Electronic Supplementary Material (ESI) for Green Chemistry.

Supplementary Information

Coupled Immobilized Bi-enzymatic Flow Reactor Employing Cofactor Regeneration of NAD^+ using a Thermophilic Aldehyde Dehydrogenase and Lactate Dehydrogenase

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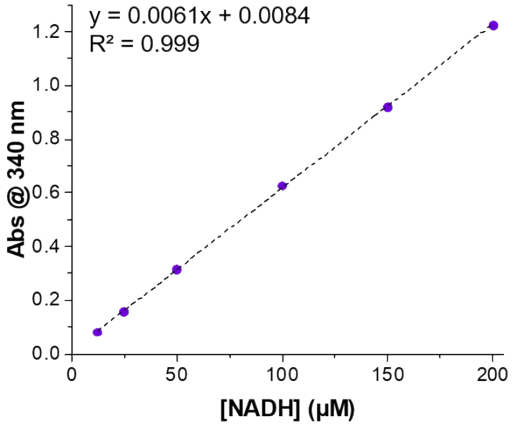

Figure S1: Standard curve of NADH in 10 mM potassium phosphate pH 8. Error bars are included (± standard deviation) obtained from triplicate measurements but are too small to be visible.

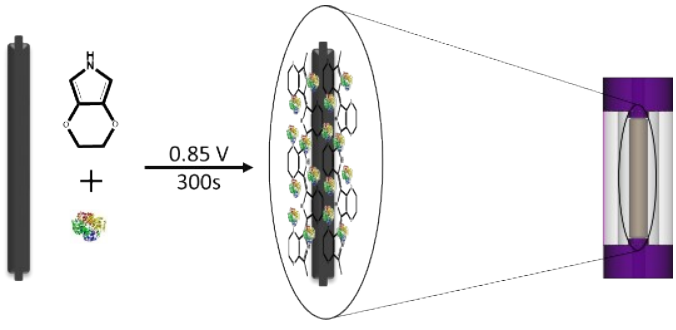

Figure S2: Schematic diagram of electrochemical enzyme encapsulation in PEDOP on GRE and the assembled reactor.

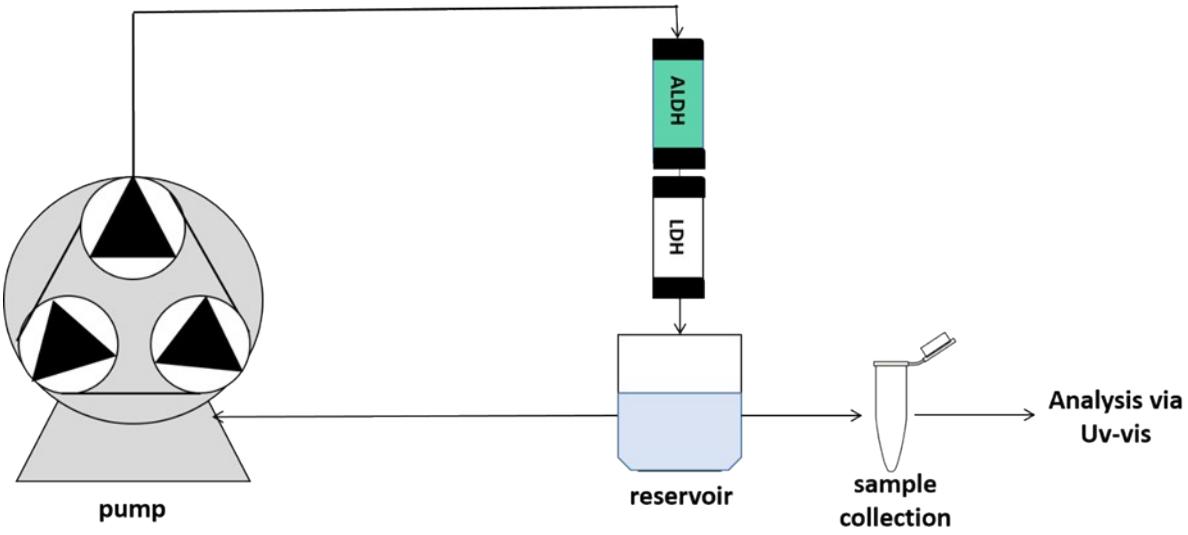

Figure S3: Schematic diagram of flow reactor.

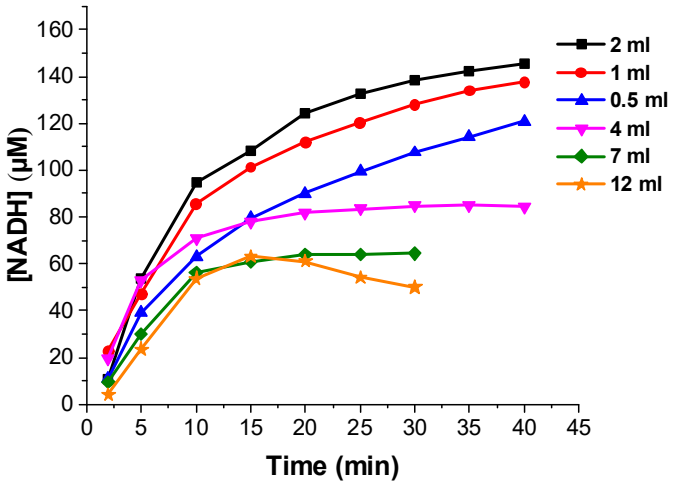

Figure S4: NADH production over time by $ALDH_{Tt}$-Ni-sepharose reactor at varying cell lysate loadings from 0.5-12 ml. Each condition was immobilized and subsequently run once for optimization purposes.

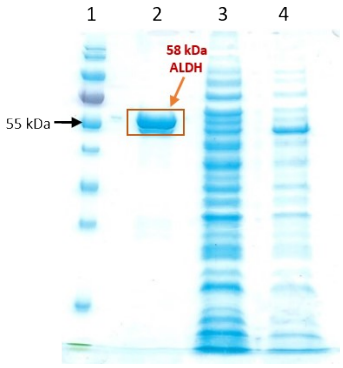

Figure S5: SDS-PAGE analysis of $ALDH_{Tt}$ on Ni^{2+} column from crude cell extract. Lane 1: Pageruler prestained protein ladder, lane 2: binding and elution of $ALDH_{Tt}$ using 200 mM imidazole, lane 3: crude cell lysate, lane 4: 10 mM imidazole wash of column.

Column Volume (ml)	Flow Rate (ml/min)	Residence Time (min)	Overall NADH Produced (µM)	% Conversion
1	0.5	2	130.7	54.7
	1	1	146.2	58.5
	2	0.5	157.6	63.0

Table S1: Flow rate, residence time, concentration of NADH produced and %conversion of NAD^+ for the $ALDH_{Tt}$-Ni-sepharose reactor.

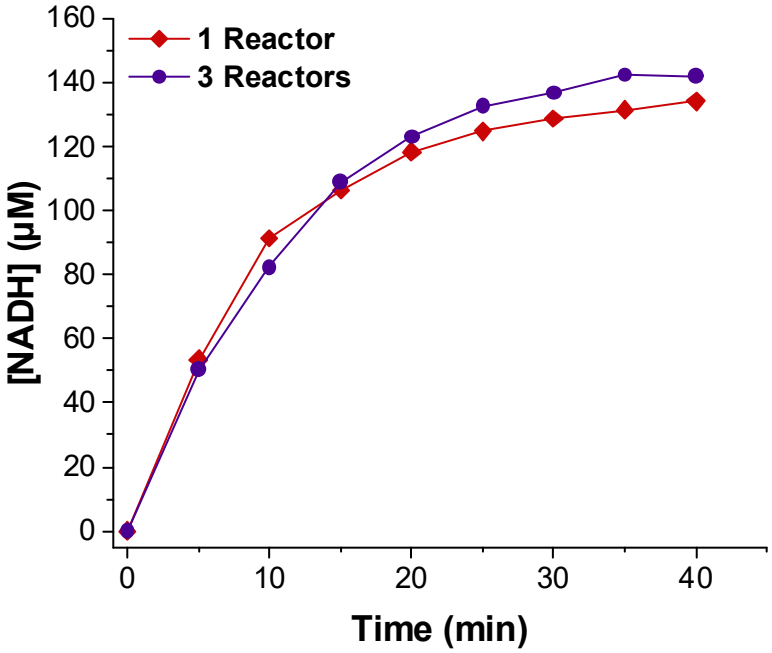

Figure S6: Concentration of NADH produced using a single and three $ALDH_{Tt}$-Ni-sepharose reactors combined in series. Each condition was immobilized and subsequently run one for optimization purposes.

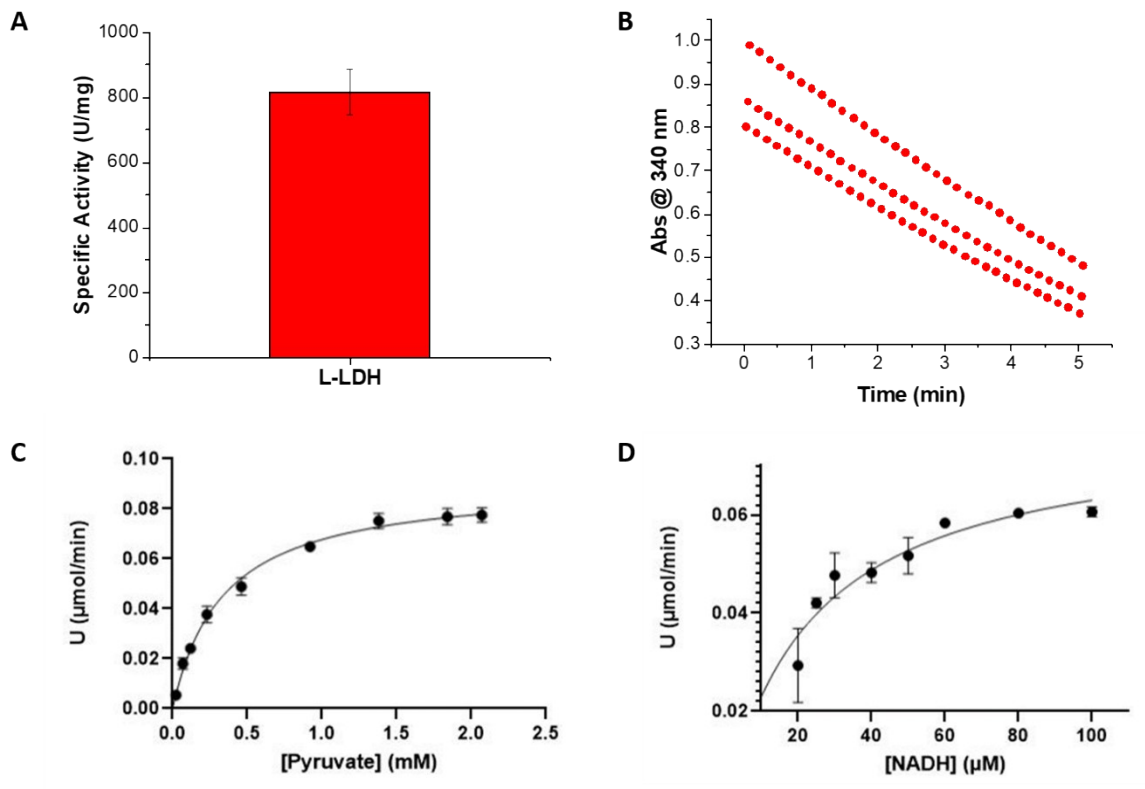

Figure S7: (A) specific activity of LDH, (B) Plot of A_{340nm} vs. time for the LDH conversion of pyruvate, (C) Michaelis-Menten plot for the LDH conversion of pyruvate, D) Michaelis-Menten plot for the LDH conversion of pyruvate with varying NADH. Error bars are ± standard deviation obtained from triplicate measurements. Some error bars are too small to be visible.

	K_M	V_{max} (µmol/min)	k_{cat} (s^{-1})
Pyruvate	0.34 mM	0.09	3750
NADH	24 µM	0.08	3333

Table S2: Michaelis-Menten parameters for LDH

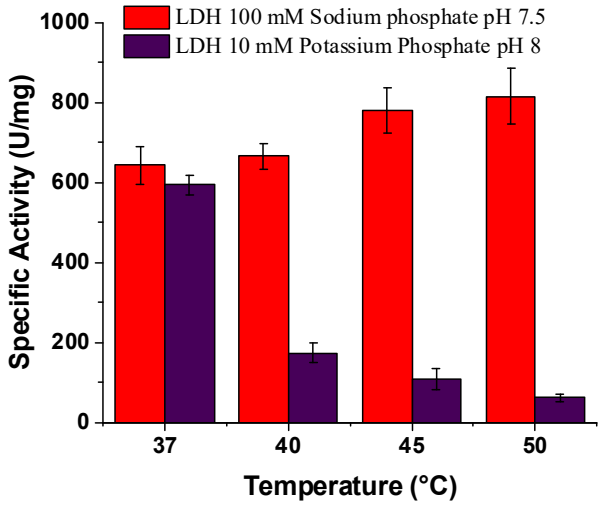

Figure S8: Specific activity of LDH in 100 mM sodium phosphate pH 7.5 and 10 mM potassium phosphate pH 8, as a function of temperature. Error bars are ± standard deviation obtained from triplicate measurements.

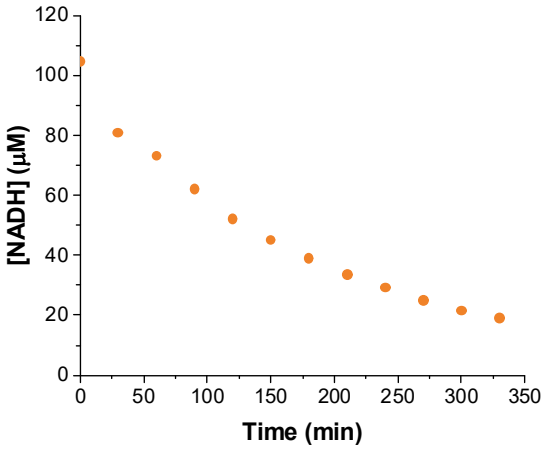

Figure S9: Time course of NADH consumption at PEDOP-LDH-GRE reactor in a batch system (2 ml volume, 0.12 mM NADH, 2.3 mM pyruvate, phosphate buffer, 10 mM, pH 8). This reactor was run once as a proof of concept prior to flow reactor set up.

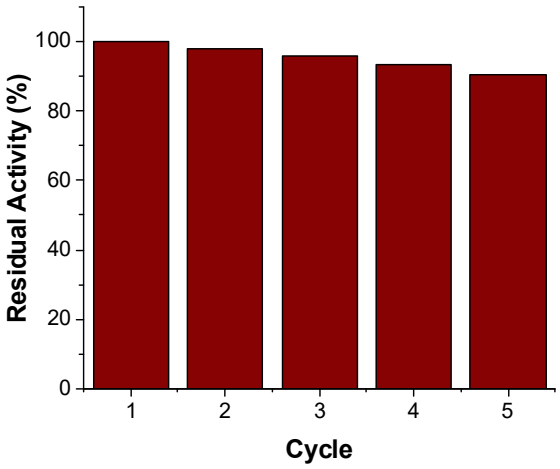

Figure S10: Residual activity obtained using a PEDOP-LDH-GRE flow reactor as a function of reaction cycle at a flow rate of 0.36 ml/min.

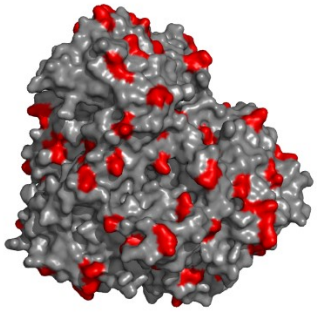

Figure S11: Surface representation of LDH using Pymol showing the surface accessible lysine residues (highlighted in red).

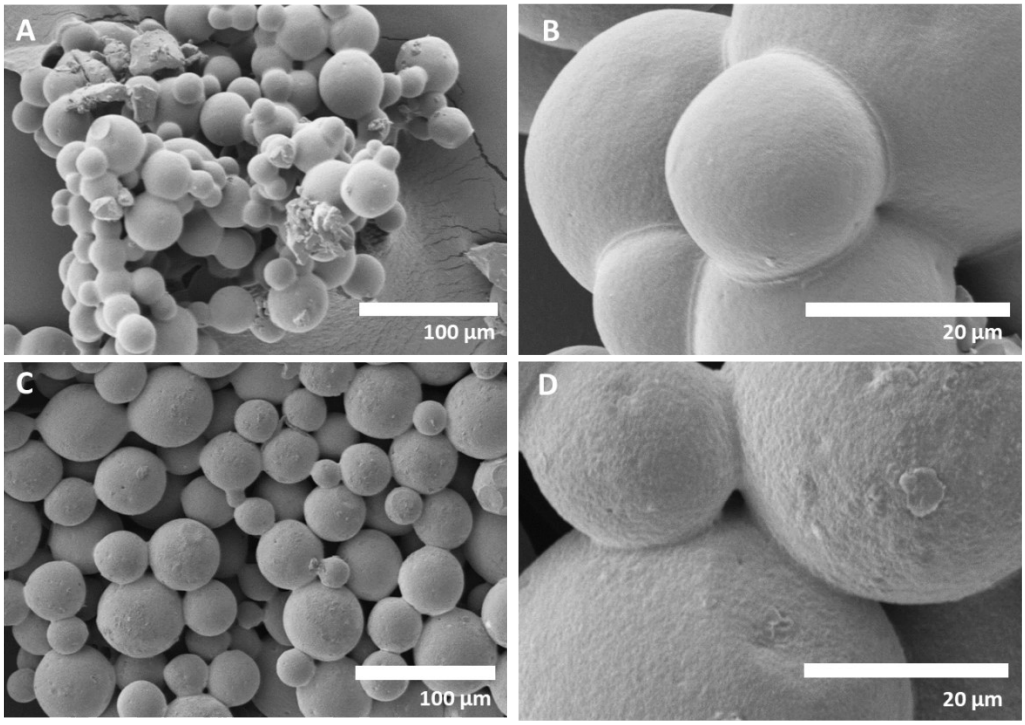

Figure S12: SEM of agarose (A) and B) and of LDH@Agarose (C) and (D).

Quantity Immobilized (mg)	Immobilization Yield (%)	Actual Quantity Immobilized (mg)	Enzyme Loading (mg/g)	Specific Activity (U/mg)
1	100	1	0.99	3.6 ± 0.27
2	77.5	1.55	1.62	2.62 ± 0.15

Table S3: Optimisation of immobilization of LDH@Agarose

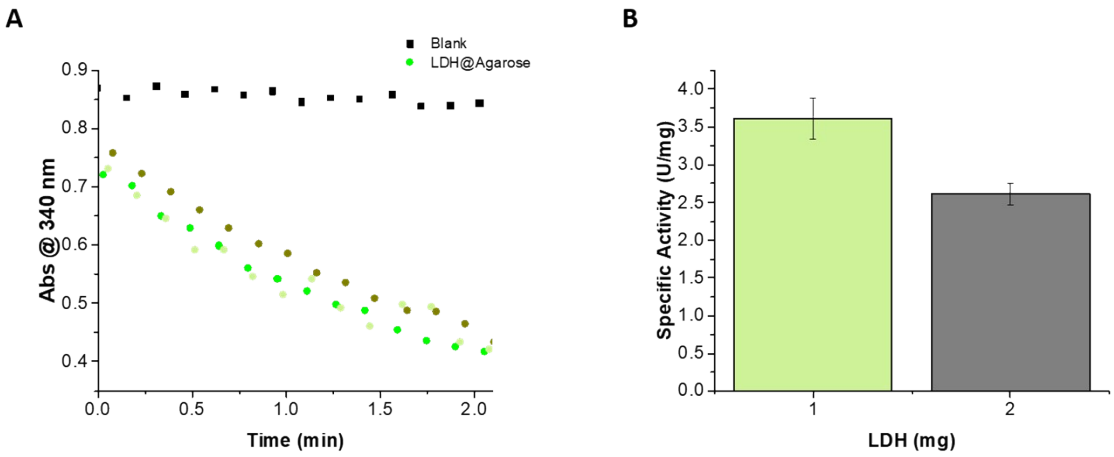

Figure S13: (A) Plot A_{340} obtained as a function of time with LDH@Agarose in batch mode performed in triplicate and (B) specific activity of LDH@Agarose obtained as a function of the amount of immobilized LDH. Error bars are ± standard deviation obtained from triplicate measurements.

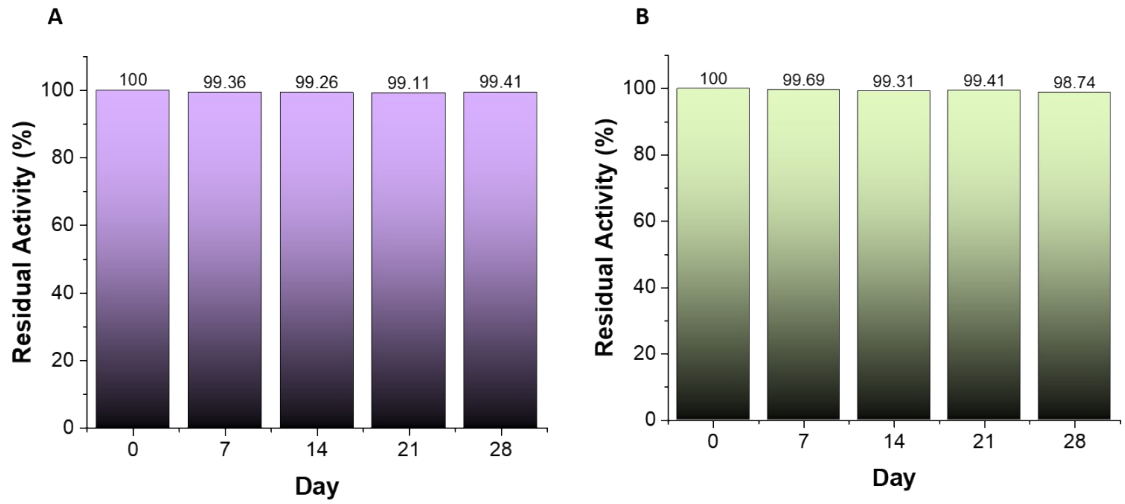

Figure S14: Residual activity of an LDH@Agarose flow reactor as a function of storage time at (A) room temperature and (B) 37°C. One reactor was immobilized and stored for each condition.

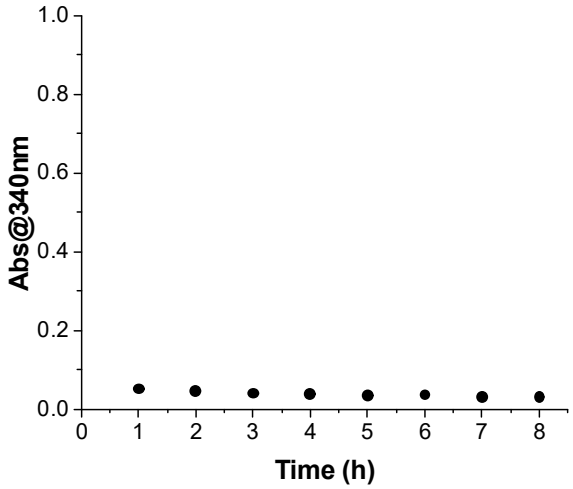

Figure S15: Plot of A_{340nm} as a function of time for an $ALDH_{Tt}$-LDH reactor operating for 8 h (1.69 mM benzaldehyde, 250 µM NAD^+, 2.3 mM sodium pyruvate in 10 mM potassium phosphate pH 8).