Electronic Supplementary Material (ESI) for Green Chemistry

Electronic Supplemental Information
Enantioselective reduction of sparingly water-soluble ketones: Continuous process and recycle of the aqueous buffer system

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The supplemental information consists mainly of the kinetic constants used in the model and figures illustrating activity and stability.

Abbreviation	Explanation
API	active pharmaceutical ingredient
CBS	names of Corey, Bakshi and Shibata
Da	Dalton, equivalent to 1 u
DSP	down stream processing
ee	enantiomeric excess
EMR	enzyme membrane reactor
GC	gas chromatograph(y)
GDL	gluconic-acid-δ-lactone
ID	inner diameter
IL	ionic liquid
MSDS	material safety data sheet
MSTFA	*N*-methyl-*N*-trimethylsilyl-trifluoracetamid
SPE	solid phase extraction
STY	space time yield
t	time
TON	turnover number ($mol_{product}/mol_{catalyst}$)
X	conversion

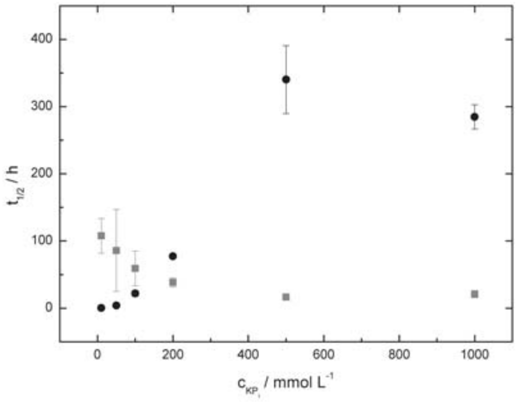

Figure 1: Stability of *Lb*ADH (grey) and GDH (black) in presence of different KP_i-concentrations; pH = 7.0; T = 25 °C ; 300 rpm; c_{MgCl_2} = 1 mmol L^{-1}

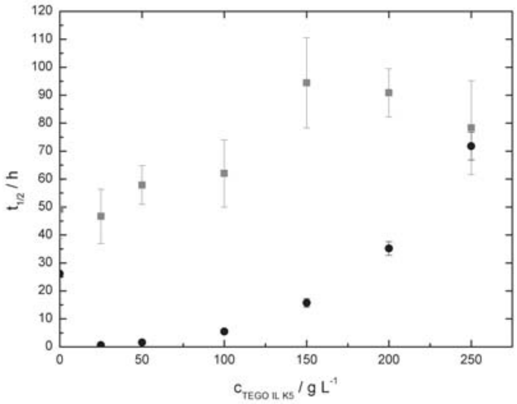

Figure 2: Stability of *Lb*ADH (grey) and GDH (black) in presence of different TEGO IL K5-concentrations; pH = 7.0; T = 25 °C ; 300 rpm; c_{KP_i} = 100 mmol L^{-1} ; c_{MgCl_2} = 1 mmol L^{-1}

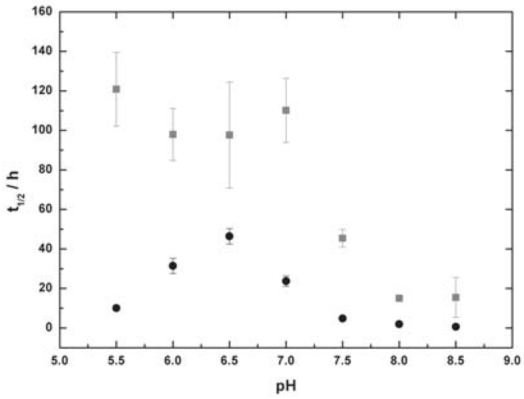

Figure 3: Stability of *Lb*ADH (grey) and GDH (black) at different pH; T = 25 °C ; 300 rpm; c_{KP_i} = 100 mmol L^{-1} ; c_{MgCl_2} = 1 mmol L^{-1}

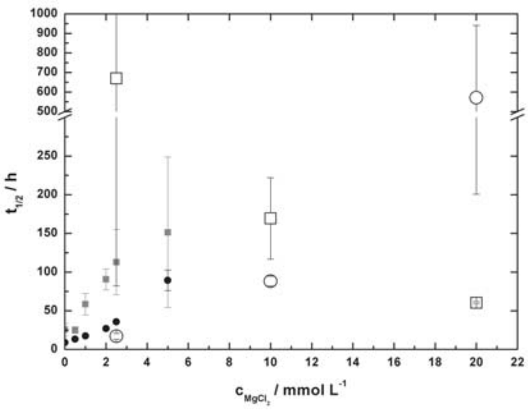

Figure 4: Stability of *Lb*ADH (squares) and GDH (circles) in presence of different $MgCl_2$-concentrations in KP_i-buffer (full symbols) and ADA-buffer (open symbols); pH = 7.0; T = 25 °*C* ; 300 rpm; c_{KP_i} = 100 mmol L^{-1}

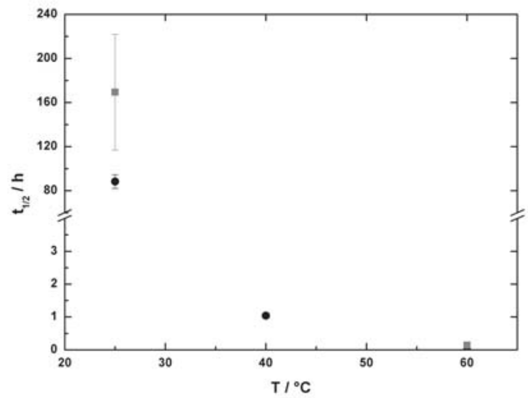

Figure 5: Stability of *Lb*ADH (squares) and GDH (circles) as a function of temperature (ADA-buffer=100 mmol L^{-1}; pH=7)

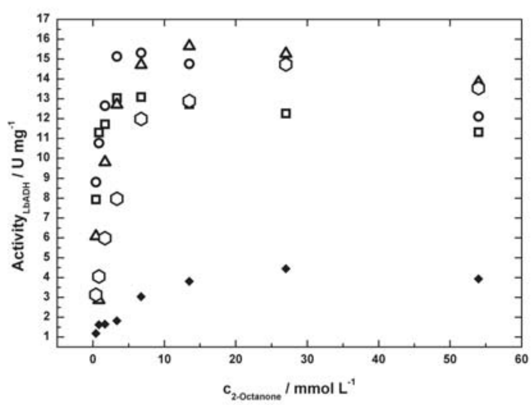

Figure 6: Activity of *Lb*ADH as a function of temperature and substrate-concentration; squares: 23 °*C*, circles: 27 °*C*, triangles:33 °*C*, hexagons: 38 °*C*, diamonds:44 °*C*; c_{ADA} = 100 mmol L^{-1} ; c_{MgCl_2} = 10 mmol L^{-1}; pH=7.0; c_{NADPH}= 0.05 mmol L^{-1}

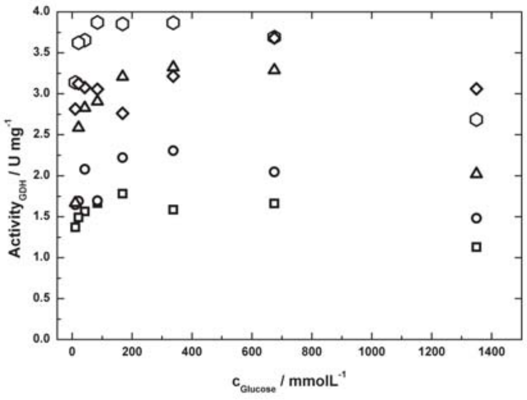

Figure 7: Activity of GDH as a function of temperature and substrate-concentration; squares: 22 °C, circles: 29 °C, triangles:33 °C, hexagones: 38 °C, diamonds:43 °C; c_{ADA} = 100 mmol L^{-1} ; c_{MgCl_2} = 10 mmol L^{-1}; pH=7.0; c_{NADPH}= 0.05 mmol L^{-1}

Table 1: Kinetic constants for GDH measured with fluorescence spectrometry at 25 °C . c_{GDH} = 5 mg L^{-1}; $c_{ADA\text{-}buffer}$= 100 mmol L^{-1}; c_{MgCl_2} = 10 mmol L^{-1}; $c_{TEGO\ IL\ K5}$ = 0,100,200 g L^{-1}; pH=7.0

cIL / gL^{-1}-1	Parameter	Unit	Value	
0	Vmax	U mg^{-1}	5.4	± 0.24
100	Vmax	U mg^{-1}	5.3	± 0.32
200	Vmax	U mg^{-1}	3.1	± 0.15
0	KM1	mmol L^{-1}	6.756	±1.274
100	KM1	mmol L^{-1}	1.931	±0.336
200	KM1	mmol L^{-1}	1.313	±0.312
0	KM2	mmol L^{-1}	0.027	±0.002
100	KM2	mmol L^{-1}	0.026	±0.004
200	KM2	mmol L^{-1}	0.017	±0.003
0	KP1	mmol L^{-1}	≥ 1E+4	
100	KP1	mmol L^{-1}	≥ 1E+4	
200	KP1	mmol L^{-1}	≥ 1E+4	
0	KP2	mmol L^{-1}	0.036	±0.004
100	KP2	mmol L^{-1}	0.027	±0.006
200	KP2	mmol L^{-1}	0.046	±0.011
0	KS1	mmol L^{-1}	4210	± 1068
100	KS1	mmol L^{-1}	8366	± 3860
200	KS1	mmol L^{-1}	9128	± 4115

Table 2: Kinetic constants for *Lb*ADH measured with fluorescence spectrometry at 25 °*C* . c_{LbADH} = 1.25 mg L^{-1} $c_{ADA\text{-}buffer}$ = 100 mmol L^{-1}; c_{MgCl_2} = 10 mmol L^{-1}; $c_{TEGO\ IL\ K5}$ = 0,100,200 g L^{-1}; pH=7.0

cIL/g L^{-1}	Parameter	Unit	2ON	2OL	2NN	2NL	2DN	2DL	3ON	3OL
0	Vmax	U mg^{-1}	13.7 ± 0.77	7.8 ± 0.17	–	–	–	–	–	–
100	Vmax	U mg^{-1}	17.3 ± 1.46	9.8 ± 1.28	17.7 ± 0.81	8.5 ± 0.73	17.3 ± 0.58	10.6 ± 0.63	12.7 ± 0.94	4.0 ± 0.85
200	Vmax	U mg^{-1}	13.2 ± 1.07	8.0 ± 0.31	9.5 ± 0.68	6.1 ± 0.58	9.0 ± 0.94	6.4 ± 0.65	5.1 ± 0.17	2.3 ± 1.18
0	KM1	mmol L^{-1}	0.054 ±0.008	0.115 ±0.010	–	–	–	–	–	–
100	KM1	mmol L^{-1}	0.209 ±0.045	0.847 ±0.222	0.534±0.061	1.438 ± 0.261	1.137 ±0.082	3.937 ±0.543	2.846 ±0.491	3.154 ± 1.145
200	KM1	mmol L^{-1}	0.628 ±0.139	1.292 ±0.188	0.780±0.192	4.253 ± 0.875	1.797 ±0.499	8.778 ±2.212	3.217 ±0.523	17.083 ±13.506
0	KM2	mmol L^{-1}	0.027 ±0.003	0.013 ±0.001	–	–	–	–	–	–
100	KM2	mmol L^{-1}	0.036 ±0.007	0.034 ±0.007	0.021±0.002	0.014 ± 0.003	0.032 ±0.003	0.027 ±0.002	0.021 ±0.005	0.015 ± 0.002
200	KM2	mmol L^{-1}	0.035 ±0.006	0.018 ±0.003	0.015±0.003	0.014 ± 0.002	0.006 ±0.001	0.006 ±0.001	0.005 ±0.001	0.004 ± 0.001
0	KP1	mmol L^{-1}	0.030 ±0.005	0.055 ±0.006	–	–	–	–	–	–
100	KP1	mmol L^{-1}	0.186 ±0.060	0.501 ±0.119	0.485±0.073	0.361 ± 0.057	2.506 ±0.462	0.971 ±0.123	1.905 ±0.463	6.129 ± 1.701
200	KP1	mmol L^{-1}	0.684 ±0.201	0.119 ±0.022	1.003±0.362	0.576 ± 0.096	2.684 ±1.067	1.596 ±0.359	3.337 ±1.158	4.642 ± 1.710
0	KP2	mmol L^{-1}	0.110 ±0.017	0.008 ±0.001	–	–	–	–		–
100	KP2	mmol L^{-1}	0.185 ±0.040	0.013 ±0.003	0.045±0.005	0.014 ± 0.003	0.116 ±0.010	0.011 ±0.001	0.102 ±0.102	0.024 ± 0.005
200	KP2	mmol L^{-1}	0.135 ±0.024	0.027 ±0.005	0.043±0.010	0.007 ± 0.001	0.008 ±0.002	0.005 ±0.001	0.035 ±0.012	0.009 ± 0.003
0	KIuGluc	mmol L^{-1}	2985 ± 739	5992 ± 2268	–	–	–	–	–	–
100	KIuGluc	mmol L^{-1}	≥ 1E+4	4019 ± 2343	4522 ± 1181	5.E+03 ±3.E+03	≥1E+4	≥1E+4	≥1E+ 4	≥1E+4
200	KIuGluc	mmol L^{-1}	7517 ± 5107	≥ 1E+4	2752 ± 1010	≥ 1E+4 ´	≥1E+4	≥1E+4	≥1E+ 4	≥1E+4
0	KIuGDL	mmol L^{-1}	3818 ± 1563	≥ 1E+4	–	–	–	–	–	–
100	KIuGDL	mmol L^{-1}	1631 ± 430	391 ± 82	726 ± 78	667 ± 173	4321 ± 1413	≥1E+4	≥1E+ 4	≥1E+4
200	KIuGDL	mmol L^{-1}	1437 ± 378	1566 ± 477	1826 ± 668	≥ 1E+4	1179 ± 584	2438 ± 1853	3255 ± 2484	≥1E+4
0	KS1	mmol L^{-1}	60 ± 47	≥ 1E+4	–	–	–	–	–	–
100	KS1	mmol L^{-1}	192 ± 55	192 ± 314	178 ± 38	119 ± 48	≥1E+4	≥1E+4	≥1E+ 4	19 ± 11
200	KS1	mmol L^{-1}	287 ± 81	≥ 1E+4	277 ± 94	153 ± 79	276 ± 285	≥1E+4	≥1E+ 4	76 ± 119