Electronic Supplementary Material (ESI) for Reaction Chemistry & Engineering.
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Supporting information

Biocatalytic fumarate synthesis from pyruvate and CO_2 as a feedstock

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1. **Enzymatic activity of malate dehydrogenase decarboxylating type (EC 1.1.1.38 code: MDH-73-01 obtained from *Sulfobus tokodaii*)**

Malate dehydrogenase decarboxylating type (ME, EC 1.1.1.38 code: MDH-73-01 obtained from *Sulfobus tokodaii*; commercially available reagent, 14 mg mL^{-1}; 7.62 units mL^{-1}) was purchased from Thermostable Enzyme Laboratory Co., Ltd. One activity unit of ME convert 1.0 μmol of NADH to NAD^+ in the presence of 10 mM sodium pyruvate,0.3 mM NADH,10 mM sodium bicarbonate and 10 mM $MgCl_2$ in 50 mM 1,4-piperazinediethanesulfonic acid-KOH buffer per min at pH 6.5 at 37 °C according to the data sheet provided by Thermostable Enzyme Laboratory Co., Ltd.

2. **Enzymatic activity of fumarase from porcine heart (EC 4.2.1.2)**

Commercially available Fumarase (FUM) from porcine heart (EC 4.2.1.2) was purchased from Merck Co., Ltd. One activity unit of FUM convert 1.0 μmol of L-malate to fumarate in potassium phosphate buffer per min at pH 7.6 at 25 °C.

3. **Detection for pyruvate, L-malate and fumarate using ion chromatography**

The amount of pyruvate, L-malate or fumarate was detected 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 pyruvate was detected at 8.71-9.20 min. The electrical conductivity changes in the various pyruvate concentrations (0 - 10 mM) were shown in

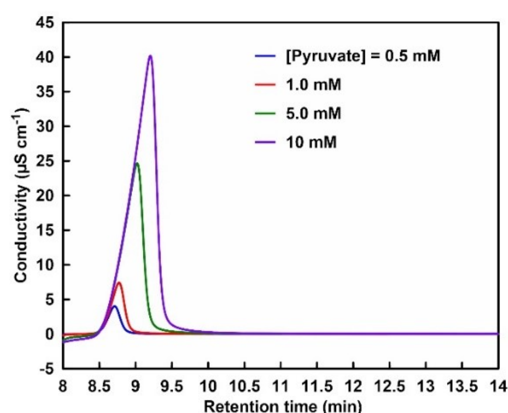

Figure S1.

Figure S1. Chromatogram of sodium pyruvate (0 - 10 mM) in 50 mM-HEPES buffer (pH 7.0).

Figure S2 shows the relationship between the pyruvate concentration and the detection peak area using ion chromatograph.

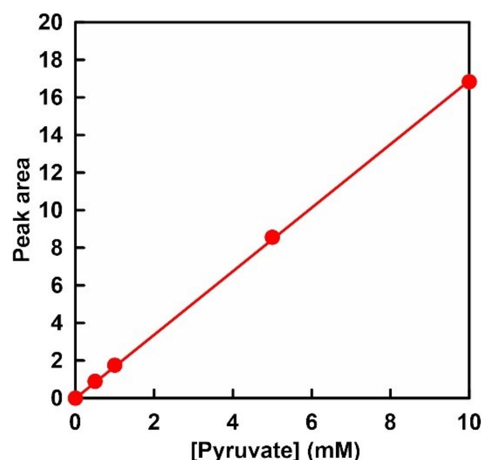

Figure S2. Relationship between the pyruvate concentration and the detection peak area.

As shown in Figure S2, the pyruvate concentration and the detected peak area showed a good linear relationship (correlation coefficient: r^2=0.999) as following equation (1).

$$\text{Peak area} = 1.69 \times [\text{Pyruvate}](\text{mM}) \quad (1)$$

The retention time for L-malate was detected at 10.11-10.13 min. The electrical conductivity changes in the various L-malate concentrations (0 – 1.0 mM) were shown in Figure S3.

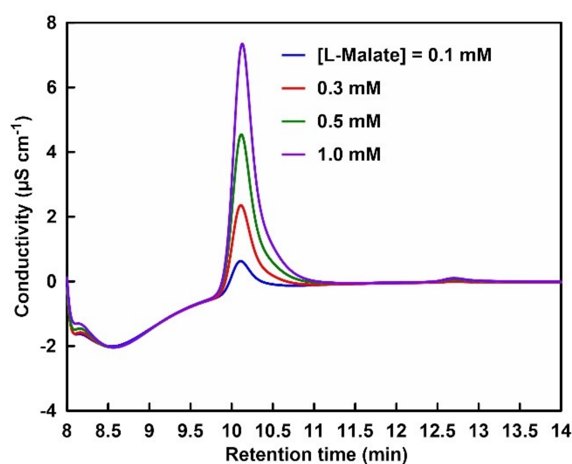

Figure S3. Chromatogram of sodium L-malate (0 - 1.0 mM) in 50 mM-HEPES buffer (pH 7.0).

Figure S4 shows the relationship between the L-malate concentration and the detection

peak area using ion chromatograph.

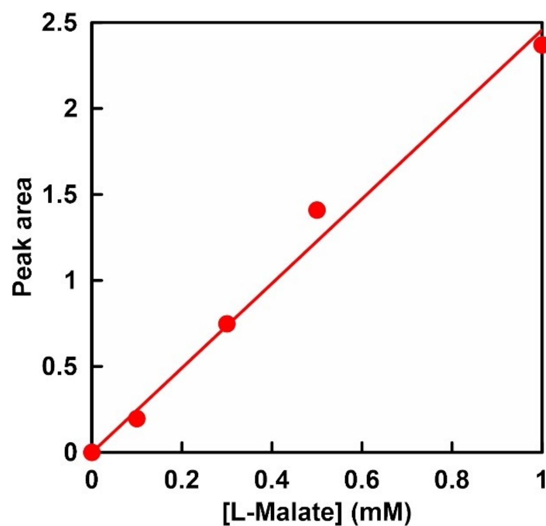

Figure S4. Relationship between the L-malate concentration and the detection peak area.

As shown in Figure S4, the L-malate concentration and the detected peak area showed a good linear relationship (correlation coefficient: r^2=0.999) as following equation (2).

$$\text{Peak area} = 2.46\times[\text{L-malate}](\text{mM}) \quad (2)$$

The retention time for fumarate was detected at 12.28-12.37 min. The electrical conductivity changes in the various fumarate concentrations (0 – 1.0 mM) were shown in

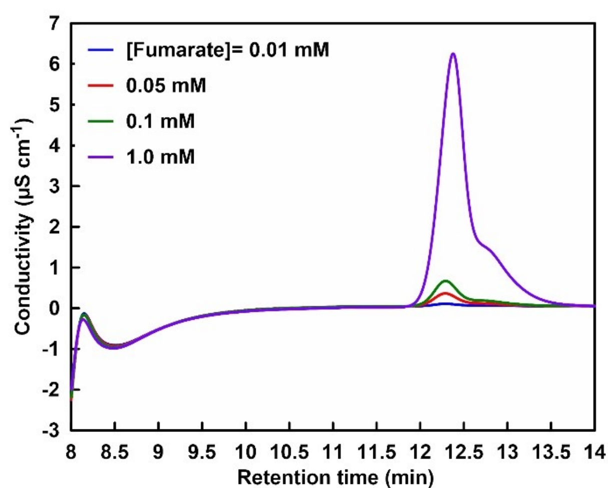

Figure S5.

Figure S5. Chromatogram of sodium fumarate (0 - 1.0 mM) in 50 mM-HEPES buffer (pH 7.0).

Figure S6 shows the relationship between the fumarate concentration and the detection peak area using ion chromatograph.

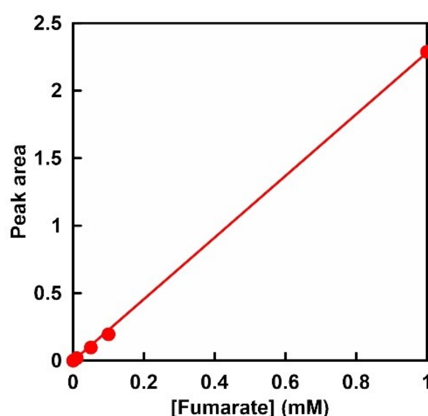

Figure S6. Relationship between the fumarate concentration and the detection peak area.

As shown in Figure S6, the fumarate concentration and the detected peak area showed a good linear relationship (correlation coefficient: r^2=0.999) as following equation (3).

$$\text{Peak area} = 2.28 \times [\text{fumarate}](\text{mM}) \quad (3)$$

4. The effect of bicarbonate concentration on the L-malate production with ME in the presence of NADH

The reaction mixture consisted of sodium pyruvate (5.0 mM), NADH (5.0 mM), magnesium chloride (10 mM) and sodium bicarbonate (10 ~ 100 mM) in 5.0 mL of 500 mM HEPES buffer (pH 7.0). The reaction vessel is a clear glass vial, and the reaction is a sealed system. The total volume of reaction vessel is 11.0 mL. The reaction was started by adding ME (0.7 units) to above mixture in the agitating water baths set at a temperature of 30.5 °C. A sample (0.5 mL) was collected from the reaction solution by syringe and the contents were analyzed by ion chromatography.

5. The effect of introducing CO_2 into the system for the L-malate production with ME in the presence of NADH

The reaction mixture consisted of sodium pyruvate (5.0 mM), NADH (5.0 mM), magnesium chloride (10 mM) and sodium bicarbonate (100 mM) in 5.0 mL of 500 mM HEPES buffer (pH 7.0). The reaction vessel is a clear glass vial, and the reaction is a sealed system. The total volume of reaction vessel is 11.0 mL. The gas phase of the reaction vessel and sample solution were replaced by flowing CO_2 gas at a flow rate of 0.1 L min^{-1} for 10 min. The reaction was started by adding ME (0.7 units) to above

mixture in the agitating water baths set at a temperature of 30.5 °C. A sample (0.5 mL) was collected from the reaction solution by syringe and the contents were analyzed by ion chromatography. At the time of sampling, CO_2 gas was flowed into the gas phase of the reaction vessel.

6. The fumarate production from L-malate with FUM

The reaction was started by adding FUM (0.5 units) to the solution of sodium L-malate (1.0 mM) in 5.0 mL of 500 mM HEPES buffer (pH 7.0) in the agitating water baths set at a temperature of 30.5 °C. The reaction vessel is a clear glass vial, and the reaction is a sealed system. The total volume of reaction vessel is 11.0 mL. A sample (0.5 mL) was collected from the reaction solution by syringe and the contents were analyzed by ion chromatography.

7. The effect of Mg^{2+} on the FUM catalyzes the production of fumarate from L-malate

The reaction was started by adding FUM (0.5 units) to the solution of sodium L-malate (1.0 mM) and magnesium chloride (0 ~ 10 mM) in 5.0 mL of 500 mM HEPES buffer (pH 7.0) in the agitating water baths set at a temperature of 30.5 °C. The reaction vessel is a clear glass vial, and the reaction is a sealed system. The total volume of reaction vessel is 11.0 mL. A sample (0.5 mL) was collected from the reaction solution by syringe and the contents were analyzed by ion chromatography.

8. The fumarate production from pyruvate and CO_2 with ME and FUM in the presence of NADH

The reaction mixture consisted of sodium pyruvate (5.0 mM), NADH (5.0 mM), magnesium chloride (5.0 mM) and sodium bicarbonate (100 mM) in 5.0 mL of 500 mM HEPES buffer (pH 7.0). The reaction vessel is a clear glass vial, and the reaction is a sealed system. The total volume of reaction vessel is 11.0 mL. The gas phase of the reaction vessel and sample solution were replaced by flowing CO_2 gas at a flow rate of 0.1 L min^{-1} for 10 min. The reaction was started by adding ME (0.7 units) and FUM (0.5 units) to above mixture in the agitating water baths set at a temperature of 30.5 °C. A sample (0.5 mL) was collected from the reaction solution by syringe and the contents were analyzed by ion chromatography.