Electronic Supplementary Material (ESI) for Green Chemistry.

Supporting information

Visible-light driven 3-hydroxybutyrate production from acetone and low concentrations of CO_2 with the system of hybridized photocatalytic NADH regeneration and multi-biocatalysts

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1. Experimental procedure

1.1 Determination of enzyme activities of acetone carboxylase (AC) and 3-hydroxybutyrate dehydrogenase (HBDH)

The enzyme unit for enzyme is defined a catalytic activity. The one unit (U; μmol min^{-1}) is defined as the amount of the enzyme that catalyzes the conversion of 1.0 μmol of substrate per minute. For determination of enzymatic activity of AC, the reaction was started by adding enzyme extract (0.2 mL) to the solution of acetone (10 μmol), sodium bicarbonate (0.25 mmol), ATP disodium salt hydrate (10 μmol) and magnesium chloride (25 μmol) in 5.0 mL of 500 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) buffer (pH 8.2) thermostatic chamber set at a temperature of 30.5 °C. The amount of acetoacetate produced was detected by ion chromatography (Metrohm, Eco IC; electrical conductivity detector) with ion exclusion column (Metrosep Organic Acids 250/7.8 Metrohm; column size: 7.8 × 250 mm; composed of 9 μm polystyrene-divinylbenzene copolymer with sulfonic acid groups). Under this condition, the amount of acetoacetate produced per minute of incubation time was estimated to be 46~62 nmol. Thus, the enzyme activity of AC in 0.2 mL of enzyme extract was calculated to be 0.046~0.062 U.

For determination of enzymatic activity of HBDH, the reaction was started by adding cell extract (0.2 mL) to the solution of lithium acetoacetate (10 μmol) and NADH (25 μmol) in 5.0 mL of 500 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) buffer (pH 7.5) thermostatic chamber set at a temperature of 30.5 °C. The concentration of NADH was monitored by absorption spectrum change using UV-visible absorption spectroscopy (SHIMADZU, MaltiSpec-1500) with the molar coefficient at 340 nm (ε=6220 cm^{-1} M^{-1}). The NADH consumption could be calculated as a 3-hydroxybutyrate concentration. Under this condition, the amount of 3-hydroxybutyrate produced per minute of incubation time was estimated to be 0.31~0.70 μmol. Thus, the enzyme activity of HBDH in 0.2 mL of enzyme extract was calculated to be 0.31~ 0.70 U.

1.2 Detection for acetoacetate using ion chromatography (1)

The amount of acetoacetate 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 × 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 0.25 mL min^{-1}. The retention time for acetoacetate was detected at 26.5-28.5 min. The electrical conductivity changes in the various acetoacetate concentrations (0 – 2.0 mM) were shown in Figure S1(a). Figure S1(b) shows the relationship between the acetoacetate concentration and the detection peak area using ion chromatograph.

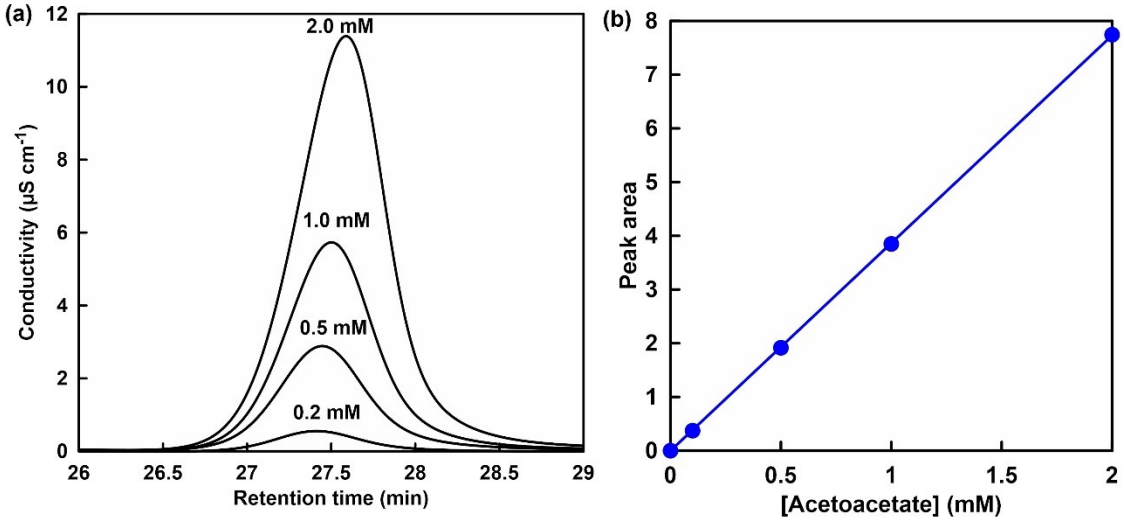

Figure S1. Chromatogram of lithium acetoacetate (0 – 2.0 mM) in 500 mM-HEPES buffer (pH 7.0) (a) and the relationship between the acetoacetate concentration and the detection peak area (b).

As shown in Figure S1(b), the acetoacetate concentration and the detected peak area showed a good linear relationship (correlation coefficient: r^2=1.0) as following equation (S1).

$$\text{Peak area} = 3.87 \times [\text{Acetoacetate}](\text{mM}) \quad \text{(S1)}$$

1.3 Detection for acetoacetate and 3-hydroxybutyrate using ion chromatography (2)

The amount of acetoacetate 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 × 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 acetoacetate was detected at 13.5-14.5 min. The electrical conductivity changes in the various acetoacetate concentrations (0 – 2.0 mM) were shown in Figure S2(a). Figure S2(b) shows the relationship between the acetoacetate concentration and the detection peak area using ion chromatograph.

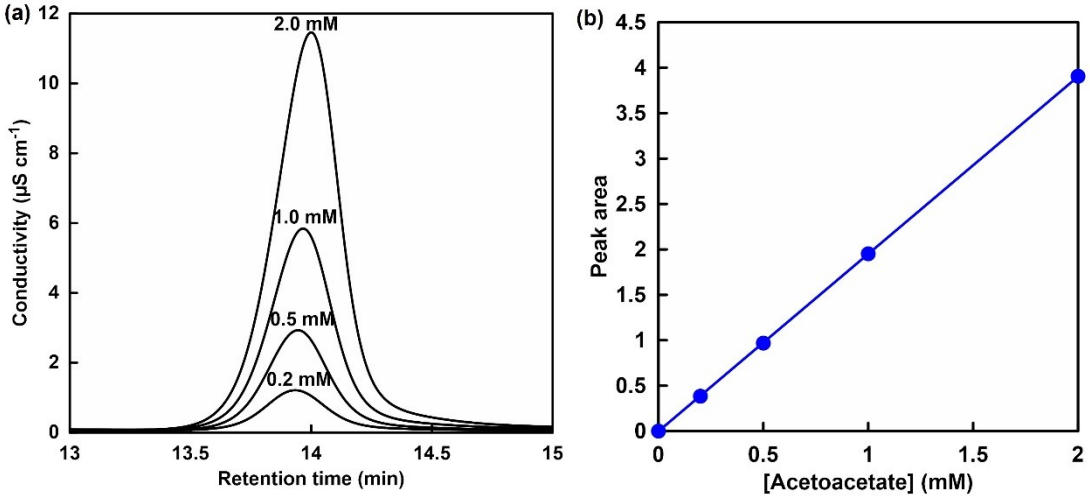

Figure S2. Chromatogram of lithium acetoacetate (0 – 2.0 mM) in 500 mM-HEPES buffer (pH 7.0) (a) and the relationship between the acetoacetate concentration and the detection peak area (b).

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

$$\text{Peak area} = 1.95 \times [\text{Acetoacetate}](\text{mM}) \quad \text{(S2)}$$

The amount of 3-hydroxybutyrate 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 × 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 3-hydroxybutyrate was detected at 14.3 min. The electrical conductivity changes in the various 3-hydroxybutyrate concentrations (0 – 2.0 mM) were shown in Figure S3 (a). Figure S3(b) shows the relationship between the 3-hydroxybutyrate concentration and the detection peak area using ion chromatograph.

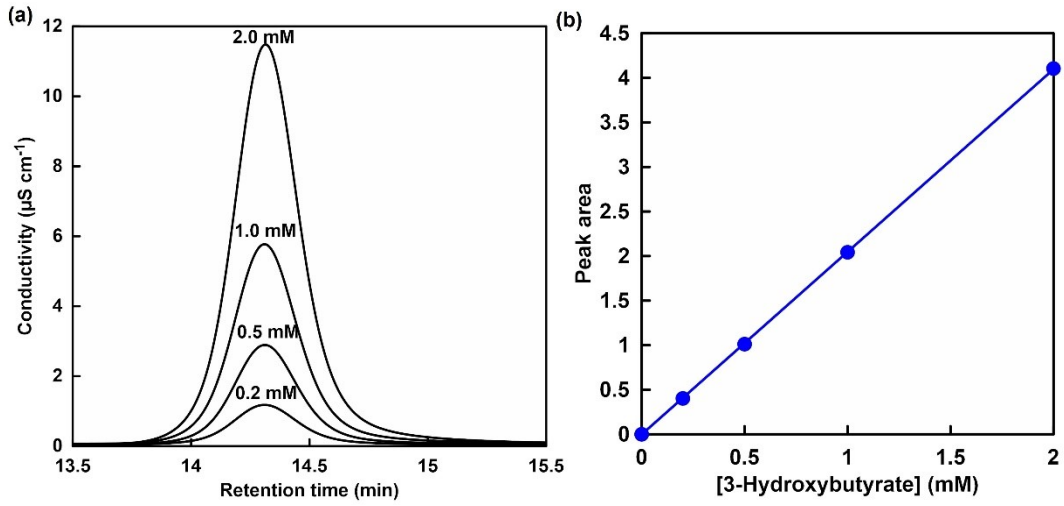

Figure S3. Chromatogram of sodium 3-hydroxybutyrate (0 – 2.0 mM) in 500 mM-HEPES buffer (pH 7.0) (a) and the relationship between the 3-hydroxybutyrate concentration and the detection peak area (b).

As shown in Figure S3(b), the 3-hydroxybutyrate concentration and the detected peak area showed a good linear relationship (correlation coefficient: r^2=0.999) as following equation (S3).

$$\text{Peak area} = 2.05 \times [\text{3-Hydoxybutyrate}](\text{mM}) \quad \text{(S3)}$$

1.4 Property of halogen lamp as a visible light source

A 250 W halogen lamp (JD110V 250W PEX, Panasonic) was used as the visible-light source for all experiments in this study. Figure S4 shows the spectral distribution of a 250 W halogen lamp.

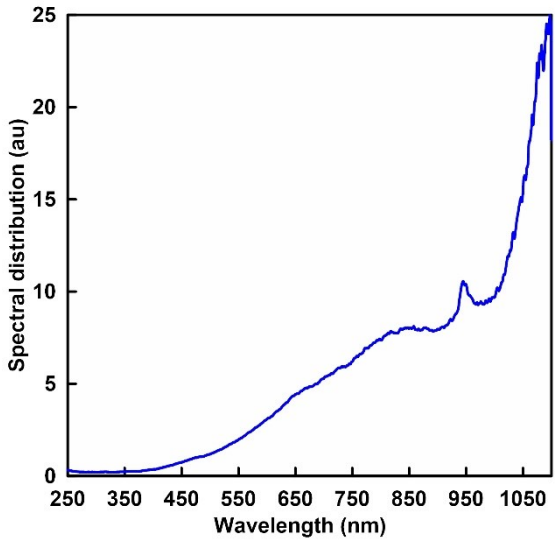

Figure S4. Spectral distribution of 250 W halogen lamp.

1.5 Detection for acetoacetate and 3-hydroxybutyrate using ion chromatography (3)

The amount of acetoacetate 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 × 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 acetoacetate was detected at 13.5-14.5 min. The electrical conductivity changes in the various acetoacetate concentrations (0 – 2.0 mM) were shown in Figure S5(a). Figure S4(b) shows the relationship between the acetoacetate concentration and the detection peak area using ion chromatograph.

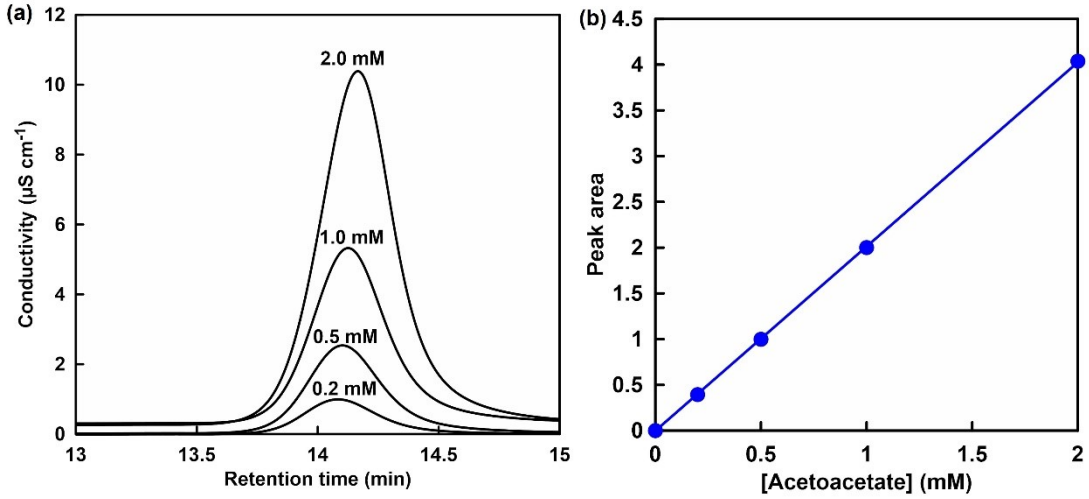

Figure S5. Chromatogram of lithium acetoacetate (0 – 2.0 mM) in 500 mM-HEPES buffer (pH 7.0) (a) and the relationship between the acetoacetate concentration and the detection peak area (b).

As shown in Figure S5(b), the acetoacetate concentration and the detected peak area showed a good linear relationship (correlation coefficient: r^2=0.999) as following equation (S4).

$$\text{Peak area} = 2.01 \times [\text{Acetoacetate}](\text{mM}) \quad \text{(S4)}$$

The amount of 3-hydroxybutyrate 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 × 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 3-hydroxybutyrate was detected at 14.3 min. The electrical conductivity changes in the various 3-hydroxybutyrate concentrations (0 – 2.0 mM) were shown in Figure S6 (a). Figure S6(b) shows the relationship between the 3-hydroxybutyrate concentration and the detection peak area using ion chromatograph.

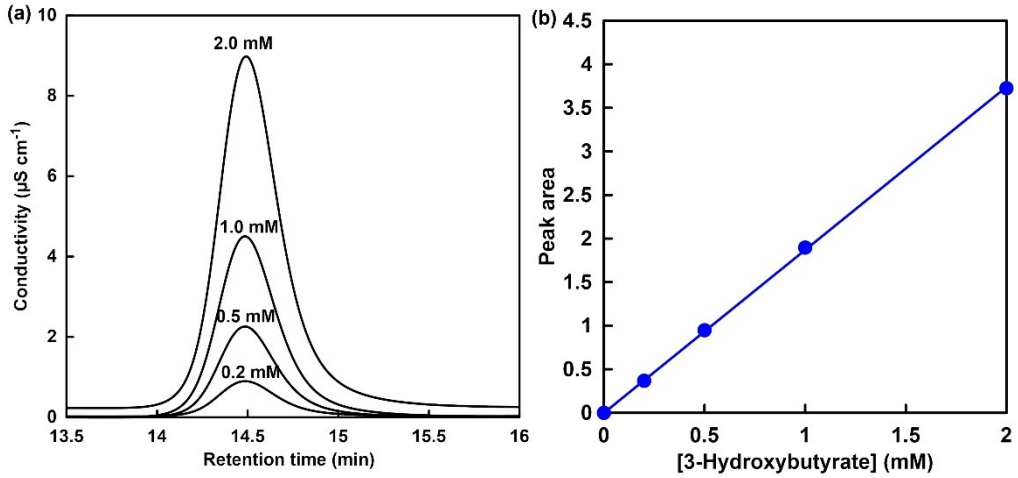

Figure S6. Chromatogram of sodium 3-hydroxybutyrate (0 – 2.0 mM) in 500 mM-HEPES buffer (pH 7.0) (a) and the relationship between the 3-hydroxybutyrate concentration and the detection peak area (b).

As shown in Figure S6(b), the 3-hydroxybutyrate concentration and the detected peak area showed a good linear relationship (correlation coefficient: r^2=0.999) as following equation (S5).

$$\text{Peak area} = 1.87 \times [\text{3-Hydoxybutyrate}](\text{mM}) \quad \text{(S5)}$$

1.6 Chromatogram changes in acetoacetate production from the acetone and direct captured CO_2 with AC in the enzyme extract

Figure S7 shows a chart of an ion chromatogram sampled from the reaction solution containing acetone (100 mM), ATP·2Na (5.0 mM), magnesium chloride (5.0 mM) and enzyme extract (AC: 0.046 U and HBDH: 0.31 U) in 5.0 mL of 500 mM HEPES buffer-NaOH (pH 8.2) with incubation time. The gas phase: The mixture gas of 85 % N_2 and 15 % CO_2.

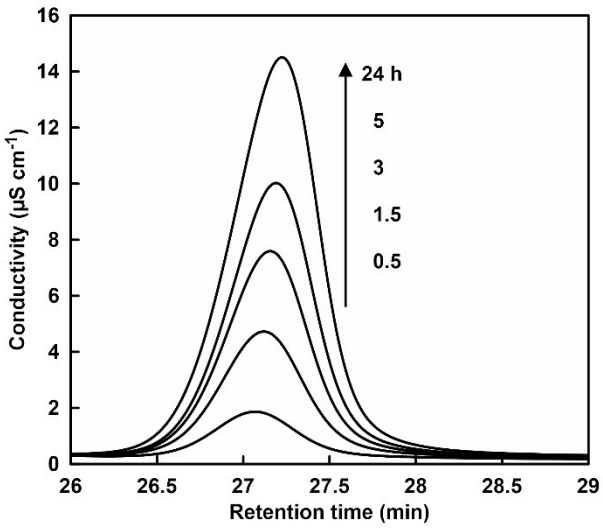

Figure S7. A chart of an ion chromatogram sampled from the reaction solution containing acetone (100 mM), ATP·2Na (5.0 mM), magnesium chloride (5.0 mM) and enzyme extract (AC: 0.046 U and HBDH: 0.31 U) in 5.0 mL of 500 mM HEPES buffer-NaOH (pH 8.2) with incubation time. The gas phase: The mixture gas of 85 % N_2 and 15 % CO_2.

1.7 Determination of respective concentrations in acetoacetate and 3-hydroxybutyrate mixtures by ion chromatograph

In the chart of the ion chromatograph, the peaks of the mixed sample of acetoacetate and 3-hydroxybutyrate overlap as shown in Figure S8. Therefore, as shown in Figure S8, the area was divided into the area (1) attributed to acetoacetate and the area (2) attributed to 3-hydroxybutyrate. The respective calibration curves for mixtures of acetoacetate and 3-hydroxybutyrate in this method were in close agreement with equations S2, S3, S4 and S5.

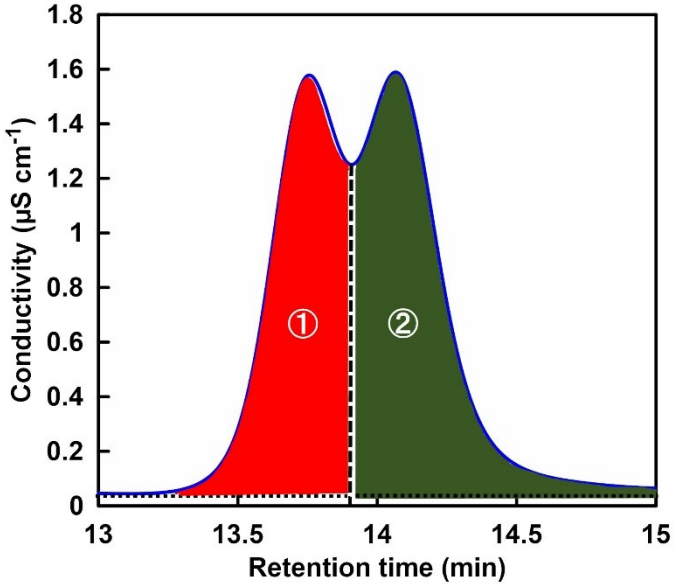

Figure S8. Peak splitting method for two overlapping chromatograms: Area (1) attributed to acetoacetate and the Area (2) attributed to 3-hydroxybutyrate.

1.8 Chromatogram changes in 3-hydroxybutyrate production from the acetone and direct captured CO_2 with AC and HBDH in the enzyme extract

Figure S9 shows a chart of an ion chromatogram sampled from the reaction solution containing acetone (2.0 mM), ATP·2Na (5.0 mM), magnesium chloride (5.0 mM), NADH (5.0 mM) and enzyme extract (AC: 0.062 U and HBDH: 0.70 U) in 5.0 mL of 500 mM HEPES-NaOH buffer (pH 8.2) with incubation time. The gas phase: The mixture gas of 85 % N_2 and 15 % CO_2.

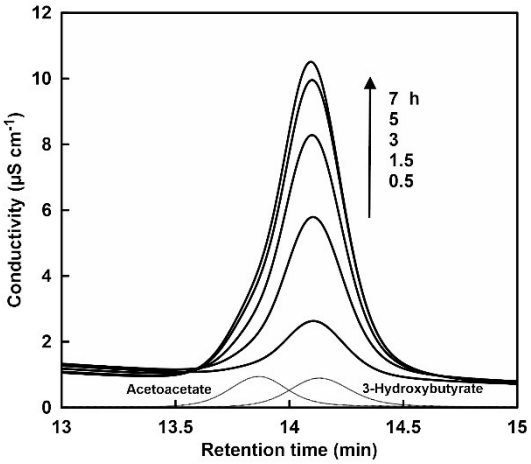

Figure S9. A chart of an ion chromatogram sampled from the reaction solution containing acetone (2.0 mM), ATP·2Na (5.0 mM), magnesium chloride (5.0 mM), NADH (5.0 mM) and enzyme extract (AC: 0.062 U and HBDH: 0.70 U) in 5.0 mL of 500 mM HEPES-NaOH buffer (pH 8.2) with incubation time. The gas phase: The mixture gas of 85 % N_2 and 15 % CO_2.

1.9 Chromatogram changes in the visible-light driven 3-hydroxybutyrate production from acetone and bicarbonate with AC and HBDH in the enzyme extract

Figure S10 shows a chart of an ion chromatogram sampled from the reaction solution containing acetone (0.5 mM), ATP·2Na (2.0 mM), magnesium chloride (5.0 mM), TEOA (0.2 M), ZnTPPS (50 μM), $[Cp^*Rh(bpy)(H_2O)]^{2+}$ (5.0 μM), NAD^+(2.0 mM), sodium bicarbonate and enzyme extract (AC: 0.062 U and HBDH: 0.7 U) in 5 mL of 500 mM HEPES-NaOH buffer (pH 7.0) with irradiation. The bicarbonate concentration was changed between 0.1 and 5.0 mM.

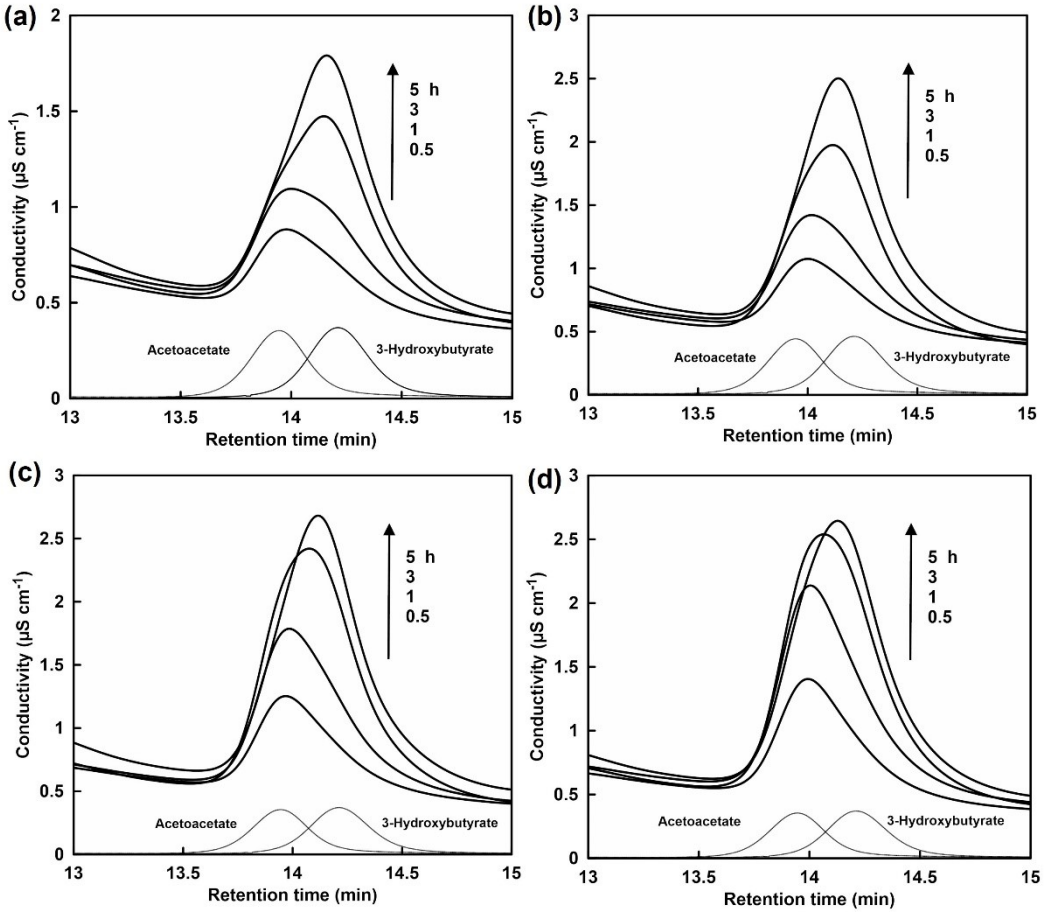

Figure S10. A chart of an ion chromatogram sampled from the reaction solution containing acetone (0.5 mM), ATP·2Na (2.0 mM), magnesium chloride (5.0 mM), TEOA (0.2 M), ZnTPPS (50 μM), $[Cp^*Rh(bpy)(H_2O)]^{2+}$ (5.0 μM), NAD^+(2.0 mM), sodium bicarbonate and enzyme extract (AC: 0.062 U and HBDH: 0.7 U) in 5 mL of 500 mM HEPES-NaOH buffer (pH 7.0) with irradiation. The concentration of bicarbonate: (a) 0.5, (b) 1.0, (c) 10 and (d) 50 mM.

1.10 UV-vis absorption spectra changes in the solution containing TEOA, ZnTPPS, $[Cp^*Rh(bpy)(H_2O)]^{2+}$, NAD^+ in HEPES-NaOH buffer with irradiation.

Figure S11 shows UV-vis absorption spectra changes in the solution containing TEOA (0.2 M), ZnTPPS (50 μM), $[Cp^*Rh(bpy)(H_2O)]^{2+}$ (5.0 μM), NAD^+(2.0 mM) in 5.0 mL of 500 mM HEPES-NaOH buffer with irradiation under the condition of Ar, N_2, CO_2 or the mixture of N_2 and CO_2 gas. Figure S12 shows the difference UV-vis absorption spectra changes in each sample solution under the condition of Ar, N_2, CO_2 or the mixture of N_2 and CO_2 gas.

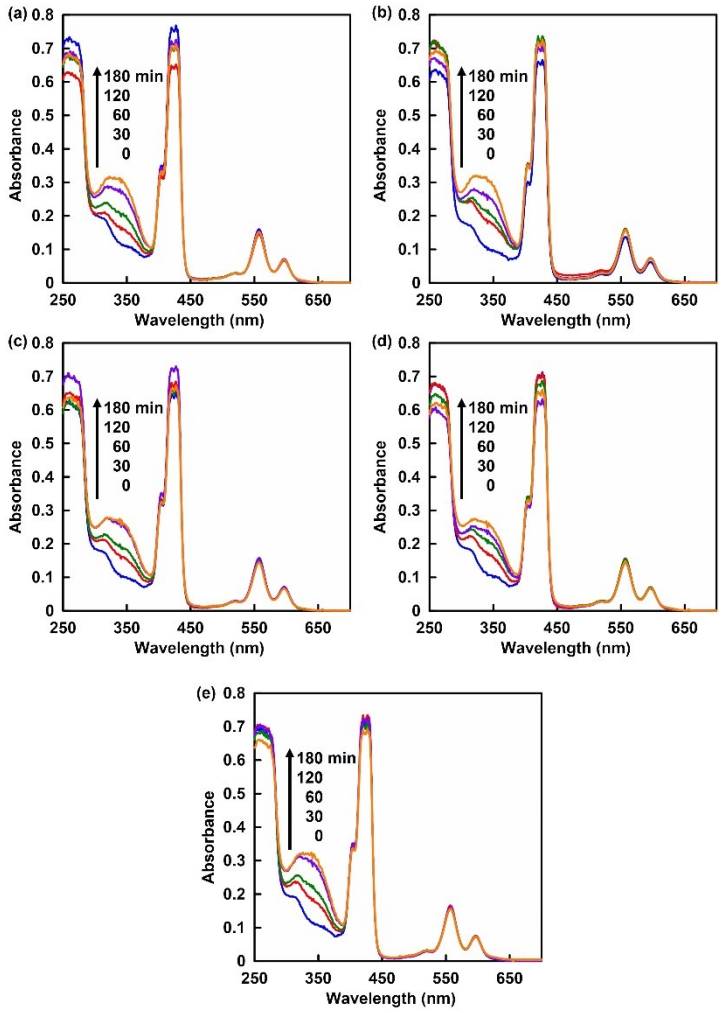

Figure S11. UV-vis absorption spectra changes in the solution containing TEOA (0.2 M), ZnTPPS (50 μM), $[Cp^*Rh(bpy)(H_2O)]^{2+}$ (5.0 μM), NAD^+(2.0 mM) in 5.0 mL of 500 mM HEPES-NaOH buffer (pH 7.0) with irradiation under the condition of N_2(a), N_2 and bicarbonate (b), CO_2 (c), the mixture of N_2 85 % and CO_2 15% gas (d) and Ar gas (e) . After 25 times diluting a sample collected from the reaction solution, the UV-vis absorption spectrum was measured.

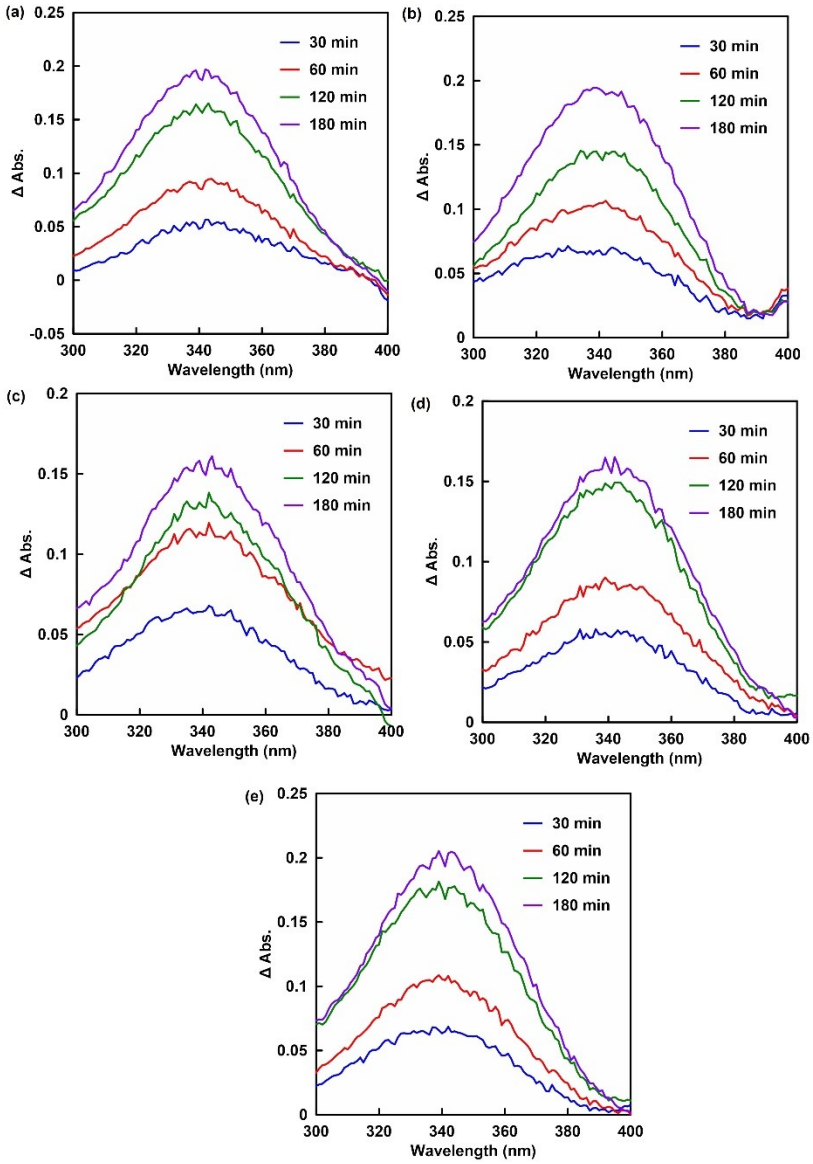

Figure S12. Difference UV-vis absorption spectra changes in the solution containing TEOA (0.2 M), ZnTPPS (50 μM), $[Cp^*Rh(bpy)(H_2O)]^{2+}$ (5.0 μM), NAD^+(2.0 mM) in 5.0 mL of 500 mM HEPES-NaOH buffer (pH 7.0) with irradiation under the condition of N_2(a), N_2 and bicarbonate (b), CO_2 (c), the mixture of N_2 85 % and CO_2 15% gas (d) and Ar gas (e) . After 25 times diluting a sample collected from the reaction solution, the UV-vis absorption spectrum was measured.

1.11 Chromatogram changes in the visible-light driven 3-hydroxybutyrate production from acetone and direct captured CO_2 with AC and HBDH in the enzyme extract

Figure S13 shows a chart of an ion chromatogram sampled from the reaction solution of acetone (0.5 mM), ATP·2Na (2.0 mM), magnesium chloride (5.0 mM), TEOA (0.2 M), ZnTPPS (50 μM), $[Cp^*Rh(bpy)(H_2O)]^{2+}$ (5.0 μM), NAD^+(2.0 mM) and enzyme extract (AC: 0.062 U and HBDH: 0.7 U) in 5 mL of 500 mM HEPES-NaOH buffer (pH 7.0) under conditions with varying ratios of CO_2 and N_2 in the gas phase with irradiation.

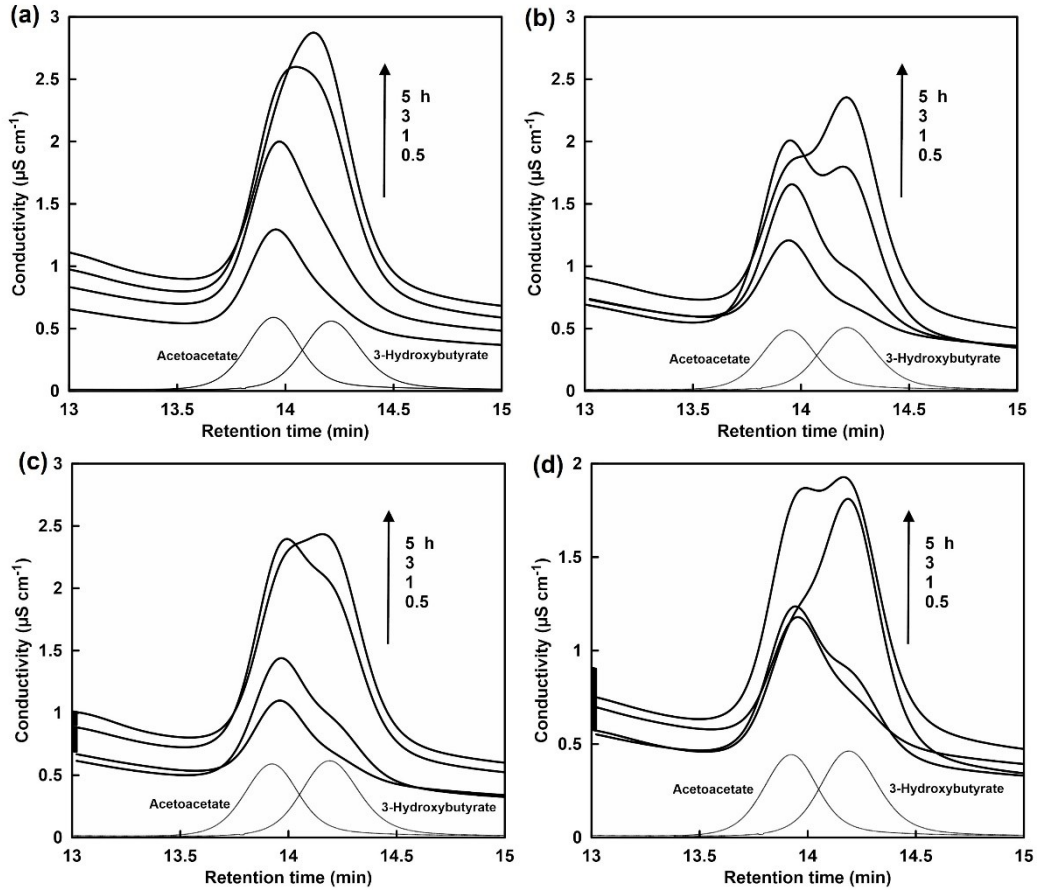

Figure S13. A chart of an ion chromatogram sampled from the reaction solution containing acetone (0.5 mM), ATP·2Na (2.0 mM), magnesium chloride (5.0 mM), TEOA (0.2 M), ZnTPPS (50 μM), $[Cp^*Rh(bpy)(H_2O)]^{2+}$ (5.0 μM), NAD^+(2.0 mM) and enzyme extract (AC: 0.062 U and HBDH: 0.7 U) in 5 mL of 500 mM HEPES-NaOH buffer (pH 7.0) with irradiation. The ratio of CO_2 and N_2: (a) 5.0, (b) 15, (c) 50 and (d) 100 %.

1.12 Chromatogram changes in the visible-light driven 3-hydroxybutyrate production from the waste acetone and direct captured CO_2 with AC and HBDH in the enzyme extract

Figure S14 shows a chart of an ion chromatogram sampled from the reaction solution of the supernatant containing acetone (*ca*. 0.5 mM), ATP·2Na (2.0 mM), magnesium chloride (5.0 mM), TEOA (0.2 M), ZnTPPS (50 μM), $[Cp^*Rh(bpy)(H_2O)]^{2+}$ (5.0 μM), NAD^+(2.0 mM) and enzyme extract (AC: 0.062 U and HBDH: 0.7 U) in 5 mL of 500 mM HEPES-NaOH buffer (pH 7.0) under conditions with a mixed gas of a CO_2 to N_2 ratio of 0.15 in the gas phase with irradiation.

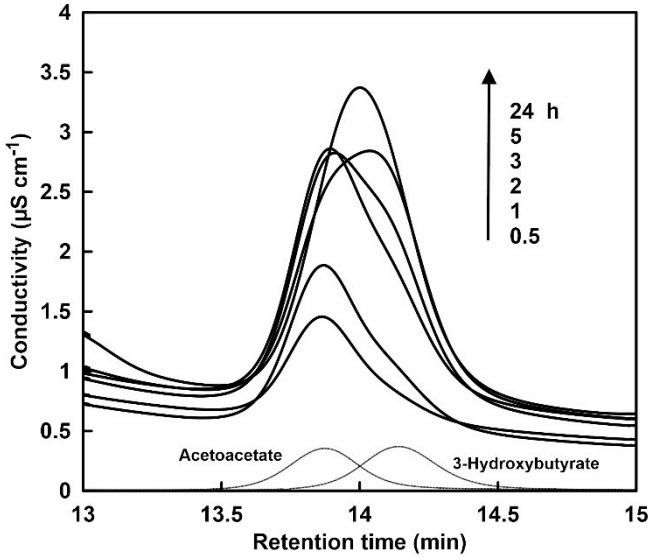

Figure S14. A chart of an ion chromatogram sampled from the reaction solution containing the supernatant containing acetone (*ca*. 0.5 mM), ATP·2Na (2.0 mM), magnesium chloride (5.0 mM), TEOA (0.2 M), ZnTPPS (50 μM), $[Cp^*Rh(bpy)(H_2O)]^{2+}$ (5.0 μM), NAD^+(2.0 mM) and enzyme extract (AC: 0.062 U and HBDH: 0.7 U) in 5 mL of 500 mM HEPES-NaOH buffer (pH 7.0) under conditions with a mixed gas of a CO_2 to N_2 ratio of 0.15 in the gas phase with irradiation.