Electronic Supporting Information

Positive Impact of Natural Deep Eutectic Solvents on the Biocatalytic Performance of 5-Hydroxymethyl Furfural Oxidase

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1. Gas chromatography analyses

GC/MS analysis for compounds **1**–**12** were performed on a GC Hewlett Packard 6890 Series II and MS Hewlett Packard 5973 chromatograph (Agilent Technologies) equipped with HP-5 cross-linked methyl siloxane column (30 m × 0.32 mm × 0.25 μm, 1.0 bar N_2). The injector temperature was 225 °C and the FID temperature was 250 °C. The temperature program performed for all the substrates was: 50ºC/ 5 min/ 10ºC/min/ 200ºC/ 2 min. Conversions were determined by GC/MS by performing a calibration of the substrates and the products at different concentrations, in order to obtain the response of each compound to the detector and then make the calculations with these values

Table S1. Retention times at GC/MS analyses in the biooxidations of alcohols **1-12a**.

Compound	t_R **a** (min)	t_R **b** (min)
1	9.9	8.1
2	14.6	12.9
3	20.6	19.5
4	17.2	14.7
5	13.4	11.5
6	17.2	15.1
7	8.9	8.2
8	18.1	16.4
9	12.3	10.7
10	10.5	9.3
11	11.7	10.2
12	13.2	13.9

2. HPLC analyses

The optical purity of (*R*)-1-indanol (**12a**) was determined using a Thermo-Fischer UltiMate chromatograph equipped with a Thermo UltiMate detector. This compound was analysed employing a Chiralcel OD column (Daicel, 0.46 cm x 25 cm), 35ºC, *n*-hexane/*i*-propanol 98:2, 1.0 mL/min: t_R (*S*)= 15.2 min; t_R (*R*)= 17.5 min.

The products formed by HMFO using **13a** as a substrate were analysed using an HPLC Jasco MD-2010 Plus , equipped with a Zorbax Eclipse XDB-C8 column, 5 μm (Agilent). The mobile phase used was 12 mM phosphate buffer pH 7.0 (A) and

acetonitrile (B) at a flow rate of 1.2 mL/min. After 1 min 100% A, B was increased to 5 % in 3.5 min and then to 40% in 2.5 min. After 0.5 min at 40% B, the eluent returned to 100% A in 0.5 min and this was maintained for 2 minutes. Detection was done at 268 nm. The retention times of **13a**, **13b** and **13c** were 6.4, 2.1 and 1.2 minutes respectively.

3. General procedure for the HMF oxidation catalysed by HMFO in presence of DES.

HMF (**13a**, 5 mM) were dissolved in the corresponding mixtures 50 mM Tris/HCl buffer pH 8.0/DES at different concentrations (1.0 mL), containing HMFO (2.0 μM). Reactions were shaken at 220 rpm and 30ºC for 24 hours. The reactions were stopped by incubation at 95ºC for 10 min to inactivate the enzyme. Subsequently the reactions were centrifuged at 13.000 rpm for 10 min to remove the precipitated enzyme before HPLC analysis.

Table S2. Percentage of products formed during the oxidation of HMF catalysed by HMFO in Tris/HCl 50 mM pH 8.0 containing Glu:Fru:H_2O (1:1:6).[1] Average values of two experiments.

% Glu:Fru:H_2O	% HMF (**13a**)[2]	% FFA (**13b**)[2]	% FDCA (**13c**)[2]
None	≤3.0	84.4	15.6
30	≤3.0	69.2	30.8
60	≤3.0	75.0	25.0
90	13.3	78.2	5.9

[1] Average values for two experiments. [2] Determined by HPLC.