Electronic supporting information

Heterogeneous biocatalytic reduction of 5-(hydroxy)methyl furfural using two co-immobilised alcohol dehydrogenases

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1. LIST OF ABBREVIATIONS

- HMF: 5-hydroxymethylfurfural
- BHMF: 2,5-bis(hydroxymethyl) furan
- ADH: Alcohol dehydrogenase
- GDH: Glucose dehydrogenase
- FDH: Formate dehydrogenase
- PDH: Phosphite dehydrogenase
- EcADHZ3: ADH from *Escherichia coli*
- BsGDH-2M: Thermostable glucose dehydrogenase from *Bacillus subtilis* developed by Figueroa et al. (BsGDH-2M)[1]
- CbFDH: FDH from *Candida boidinii*
- PsPDH: PDH from *Pseudomonas stutzeri*
- Pu-G: Purolite™ ECR8204F functionalized with glyoxyl groups
- Pu-E/Co^{2+}: Purolite™ ECR8204F functionalized with epoxy and cobalt (II)-chelates
- TTN: Total turnover number

2. SUPPLEMENTARY TABLES

Supporting Table 1. Specific activity of different NAD^+-dependent alcohol dehydrogenases. Reactions were performed at room temperature with 25 mM of the corresponding substrate (sodium formate, sodium phosphite and D-glucose) and 0.5 mM NAD^+ in 50 mM TRIS, pH 7.0.

Type	Organism	U·mg^{-1}
Formate dehydrogenase	*Candida biodinii*	0.7
Phosphite dehydrogenase	*Pseudomonas stutzeri*	0.5
Glucose dehydrogenase	*Bacillus subtilis*-2M*	77.5
	Bacillus subtilis-7M*	17.7
	Sulfolobus solfataricus	4.0

Supporting Table 2. Kinetic parameters of different dehydrogenases towards NAD^+. Reactions were performed at room temperature in 50 mM TRIS pH 7.0 and in presence of 0.5 mM NAD^+ as well as gradient of the corresponding substrate.

Enzyme	K_M (mM)	Vmax (U mg^{-1})
BsGDH-2M	1.40	110.1
CbFDH[2]	6.0	0.81
PsPDH[3]	0.06	0.2

Supporting Table 3. Enzyme immobilization carrier screening. Immobilization yield (Y) (%) representing the proportion of enzyme that is retained within the carrier respect to the one left in the supernatant. Recovered activity (RA) ($U \cdot g^{-1}$) describes the amount of units enzymatic activity recovered posterior to the immobilization and Effectiveness means the proportion of enzymatic activity recovered respect to the amount of total units offered to the carrier. Overnight (ON).

Entry	Carrier	EcADH			BsGDH-2M		
		Y	RA	Effectiveness	Y	RA	Effectiveness
1	EziG1™	100	0.0	0.00	100	0.3	0.10
2	YSZ	100	0.0	0.00	100	0.3	0.16
3	Agarose 4% BCL	58	0.6	0.03	100	0.4	0.18
4		53	0.0	0.04	100	1.3	0.58
5		62	0.1	0.02	100	0.3	0.14
6		100	0.1	0.09	52	0.0	0.00
7		91	0.2	0.07	50	0.1	0.04
8		57	0.2	0.00	28	0.1	0.03
9		0	0.0	0.00	0	0.1	0.04
10		0	0.0	0.00	0	0.0	0.01
11	Relizyme 112/S	78	0.4	0.08	99	1.3	0.28
12		100	0.4	0.13	99	0.9	0.33
13		98	0.3	0.06	99	0.1	0.03
14		97	0.2	0.10	100	0.0	0.00
15		95	0.2	0.09	100	0.0	0.00
16		95	0.2	0.10	100	0.0	0.00
17		99	0.2	0.23	100	0.0	0.0
18		98	0.7	0.47	100	0.1	0.05
19	Sunresin LX1000EP	100	0.0	0.00	100	0.0	0.00
20		100	0.0	0.00	100	0.0	0.00
21		100	0.0	0.00	100	0.0	0.00
22	Purolite™ ECR8204F	99	0.5	0.02	90	0.6	0.02
23		99	1.8	0.05	90	1.0	0.02
24		100	0.2	0.08	100	0.8	0.23
25		100	0.0	0.00	65	0.1	0.04
26		100	0.4	0.01	100	0.4	0.01
27		100	1.3	0.02	100	2.2	0.05

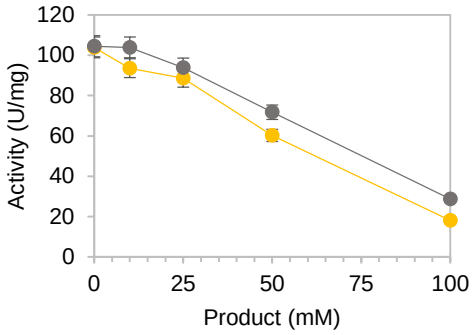
Supporting Table 4. Half-life of co-immobilized and individually immobilized biocatalysts all blocked with glutamic acid. Half-life times were calculated according to a first-order two-step inactivation mechanism[4].

Carrier		Enzyme	
		EcADHZ3	BsGDH-2M
		$t_{1/2}$ (min)	$t_{1/2}$ (min)
Soluble		21.4	6.1
ECo^{2+}	Individual	>45	8.2
	Combi-catalyst	>120	23
Glyoxyl	Individual	>120	>5
	Combi-catalyst	>120	>5

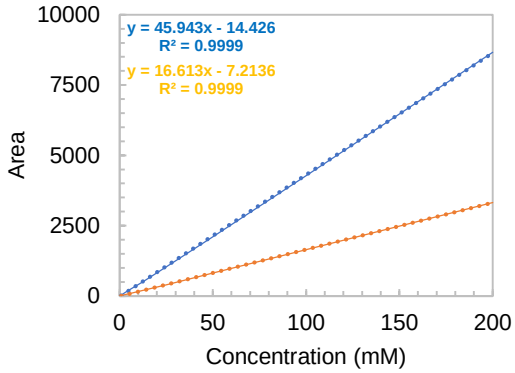
Supporting Table 5. Theoretical BHMF production via co-immobilized systems of. HMF 10 mM, NADH 1 mM, glucose 25 mM and TRIS pH 7.0, 50 mM.

Carrier	Block	BHMF ($g \cdot L^{-1} \cdot h^{-1}$)
Amino	-	4.5
A-glut.	Glycine	3.1
Eco^{2+}	Glycine	4.9
	Glutamate	5.2
Glyoxyl	Glycine	4.5
	Glutamate	5.2

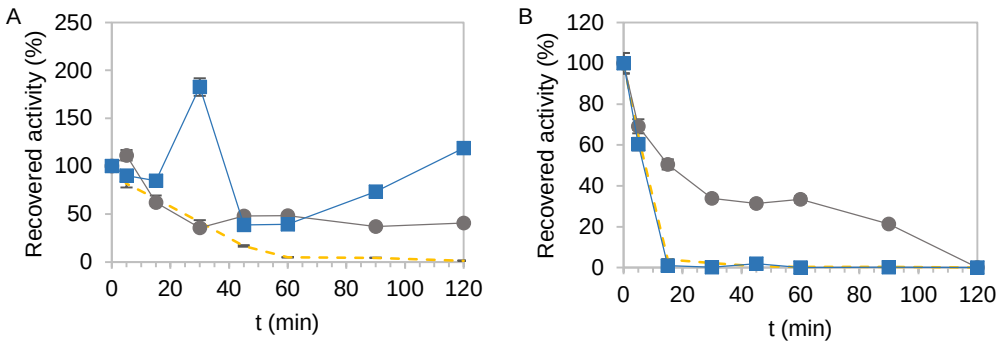
3. SUPPLEMENTARY FIGURES



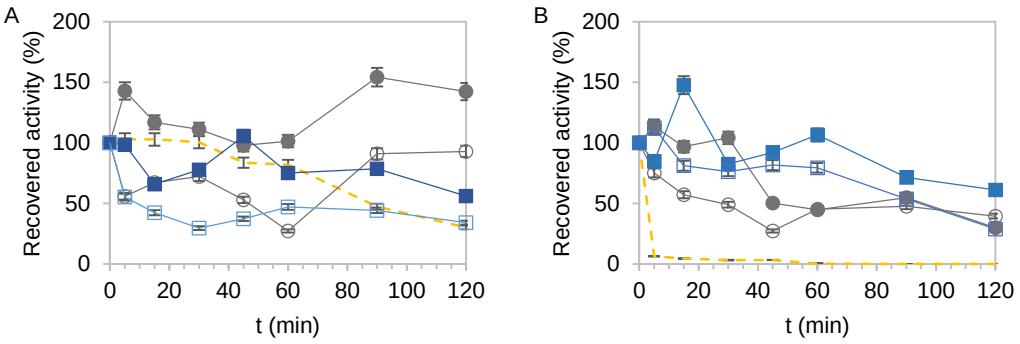
Supporting Figure 1. Recovered activity of BsGDH-2M in presence of increasing concentrations of HMF (orange) and BHMF (grey). Assay conditions, 0-100 mM HMF or BHMF were mixed with 25 mM glucose and 0.5 mM NAD+ in 50 mM Tris-HCl at pH 7 and 25 C. All reactions were triggered with BsGDH-2M (0.2 µg mL^{-1})



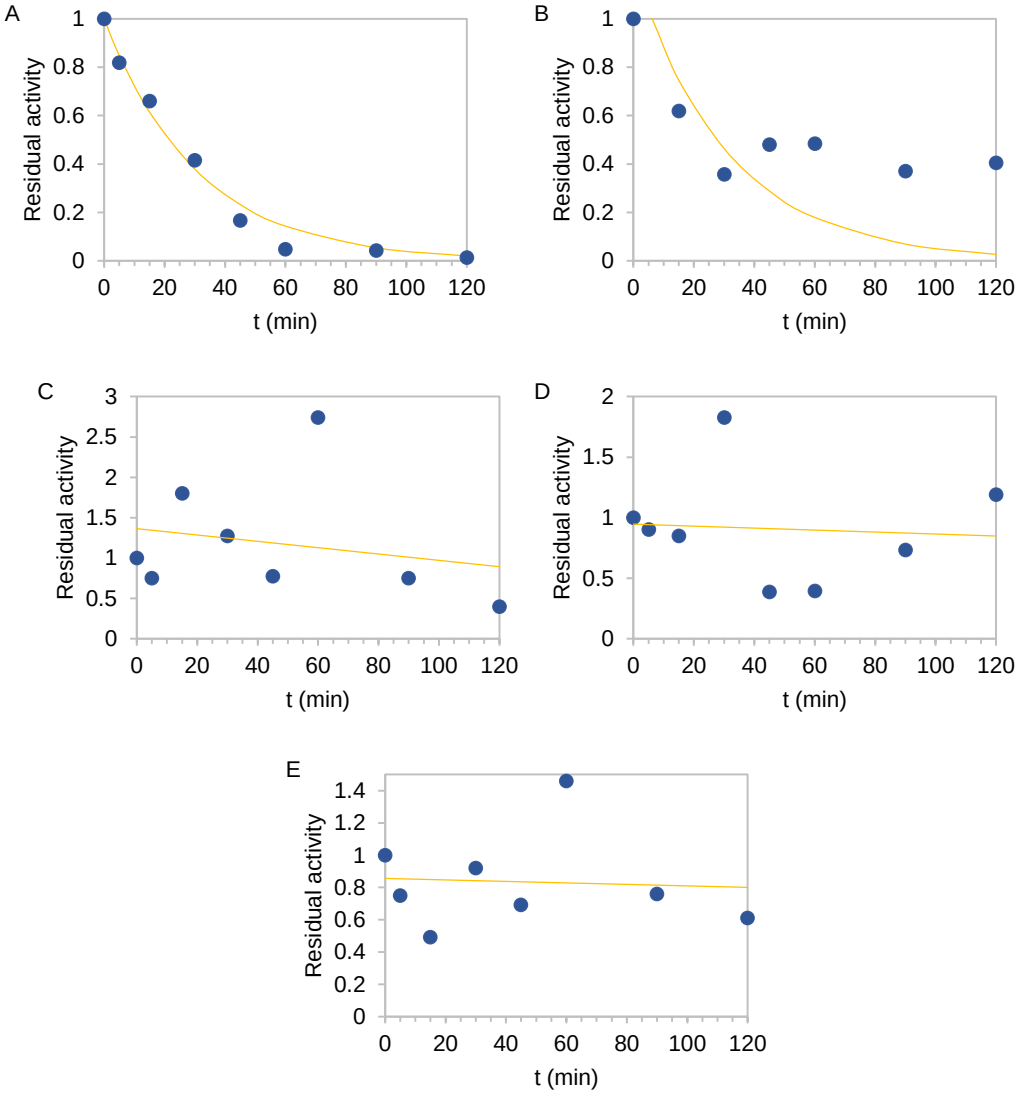
Supporting Figure 2. Calibration curve for substrate/HMF (blue) and product/BHMF (orange).



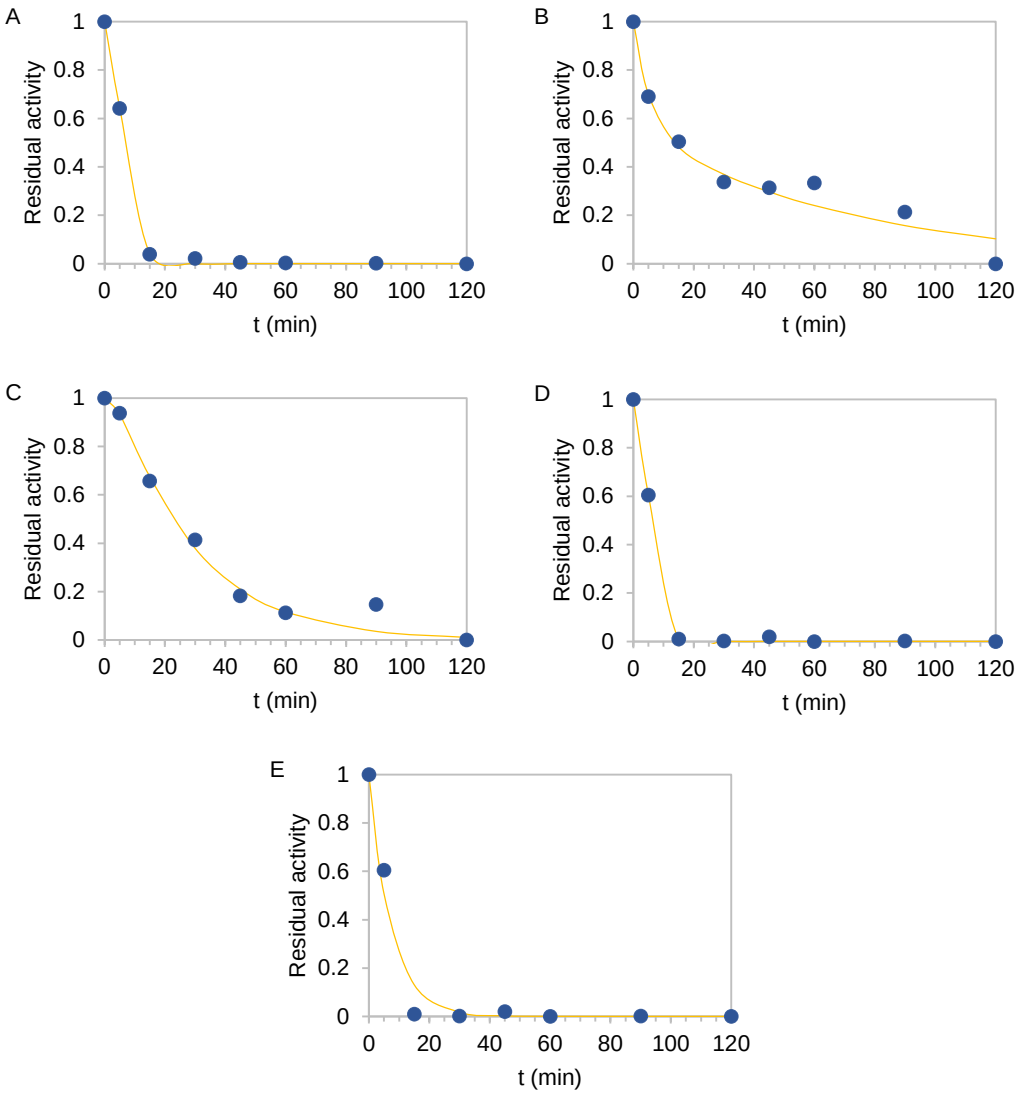
Supporting Figure 3. Recovered activity of EcADHZ3 at 70°C (A) and BsGDH-2M at 80°C (B) immobilized on Purolite™ EC8204F E-Co^{2+} (grey circles) and glyoxyl (blue squares) compared against the free counterpart (orange dashed). Empty symbols represent co-immobilized enzymes, while filled ones do individually immobilized enzymes.



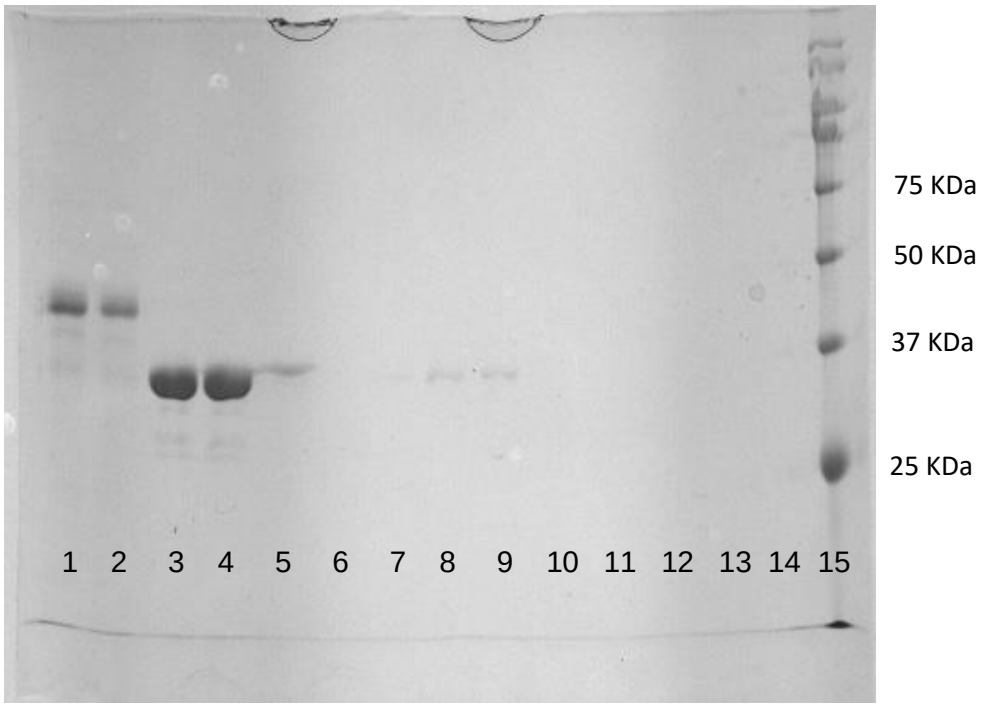
Supporting Figure 4. Recovered activity of EcADHZ3 at 65°C (A) and BsGDH-2M at 75°C (B) immobilized individually (full markers) and co-immobilized with each other (empty markers) over Purolite™ EC8204F E-Co^{2+} (grey circles) and glyoxyl (blue squares) compared against the free counterpart (orange dashed). TRIS pH 7.0, 50mM.



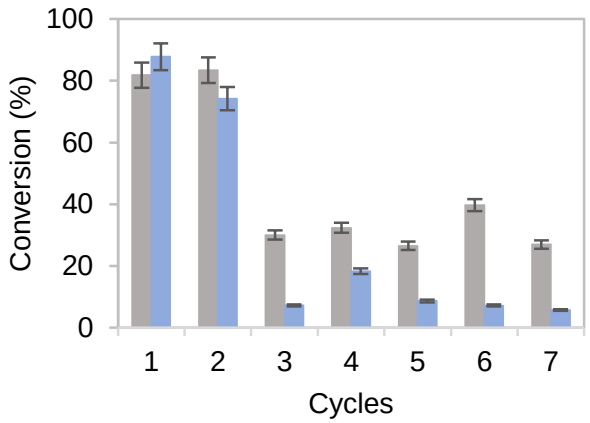
Supporting Figure 5. Residual activity of EcADHZ3 at 70°C in its soluble form (A), as well as, individually immobilized over Pu-E/Co^{2+} (B), or in the form of a co-immobilized system with BsGDH-2M (C); same for Pu-G (D), with its respective co-immobilized form (E). TRIS pH 7.0, 50 mM, 10 mM HMF, 1 mM NADH.



Supporting Figure 6. Residual activity of BsGDH-2M at 80°C in its soluble form (A), as well as, individually immobilized over Pu-E/Co^{2+} (B), or in the form of a co-immobilized system with EcADHZ3 (C); same for Pu-G (D), with its respective co-immobilized form (E). TRIS pH 7.0, 50 mM, 25 mM glucose, 1 mM NADH. All the heterogeneous biocatalysts were blocked with glutamic acid.



Supporting Figure 7. SDS. Lane 1 - 2: 0.5 mg/mL EcADHZ3; lane 3 - 4: 0.5 mg/mL BsGDH-2M; lane 5: blank; lane 6 - 7 co-immobilized enzymes on Pu-ECo^{2+} incubated 30´ at RT; lane 8 - 9: co-immobilized enzymes on Pu-G incubated 30´ at RT; lane 10 - 11: co-immobilized enzymes over Pu-ECo^{2+} incubated 30´ at 75°C; lane 12 - 13: co-immobilized enzymes on Pu-G incubated 30´ at 75°C; lane 14: blank; lane 15: marker Tris-Glycine 4~20% BlueStar Prestained Protein Marker.



Supporting Figure 8. Cycles of HMF conversion by co-immobilized systems of EcADHZ3 and BsGDH-2M over Pu-ECo^{2+} (grey) and Pu-G (blue). 40 mM HMF, 40 mM glucose, NADH 1 mM and 100 mM TRIS pH 7.0 at 25 C.

4. REFERENCES

1. E. Vázquez-Figueroa, J. Chaparro-Riggers and A. S. Bommarius, *ChemBioChem*, 2007, **8**, 2295-2301.
2. H. Slusarczyk, M. Pohl and M.-R. Kula, in *Prog. Biotechnol.*, Elsevier, 1998, vol. 15, pp. 331-336.
3. T. W. Johannes, R. D. Woodyer and H. Zhao, *Biotech. Bioeng.* 2007, **96**, 18-26.
4. J. M. Guisan, J. M. Bolivar, F. López-Gallego and J. Rocha-Martín, *Immobilization of Enzymes and Cells: Methods and Protocols*, Springer US, 2020.