

Article

# A redox-neutral, two-enzyme cascade for the production of malate and gluconate from pyruvate and glucose

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## Supplementary Materials:

**Table S1.** Atmospheric condition independent variables and their values. O<sub>2</sub> and CO<sub>2</sub> are in % and temperature is in °C.

Coded Levels	-	0	+
O <sub>2</sub> , (x <sub>1</sub> )	2.5	5	7.5
CO <sub>2</sub> , (x <sub>2</sub> )	5	10	15
Temperature, (x <sub>3</sub> )	45	55	65

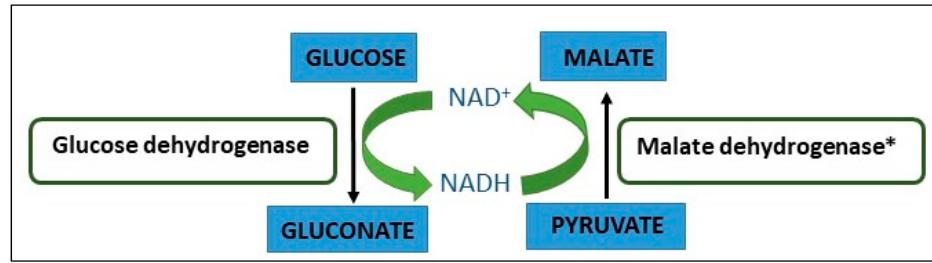
**Table S2.** Box-Behnken design for 3 factors with 3 center points. O<sub>2</sub> and CO<sub>2</sub> are in % and temperature is in °C.

Experiment number	O <sub>2</sub> (x <sub>1</sub> )	CO <sub>2</sub> (x <sub>2</sub> )	Temperature (x <sub>3</sub> )
1	-	-	0
2	+	-	0
3	-	+	0
4	+	+	0
5	-	0	-
6	+	0	-
7	-	0	+
8	+	0	+
9	0	-	-
10	0	+	-
11	0	-	+
12	0	+	+
13	0	0	0
14	0	0	0
15	0	0	0

**Table S3.** Change in kinetic parameters of GDH in the presence of different pyruvate concentrations.

Kinetic parameters.	Pyruvate concentration (mM)			
	0	10	15	20
<b>K<sub>M</sub> (mM)</b>	0.42	0.41	0.95	2.55
<b>V<sub>max</sub> (mM/min)</b>	0.04	0.03	0.02	0.03

**Figure S1.** Schematic representation of redox balanced cascade with cofactor recycling.



**Figure S2.** Effect of atmospheric conditions and the reaction temperature on the initial rate of MDH\*.

