Electronic Supplementary Material (ESI) for RSC Sustainability.

Supporting information

Multi-biocatalytic system for the effective fumarate synthesis from pyruvate and gaseous CO_2

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1. Experimental setup for the isobaric system

An overview of the experimental set-up of an isobaric system for direct use of CO_2 gas is shown in Figure S1.[1] In this system, the pressure in the reaction system is kept constant at 1.01325 x 10^5 Pa by a constant supply of CO_2 gas in the balloon.

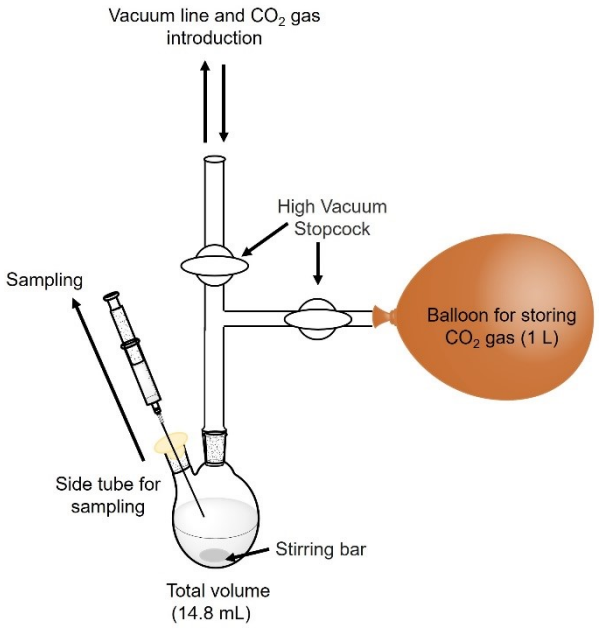

Figure S1. The experimental setup of the isobaric system for direct captured CO_2 gas and utilization to the reaction.

2. Determination for L-malate concentration using ion chromatography

The concentration of L-malate was determined using ion chromatography system (Metrohm, Eco IC; electrical conductivity detector) with an ion exclusion column (Metrosep Organic Acids 250/7.8 Metrohm; column size: 7.8 x 250 mm; composed of 9 μm polystyrene-divinylbenzene copolymer with sulfonic acid groups). The 1.0 mM perchloric acid and 50 mM lithium chloride in aqueous solution were used as an eluent and a regenerant, respectively. Flow rate of eluent solution was adjusted to be 0.5 mL min^{-1}. The retention time for L-malate was detected at 9.93-10.3 min. The electrical conductivity changes in the various L-malate concentrations (0 – 5000 μM) during the ion chromatograph analysis were shown in Figure S2. Inset of Figure S2 shows the relationship between the L-malate concentration and the detection peak area using ion chromatograph.

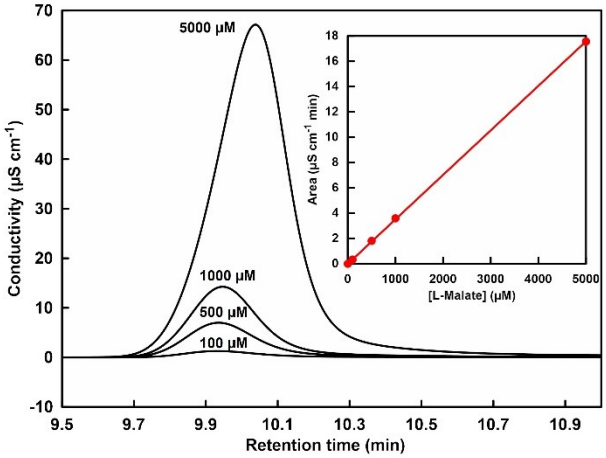

Figure S2. Chromatogram of sodium L-malate (0 - 5000 μM) in 50 mM-HEPES buffer (pH 7.0). Inset: Relationship between the L-malate concentration and the detection peak area.

As shown in the inset of Figure S2, the L-malate concentration and the detected peak area showed a good linear relationship (correlation coefficient: r^2=1.0) as following equation (S2).

$$\text{Peak area} = 0.0035 \times [\text{L-malate}](\mu M) \quad \text{(S2)}$$

3. L-Malate synthesis from the pyruvate and direct captured CO_2 with MDH

Figure S3 shows a chart of an ion chromatogram sampled from the reaction solution of sodium pyruvate (5.0 mM), manganese chloride (5.0 mM), ATP (5.0 mM), NADH (5.0 mM), acetyl-CoA (1.0 mM), PC (1.0 U) and MDH (10 U) in 5.0 mL of 500 mM HEPES buffer (pH 7.2) with CO_2 gas during the incubation.

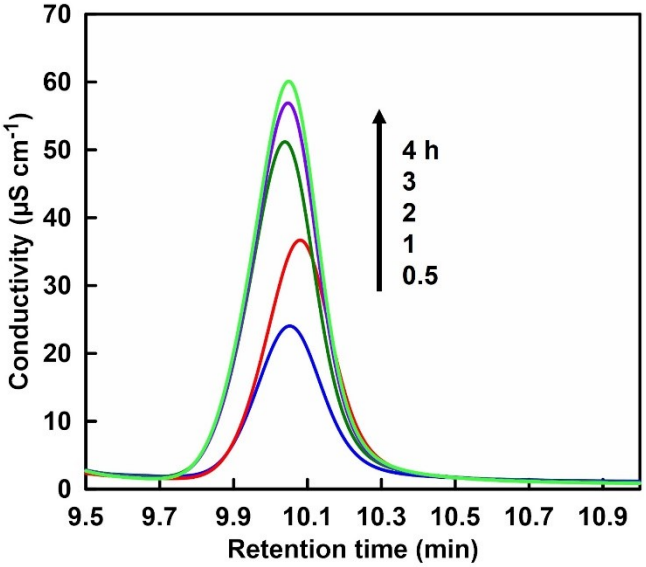

Figure S3. A chart of an ion chromatogram for L-malate concentration in the solution of sodium pyruvate (5.0 mM), manganese chloride (5.0 mM), ATP (5.0 mM), NADH (5.0 mM), acetyl-CoA (1.0 mM), PC (1.0 U) and MDH (10 U) in 5.0 mL of 500 mM HEPES buffer (pH 7.2). The gas phase: CO_2.

4. L-Malate synthesis from the pyruvate and direct captured CO_2 with ME

Figure S4 shows a chart of an ion chromatogram sampled from the reaction solution of sodium pyruvate (2.0 mM), manganese chloride (5.0 mM), NADH (2.0 mM), sodium bicarbonate (50 mM) and ME (0.7 U) in 5.0 mL of 500 mM HEPES buffer (pH 7.2) after 30 min incubation.

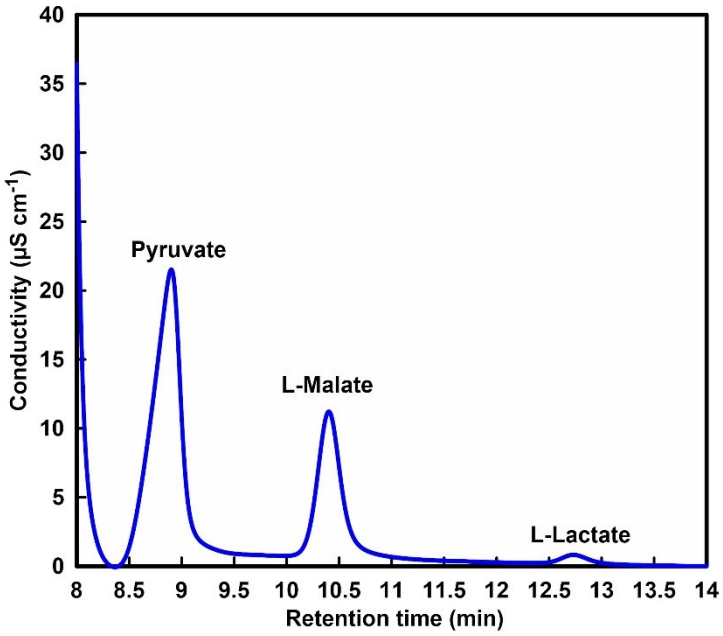

Figure S4. A chart of an ion chromatogram for L-malate concentration in the solution of sodium pyruvate (2.0 mM), manganese chloride (5.0 mM), NADH (2.0 mM), sodium bicarbonate (50 mM) and ME (0.7 U) in 5.0 mL of 500 mM HEPES buffer (pH 7.2).

The retention time for L-lactate was detected at 12.5-13.0 min using ion chromatograph system. As shown in Figure S4, the signal peak around 12.5-13.0 min was observed, thus, L-lactate was produced with the system of sodium pyruvate (2.0 mM), manganese chloride (5.0 mM), NADH (2.0 mM), sodium bicarbonate (50 mM) and ME (0.7 U) during incubation.

5. Determination for fumarate concentration using ion chromatography

The concentration of fumarate was determined using ion chromatography system (Metrohm, Eco IC; electrical conductivity detector) with an ion exclusion column (Metrosep Organic Acids 250/7.8 Metrohm; column size: 7.8 x 250 mm; composed of 9 μm polystyrene-divinylbenzene copolymer with sulfonic acid groups). The 1.0 mM perchloric acid and 50 mM lithium chloride in aqueous solution were used as an eluent and a regenerant, respectively. Flow rate of eluent solution was adjusted to be 0.5 mL min^{-1}. The retention time for fumarate was detected at 12.05-12.29 min. The electrical conductivity changes in the various fumarate concentrations (0 – 2000 μM) during the ion chromatograph analysis were shown in Figure S5. Inset of Figure S5 shows the relationship between the fumarate concentration and the detection peak area using ion chromatograph.

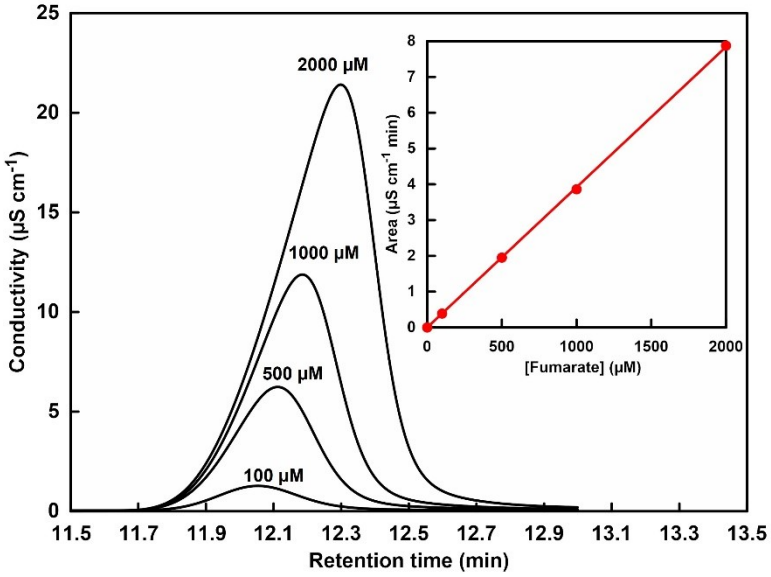

Figure S5. Chromatogram of sodium fumarate (0 – 2000 μM) in 50 mM-HEPES buffer (pH 7.0). Inset: Relationship between the fumarate concentration and the detection peak area.

As shown in the inset of Figure S5, the fumarate concentration and the detected peak area showed a good linear relationship (correlation coefficient: r^2=0.999) as following equation (S2).

$$\text{Peak area} = 0.0039 \times [\text{fumarate}](\mu M) \quad \text{(S2)}$$

6. Fumarate synthesis from the pyruvate and direct captured CO_2 with MDH and FUM

Figure S6 shows a chart of an ion chromatogram sampled from the reaction solution of sodium pyruvate (5.0 mM), manganese chloride (5.0 mM), ATP (5.0 mM), NADH (5.0 mM), Acetyl-CoA (1.0 mM), PC (1.0 U), MDH (10 U) and FUM (0.5 U) in 5.0 mL of 500 mM HEPES buffer (pH 7.2) with CO_2 gas during the incubation.

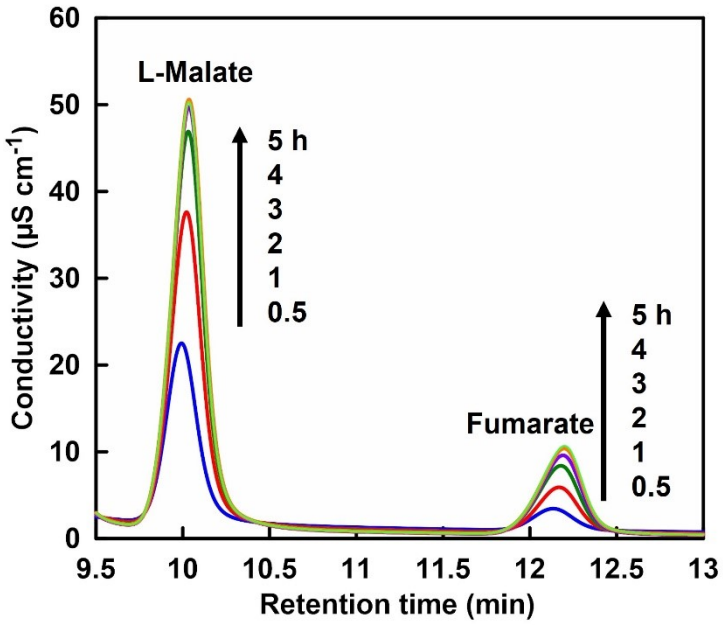

Figure S6. A chart of an ion chromatogram for L-malate or fumarate concentration in the solution of sodium pyruvate (5.0 mM), manganese chloride (5.0 mM), ATP (5.0 mM), NADH (5.0 mM), Acetyl-CoA (1.0 mM), PC (1.0 U), MDH (10 U) and FUM (0.5 U) in 5.0 mL of 500 mM HEPES buffer (pH 7.2). The gas phase: CO_2.

Reference

1. M. Takeuchi and Y. Amao, *RSC. Sustain.,* 2023, **1**, 1874.