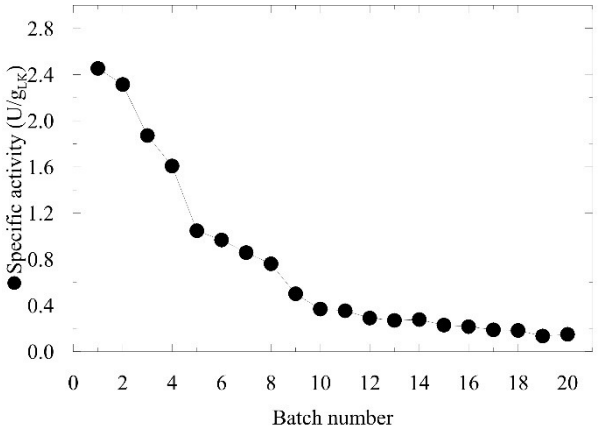

Figure S1. Repeated biotransformations with immobilized AMDase crude enzyme extract in LentiKats® with modified PVA gel- 3 g of PEG. Reaction conditions: 5 mL TrisHCl buffer (100 mM, pH = 8.5), α-phenylmalonic acid 1.8 g/L, 0.5 g of LentiKats®; 37 °C and 200 rpm.

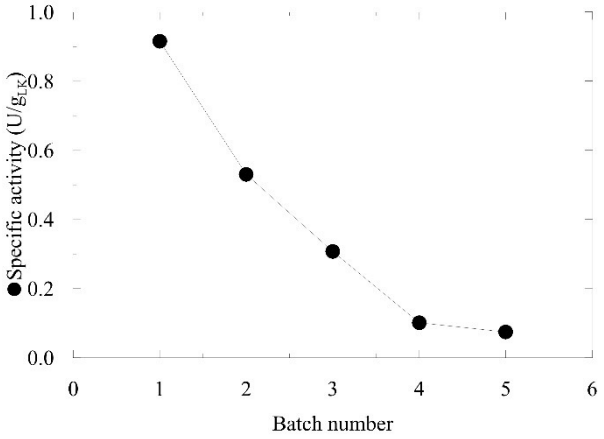

Figure S2. Repeated biotransformations with immobilized AMDase crude enzyme extract in LentiKats® with modified PVA gel- 1 g of PEG. Reaction conditions: 5 mL TrisHCl buffer (100 mM, pH = 8.5), α-phenylmalonic acid 1.8 g/L, 0.5 g of LentiKats®; 37 °C and 200 rpm.

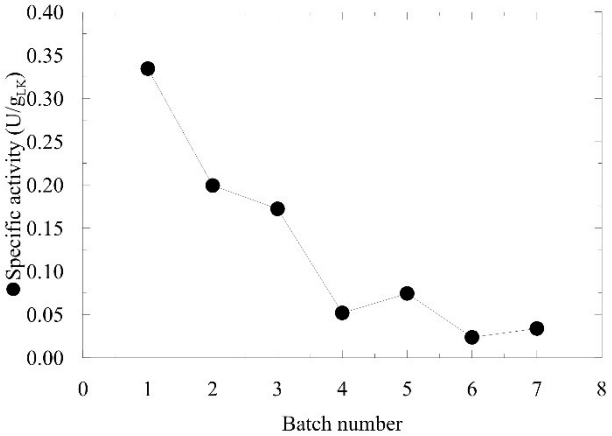

Figure S3. Repeated biotransformations with double-immobilized AMDase crude enzyme extract in CLEA and LentiKats® (CLEA- LentiKats®). Reaction conditions: 5 mL TrisHCl buffer (100 mM, pH = 8.5), α-phenylmalonic acid 1.8 g/L, 0.5 g of LentiKats®; 37 °C and 200 rpm.