Supplementary information

Biofixation of high-concentration carbon dioxide using a deep-sea bacterium: *Sulfurovum lithotrophicum* 42BKT^T

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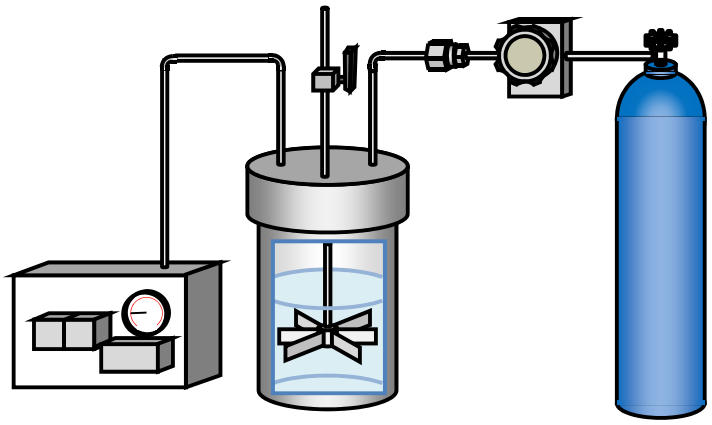

Figure S1. Schematic diagram of the 120 mL stainless steel reactor used to culture *Sulfurovum lithotrophicum* 42BKT^{T} at 10.0 atm. The three ports on the top of the reactor (1/8 inch I.D.) were connected to the gas inlet, the gas outlet, and the pressure gauge (accuracy ±0.01 atm). The inner surface of the steel reactor was coated with glass.

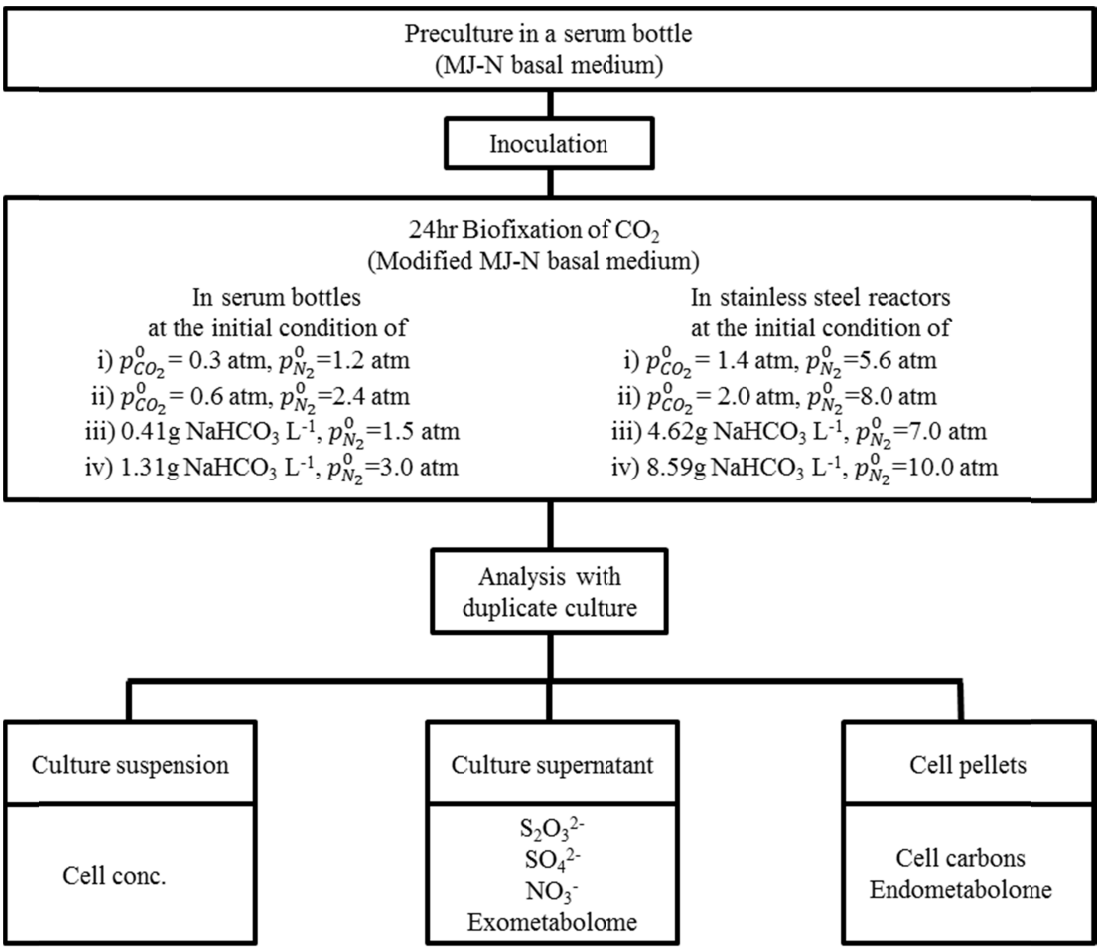

Figure S2. Flowchart of CO_2 biofixation experiments and quantitative analyses.

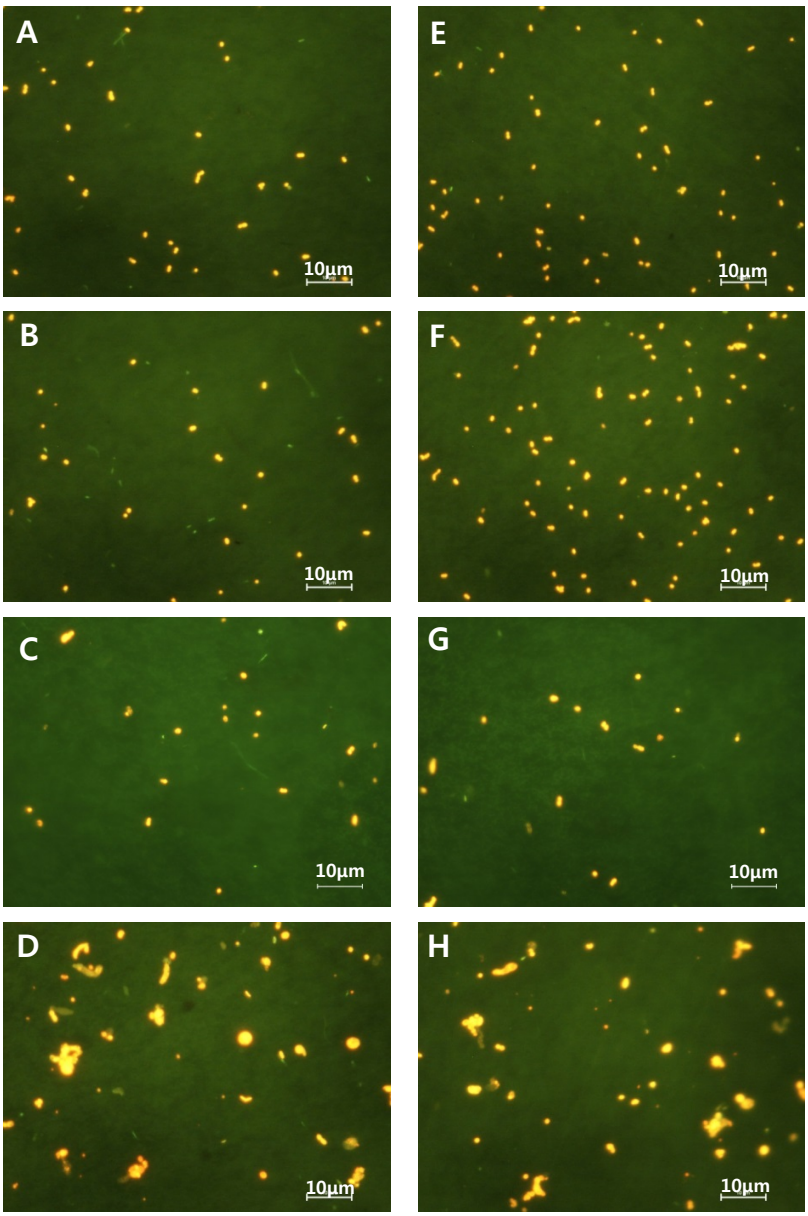

Figure S3. Images of *Sulfurovum lithotrophicum* 42BKT[T] cells grown in medium in which the inorganic carbon source was supplied as either CO_2/N_2 gas (A–D) or $NaHCO_3$ (E–H). A, B, C and D correspond to cultures in which the initial CO_2 pressures ($p^0_{CO_2}$) were 0.3, 0.6, 1.4 and 2.0 atm (P^0_{total} = 1.5, 3.0, 7.0 and 10.0 atm), respectively. E, F, G and H correspond to cultures in

which the initial concentrations of $NaHCO_3$ and the total (N_2) pressures were 0.41 g L^{-1} and 1.5 atm, 1.31 g L^{-1} and 3.0 atm, 4.62 g L^{-1} and 7.0 atm and 8.59 g L^{-1} and 10.0 atm, respectively.

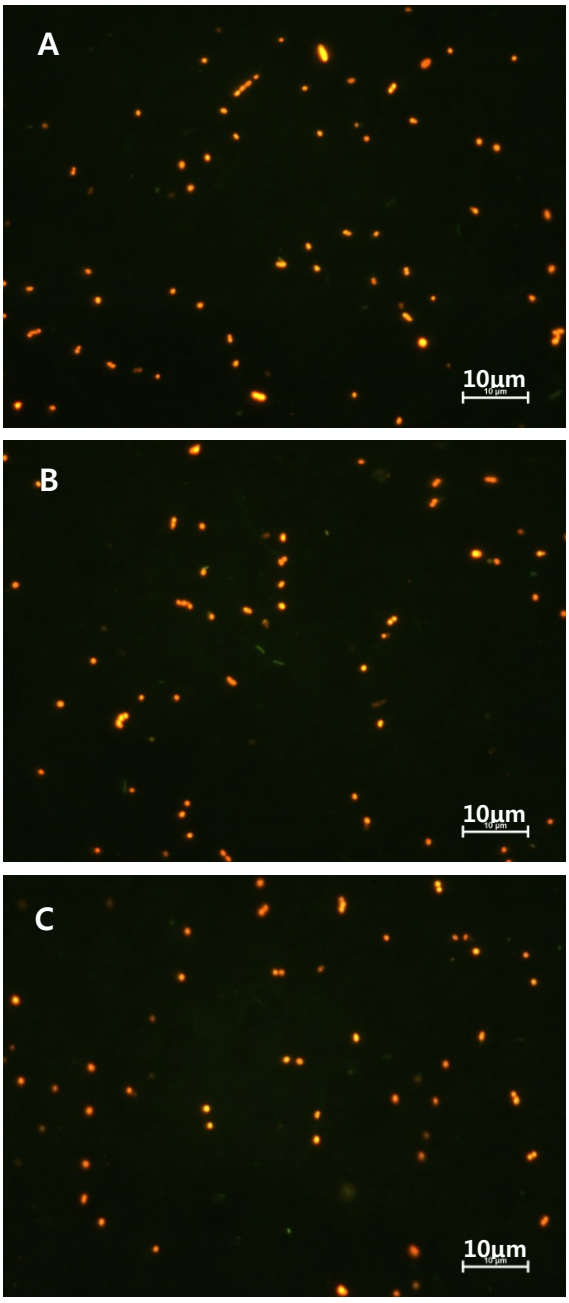

Figure S4. Images of *Sulfurovum lithotrophicum* 42BKT^{T} cells cultured with $NaHCO_3$ as the carbon source and initial total pressures [i.e., $P^0_{total}(= p^0_{N_2})$] varying from 3.0 to 30.0 atm. The initial concentration of $NaHCO_3$ was 1.31 g L^{-1} in all cultures. The values of P^0_{total} were 3.0, 15.0, and 30.0 atm for A, B, and C, respectively.

Table S1. Values of $Y_{X/S}$ and $Y_{P/X}$[a] in culture experiments performed at 29°C in which the carbon source was a CO_2/N_2 gas mixture.[b]

	1.5 atm		3 atm		7 atm		10 atm	
$Y_{X/S}$	0.535		0.592		0.556		0.572	
$Y_{P/X}$	Extracellular metabolites				Intracellular metabolites			
	1.5 atm	3 atm	7 atm	10 atm	1.5 atm	3 atm	7 atm	10 atm
Succinate	0.23	0.17	0.21	0.20	0.71	0.33	0.53	0.68
Lactate	0.00	0.04	0.07	0.15	2.96	2.12	1.87	3.65
Aspartate	0.00	0.00	0.22	1.40	14.0	9.76	5.62	8.25
Pyroglutamate	0.00	0.00	1.61	3.96	96.2	85.9	57.6	38.1
Glutamate	1.77	1.80	2.30	6.42	294	275	241	235

[a] $Y_{X/S}$ and $Y_{P/X}$ are expressed in units of g cell g CO_2^{-1} and mg metabolite g $cell^{-1}$, respectively.

[b] The pressures given in this table (i.e., 1.5, 3.0, 7.0 and 10 atm) correspond to the total pressures, which were initially controlled with a mixture of CO_2 and N_2 gases in a ratio of 2:8 (i.e., $p^0_{CO_2}$: $p^0_{N_2}$ = 2:8).

Table S2. Values of $Y_{X/S}$ and $Y_{P/X}$[a] in culture experiments performed at 29°C in which the carbon source was $NaHCO_3$.[b]

	1.5 atm		3 atm		7 atm		10 atm	
$Y_{X/S}$	0.484		0.591		0.580		0.580	
$Y_{P/X}$	Extracellular metabolites				Intracellular metabolites			
	1.5 atm	3 atm	7 atm	10 atm	1.5 atm	3 atm	7 atm	10 atm
Succinate	0.44	0.35	0.12	0.72	0.71	0.29	0.53	2.23
Lactate	0.00	0.07	0.00	0.00	3.44	1.43	4.87	6.94
Aspartate	0.00	0.00	0.03	1.03	22.8	13.7	10.7	19.7
Pyroglutamate	0.00	0.00	1.22	3.02	114	84.0	54.1	41.1
Glutamate	3.34	2.38	1.86	5.93	322	285	276	278

[a] $Y_{X/S}$ and $Y_{P/X}$ are expressed in units of g cell g CO_2^{-1} and mg metabolite g $cell^{-1}$, respectively.

[b] The pressures given in this table (i.e., 1.5, 3.0, 7.0 and 10 atm) correspond to the total pressures, which were controlled only with N_2 gas.

Table S3. Fractions of carbon in the dry cell masses obtained from cultures grown at 29°C in which the carbon source was either a gas mixture of CO_2/N_2 or $NaHCO_3$.

	CO_2/N_2 gas mixture[a]				$NaHCO_3$[b]			
	1.5 atm	3 atm	7 atm	10 atm	1.5 atm	3 atm	7 atm	10 atm
Carbon fraction (%)[c]	50.90	46.01	48.86	47.19	56.19	46.01	47.03	46.61

[a] The pressures given in this table (i.e., 1.5, 3.0, 7.0 and 10 atm) correspond to the total pressures, which were initially controlled with a mixture of CO_2 and N_2 gases in a ratio of 2:8 (i.e., $p^0_{CO_2}$: $p^0_{N_2}$ = 2:8).

[b] The pressures given in this table (i.e., 1.5, 3.0, 7.0 and 10 atm) correspond to the total pressures, which were controlled using only N_2 gas. The initial concentrations of $NaHCO_3$ were 0.41 g L^{-1}, 1.31 g L^{-1}, 4.62 g $L^{-1}$and 8.59 g L^{-1} when the total (N_2) pressures were 1.5, 3.0, and 10.0 atm, respectively.

[c] Expressed as weight percentage.

Determination of CO_2 solubility in the MJ modified medium

Once CO_2 (g) is dissolved in water, aqueous carbon dioxide (CO_2(aq)), carbonic acid (H_2CO_3(aq)), bicarbonate (HCO_3^-), and carbonate (CO_3^{2-}) are generated by the following dissolution and dissociation reactions:

$$CO_2(g) = CO_2(aq) \quad (1)$$

$$CO_2(aq) + H_2O = H_2CO_3(aq) \quad (2)$$

$$H_2CO_3(aq) = H^+ + HCO_3^- \quad (3)$$

$$HCO_3^- = H^+ + CO_3^- \quad (4)$$

Instead of differentiating between CO_2(aq) and H_2CO_3(aq), [H_2CO_3*], which corresponds to the sum of [CO_2(aq)] and [H_2CO_3(aq)], is used to describe the following dissociation equilibria of carbonic acid:

$$K_0 = [H_2CO_3^*]/f(CO_2) \quad (5)$$

$$K_1 = [H^+][HCO_3^-]/[H_2CO_3^*] \quad (6)$$

$$K_2 = [H^+][CO_3^{2-}]/[HCO_3^-] \quad (7)$$

where f(CO_2) is the fugacity of CO_2 (g).

At a given partial pressure of CO_2 (g), the corresponding f(CO_2) was calculated using the Reference Fluid Thermodynamic and Transport Properties (REFPROP) database.[1] K_0 was determined using the following equation of Weiss:[2]

$$\ln K_0 = -58.0931 + 90.5069\left(\frac{100}{T}\right) + 22.2940\ln\left(\frac{T}{100}\right) \quad (8)$$

$$+S[0.027766 - 0.025888(T/100) + 0.0050578(T/100)^2]$$

where S and T denote the salinity (g L^{-1}) and temperature (K) of the medium. K_0 is expressed in

moles L^{-1} atm^{-1}. K_1 and K_2 were determined from the following equations of Millero.[3]

$$\ln K_1 = 2.18867 - \frac{2275.0360}{T} - 1.468591 \ln(T) + (-0.138681 - 9.33291/T)S^{0.5} \quad (9)$$
$$+0.0726483S - 0.00574938S^{1.5}$$

$$\ln K_2 = 0.84226 - \frac{3741.1288}{T} - 1.437139 \ln(T) - \left(0.128417 - \frac{24.41239}{T}\right) S^{0.5} \quad (10)$$
$$+0.1195308S - 0.00912840S^{1.5}$$

Since the biofixation experiments were performed at 29°C, the value of T in Eqs. (8)-(10) was set to be 302 K. S was calculated as the sum of the mass concentrations of all soluble components in the modified MJ medium (i.e., by adding the concentrations of NH_4Cl, $Na_2S_2O_3$, $NaNO_3$, and PIPES expressed in g L^{-1} to the salinity of the MJ-N synthetic seawater). At the given partial pressure of CO_2, the value of $[H_2CO_3^*]$ was calculated from Eq. (5) with K_0 and $f(CO_2)$. Then, the values of $[HCO_3^-]$ and $[CO_3^{2-}]$ were calculated from Eqs. (6) and (7) with K_1, K_2, and pH. For these calculations, pH was measured separately using a high pressure online pH probe (high-pressure glass-based pH electrodes and Ag/AgCl reference electrodes, Corr Instrument, San Antonio, TX, USA). The solubility of CO_2 at the given partial pressure of CO_2 (g), T, S, and pH was predicted to be the sum of the values of $[H_2CO_3^*]$, $[HCO_3^-]$, and $[CO_3^{2-}]$.

To evaluate the ability of Eqs. (8)-(10) to accurately predict CO_2 solubility, CO_2 solubility (i.e., the concentrations of the total dissolved CO_2 species) was experimentally measured and compared with the predicted value. The equipment used to measure the solubility of CO_2 (g) is shown in Figure S5. The left vessel in an oven (designated as ①) was filled with high pressure CO_2, using a P-50 pump (Waters Corp., Milford, MA, USA) to prevent pressure fluctuation in the right vessel in the oven (designated as ②). The right vessel was filled with modified MJ

medium and stirred using an agitator to reduce the time needed to reach equilibrium for CO_2 dissolution. The CO_2 gas in the left vessel was supplied to the right vessel. After a 24 hr, the CO_2-saturated modified MJ medium in the right vessel was sampled using a floating piston sampler containing 8N KOH solution.[4] Hence, all of the dissolved CO_2 species (CO_2(aq), H_2CO_3(aq), HCO_3^-, and CO_3^{2-}) were converted to carbonate (CO_3^{2-}). The concentration of the converted carbonate was then determined using a TIC (Total Inorganic Carbon) analyzer (UIC Inc., Rockdale, IL, USA). The pressure of each vessel was controlled using a high pressure regulator and measured with a Heise gauge 901B digital pressure indicator (Ashcroft Inc., Stratford, CT, USA), with a total uncertainty span of ±0.035%. The temperature of the oven was maintained within ±0.5K. The temperature of each vessel was monitored using an Omega DP41-B resistance temperature detector (Omega Engineering Inc., Stamford, CT, USA), with an uncertainty of ±0.1K.

The experimentally measured and theoretically predicted concentrations of the total dissolved CO_2 species (i.e., CO_2 solubility) in modified MJ medium are shown in Figure S6. The two concentration values were almost the same at all CO_2 pressures. Therefore, Eqs. (8)−(10) were used to calculate the concentrations of the total dissolved CO_2 species when the culture medium in the biofixation reactor was in equilibrium with CO_2 gas at 0.3, 0.6, and 2.0 atm.

Figure S5. Apparatus for measuring the solubility of CO_2 in modified MJ medium.

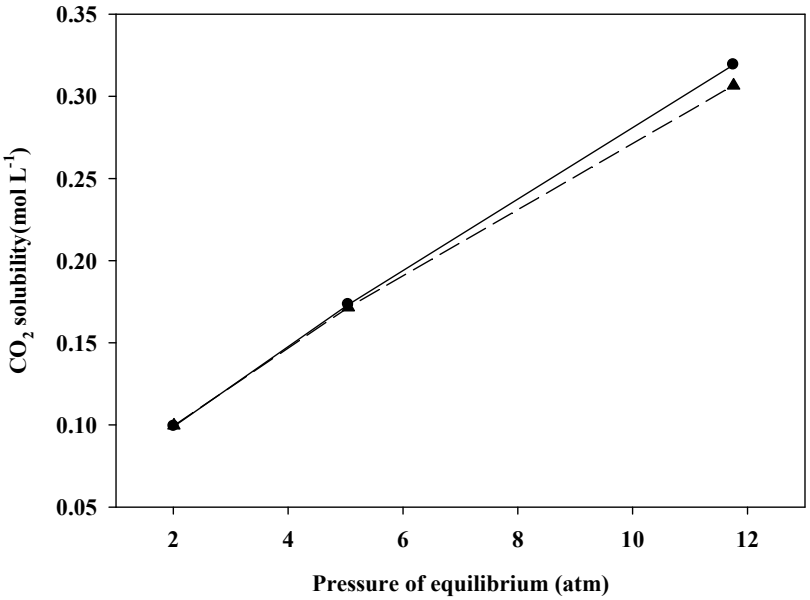

Figure S6. Experimentally measured (▲) and predicted (●) values of CO_2 solubility in modified MJ medium. Eqs. (8)-(10) were used to calculate the predicted values.

Reference

1. E. Lemmon, M. McLinden and M. Huber, REFPROP: Reference fluid thermodynamic and transport properties v. 8.0 in *NIST standard reference database*, 2007.
2. R. F. Weiss, *Marine chemistry*, 1974, **2**, 203-215.
3. F. J. Millero, *Geochimica et Cosmochimica Acta*, 1995, **59**, 661-677.
4. C. Rochelle and Y. Moore, *British Geological Survey Commissioned Report CR/02/052*, 2002.