Supplementary information

Non-photosynthetic CO_2 bio-mitigation by *Escherichia coli* harboring CBB genes†

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List of contents

Table S1. Nucleotide lengths of the rCBB genes and their molecular weights after translation

Operon	Gene	Nucleotide length (bps)	Molecular weight of protein (kDa)
cbb_{I}	*cbbF*	1,002	36.7
	prkA	1,080	39.6
	cfxA	837	30.7
	cbbL	1,461	53.6
	cbbS	390	14.3
cbb_{II}	*fbpB*	996	36.5
	prkB	879	32.2
	tklB	1,974	72.4
	gapB	1,002	36.7
	cfxB	1,065	39.1
	rbpL	1,380	50.6

Table S2. Bacterial strains and plasmids used in this study

Strains and plasmids	Characteristics [a]	Source or reference
Strains		
R. sphaeroides 2.4.1	Wild-type	KCTC 1434
S. cerevisiae s2805	Wild-type, MATa pep4::HIS3 pro 1-8.6 can 1 his3-2000 ura3-52	Korea Research Institute of Bioscience and Biotechnology, Korea
E. coli BL21	F^- *ompT* $hsdS_B$ (r_B^- m_B^-) *gal dcm* (DE3)	Novagen, Madison, WI, USA
E. coli control	*E. coli* BL21, pET-21a and pET-28b (Mock strain)	This study
E. coli ($CBB_{I/II}$)	*E. coli* BL21, pMBTLY-I/pMBTLY-II (recombinant strain)	This study
E. coli (CBB_{I})	*E. coli* BL21, pMBTLY-I (recombinant strain)	This study
E. coli (CBB_{II})	*E. coli* BL21, pMBTLY-II (recombinant strain)	This study
Plasmids		
pET-28b	Km^R, IPTG inducible T7 polymerase dependent expression vector	Novagen, Madison, WI, USA
pET-21a	Am^R, IPTG inducible T7 polymerase dependent expression vector	Novagen, Madison, WI, USA
pMBTLY-I	Km^R, pET-28b derivative carrying *cbbF-cbbS* gene (cbb_{I})	This study
pMBTLY-II	Am^R, pET-21a derivative carrying *fbpB-rbpL* gene (cbb_{II})	This study

[a] Km, kanamycin; Am, ampicillin

Table S3. Oligonucleotide used in this study

Oligo name [a]	Target gene	Sequence (5'-3') [b]	Note [b]
cbb$_{I}$ operon			
cbbI-1F	*cbbF*	taatctagagtgaagccctttcccacc	*Xba*I
cbbI-1R	*cbbF*	taacatatgtcagctccggaacaggcc	*Nhe*I
cbbI-2F	*prkA-cfxA*	gggcatatgagcaagaagcatcccatc	*Nhe*I
cbbI-2R	*prkA-cfxA*	taagctagctcaggcggctttggcggt	*Nde*I
cbbI-3F	*cbbL-cbbS*	tttgctagcatggataccaacaccacc	*Nde*I
cbbI-3R	*cbbL-cbbS*	aataagctttcagcggacgatgctgtg	*Hind*III
cbb$_{II}$ operon			
cbbII-1F	*fbpB-prkB*	taatctagaatggccatcgagctggaggac	*Xba*I
cbbII-1R	*fbpB-prkB*	taacatatgtctccgtctgtctgtcgc	*Nhe*I
cbbII-2F	*tklB-gapB*	ttacatatgaaggacattggagccgcg	*Nhe*I
cbbII-2R	*tklB-gapB*	attgctagctcagagaagccggcccat	*Nde*I
cbbII-3F	*cfxB-rbpL*	aaggctagcatggcactcatcacgctt	*Nde*I
cbbII-3R	*cfxB-rbpL*	aataagctttcaggccgcgcgatgcag	*Hind*III

[a] F, forward; R, reverse
[b] Underline, sites for restriction enzymes

Table S4. Calculation of the specific CO_2-fixtation rate of *E. coli* expressing whole rCBB ($CBB_{I/II}$) after 24 h of anaerobic cultivation in 5% CO_2 as only the carbon source [a]

Consumed CO_2 (mmol L^{-1})	CO_2-fixation rate (mg L^{-1} h^{-1})	Biomass (g DCW L^{-1})	Specific CO_2-fixation rate (mg g DCW h^{-1}) [b]
21.4 ± 0.2	39.2 ± 0.3	0.5 ± 0.0	78.4 ± 0.6

[a] Gas composition was $CO_2/H_2/Ar$ (5:60:35) in 20 mL of M9 minimal medium
[b] Calculated by the CO_2-fixation rate in the unit of mg L^{-1} h^{-1} divided by the biomass concentration in the g DCW L^{-1}

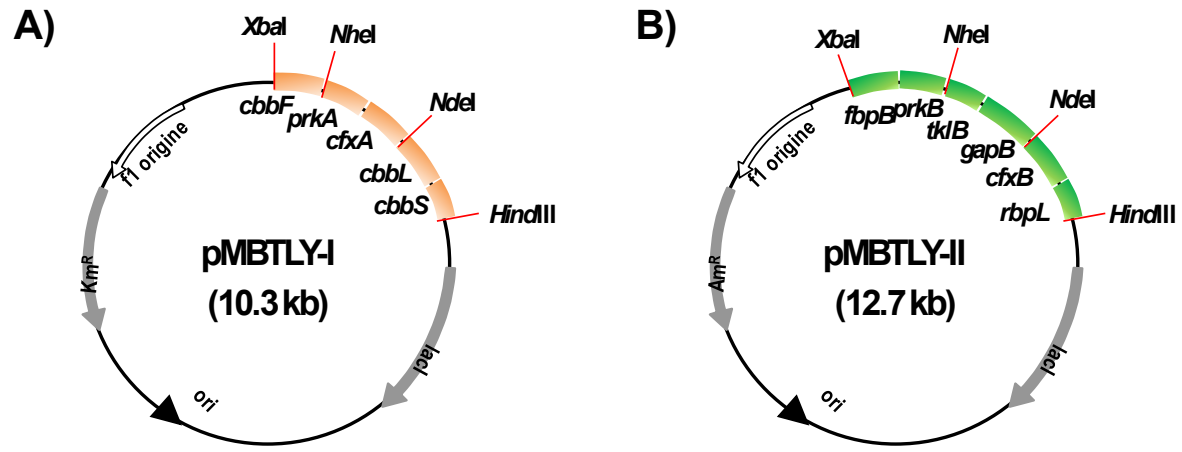

Fig. S1. Schematic diagram of the plasmids. (A) The genes on cbb_I operon (*cbbF, prkA*, *cfxA, cbbL* and *cbbS*) from *R. sphaeroides* 2.4.1 were cloned into cloned into pET-28b for the inducible expression of CBB_I enzymes in *E. coli* BL21 (DE3). (B) The genes on cbb_{II} operon (*fbpB, prkB, tklB, gapB, cfxB* and *rbpL*) from *R. sphaeroides* 2.4.1 were cloned into cloned into pET-21a for the inducible expression of CBB_{II} enzymes in *E. coli* BL21 (DE3).

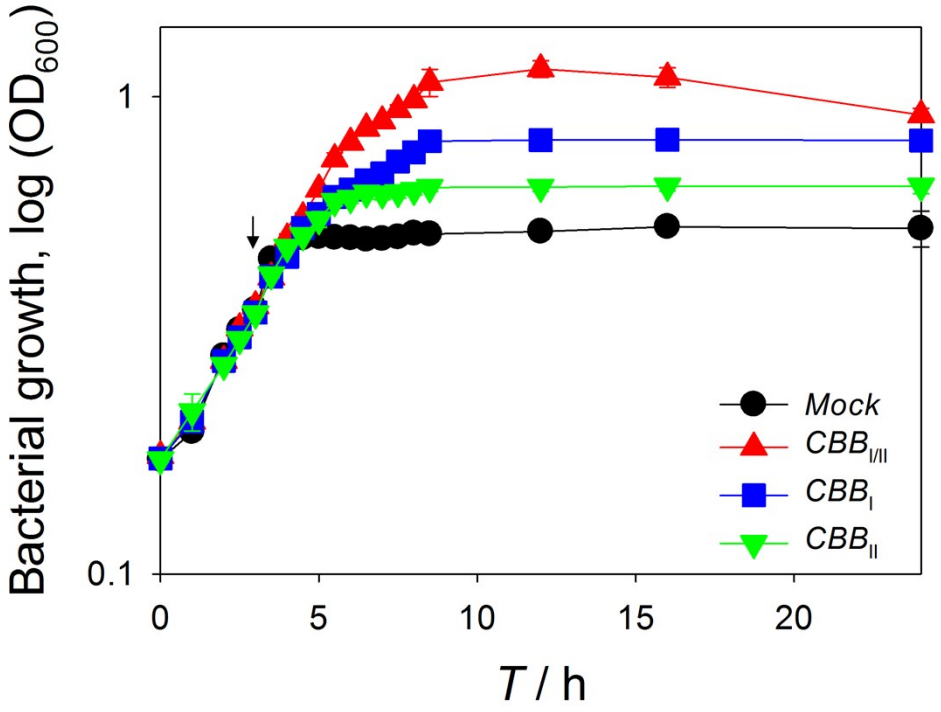

Fig. S2. Growth profiles of the *E. coli* control (Mock) and *E. coli* expressing CBB_I and CBB_{II}. Heterogeneous expressions of whole rCBB ($CBB_{I/II}$), CBB_I-only or CBB_{II}-only in *E. coli* were induced by supplementation of IPTG (1 mM) at 3-h after inoculation (arrow).

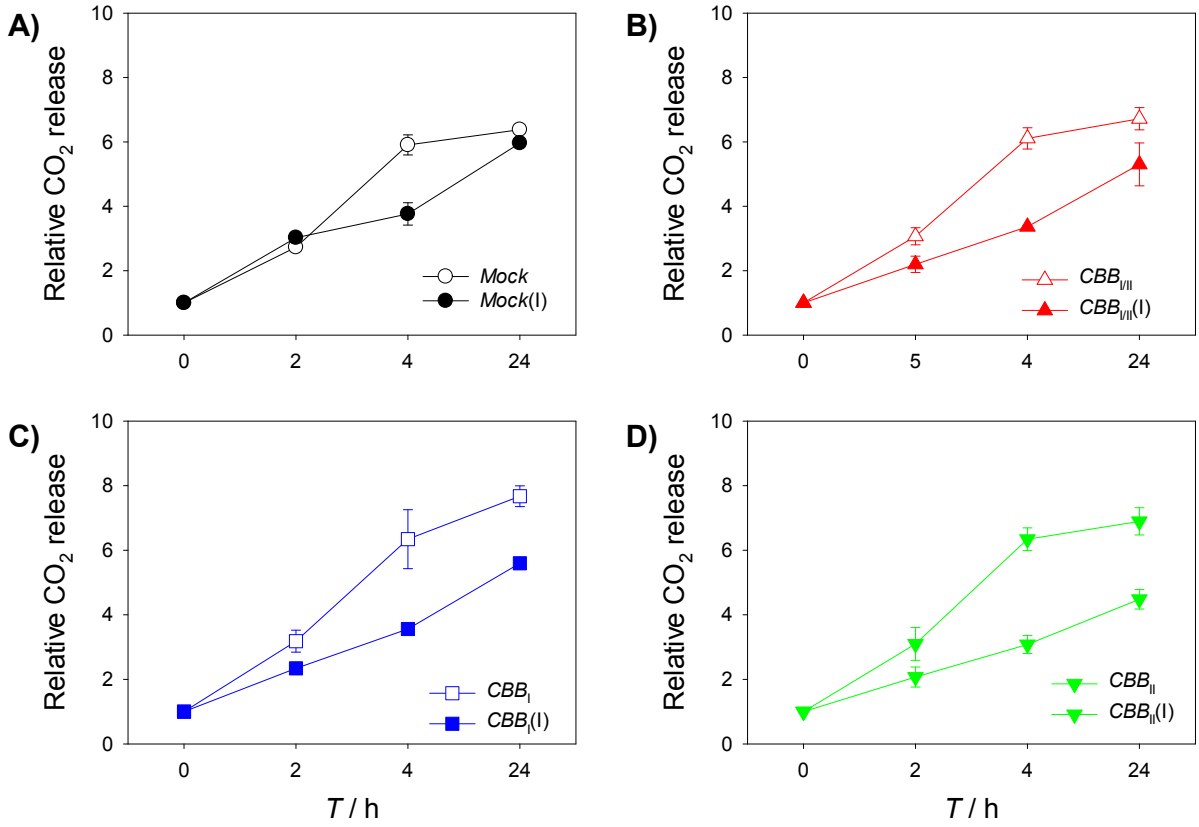

Fig. S3. Relative CO_2 release during bacterial cultivation. (A) *E. coli* control (Mock). (B) *E. coli* expressing whole rCBB ($CBB_{I/II}$). (C) *E. coli* expressing CBB_{I}-only. (D) *E. coli* expressing CBB_{II}-only. Opened symbols indicate non-induced cells. Closed symbols indicate 1 mM IPTG-supplemented cells (I) for overexpression of target genes. The ratio of relative CO_2 release was calculated that the peak area of CO_2 in gas chromatogram at T_0 in each case was 1.

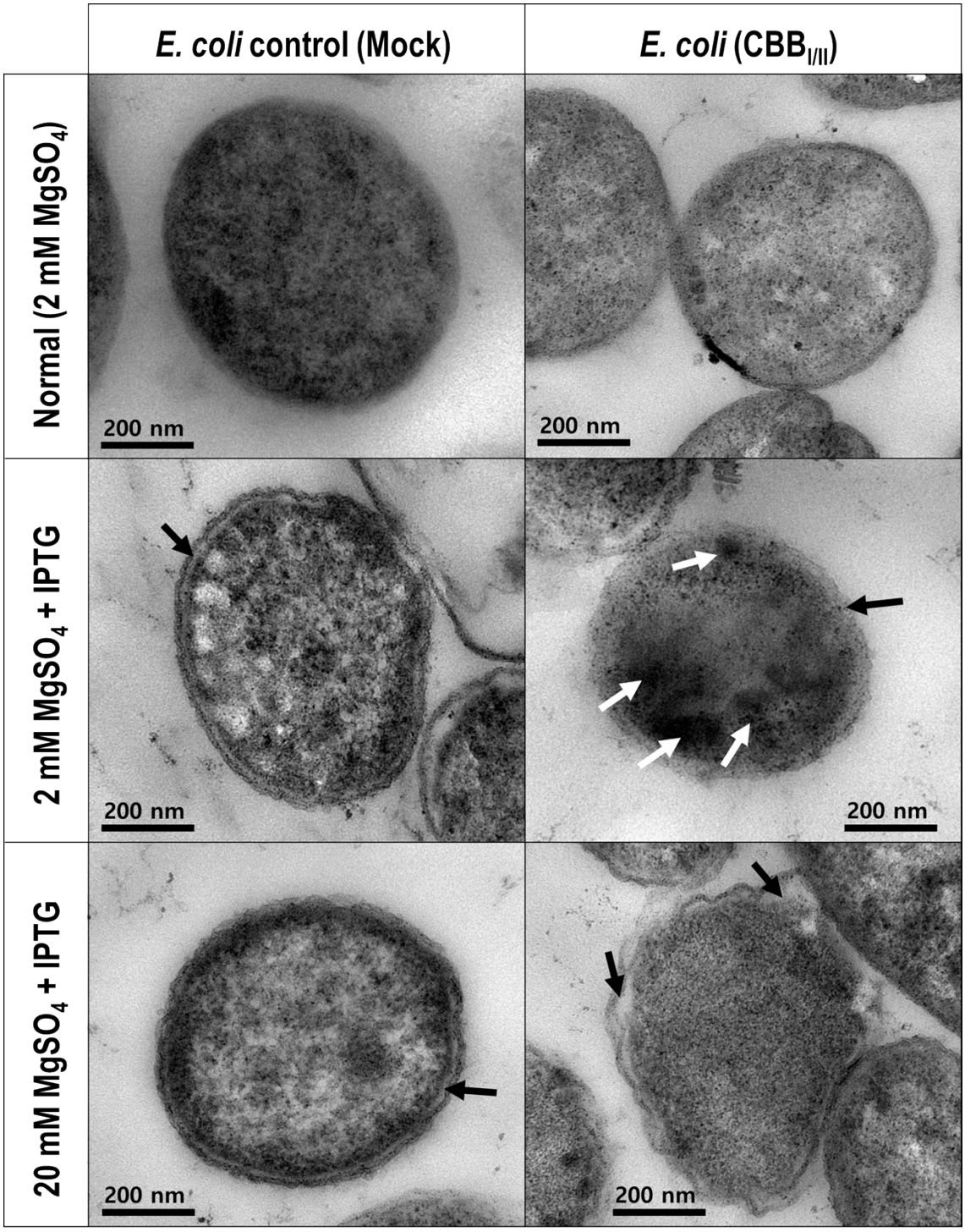

Fig. S4. Transmission electron microscope images of bacteria. Black arrows indicate the periplasmic spaces. White arrows indicate inclusion bodies.

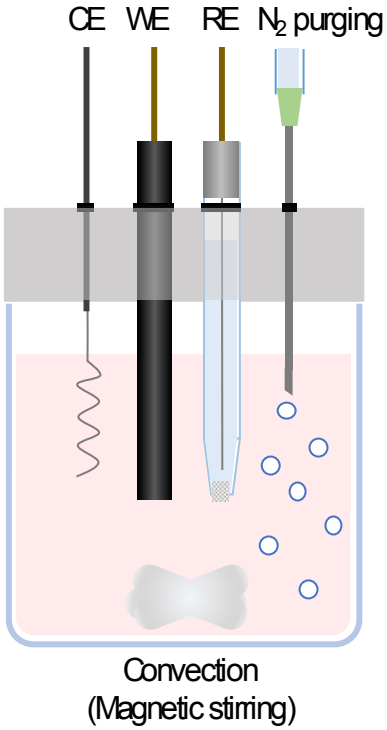

Fig. S5. Schematic illustration of the bioelectrochemical reactor for the electrochemical analysis of the *E. coli* strains. Bacterial cells attached to the glass carbon electrode (GCE) as the working electrode (WE). The reference (RE) and counter (CE) were silver/silver chloride (Ag/AgCl, NaCl saturated) and platinum wire, respectively. 0.1 mM neutral red was suspended to mediate electron transfer from WE to bacteria. M9 minimal medium with 0.4 g L^{-1} glucose was used as electrolyte. Electrochemical experiments, cyclic voltammetry and chronoamperometry were performed at 37 ^{o}C and 100 rpm.

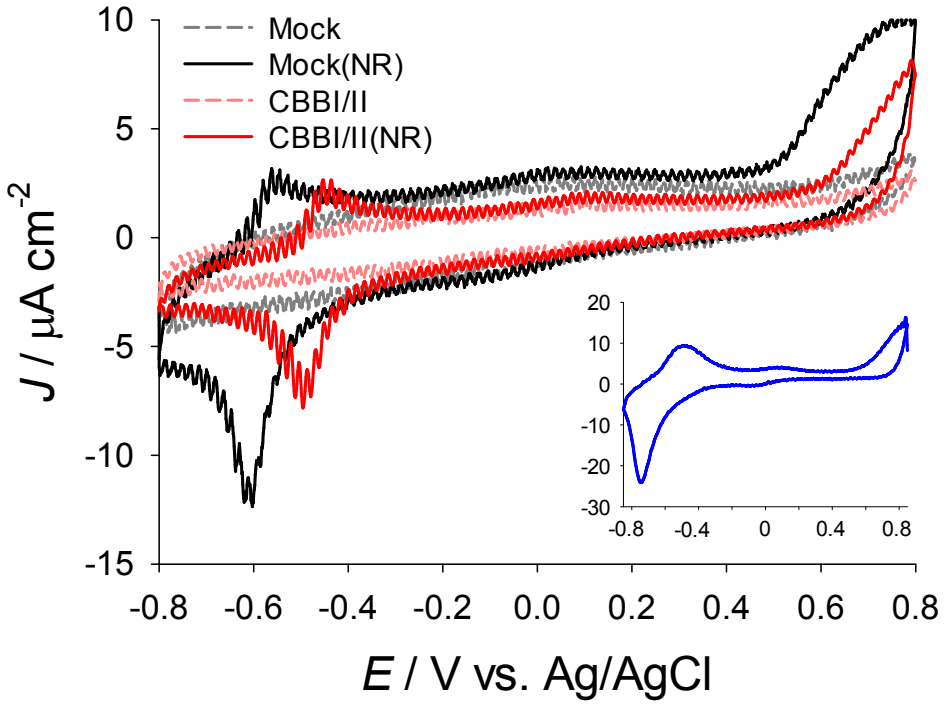

Fig. S6. Cyclic voltammograms of the *E. coli* control (Mock, black lines) and *E. coli* expressing whole rCBB ($CBB_{I/II}$, red lines) without (dotted lines) and with 0.1 mM neutral red (NR) before conducting chronoamperometry experiment (*T*(i, 0) in Figure 5a). 1 mM IPTG was supplemented with NR. Inset, NR only (blue line). Scan rate, 10 mV s^{-1}.

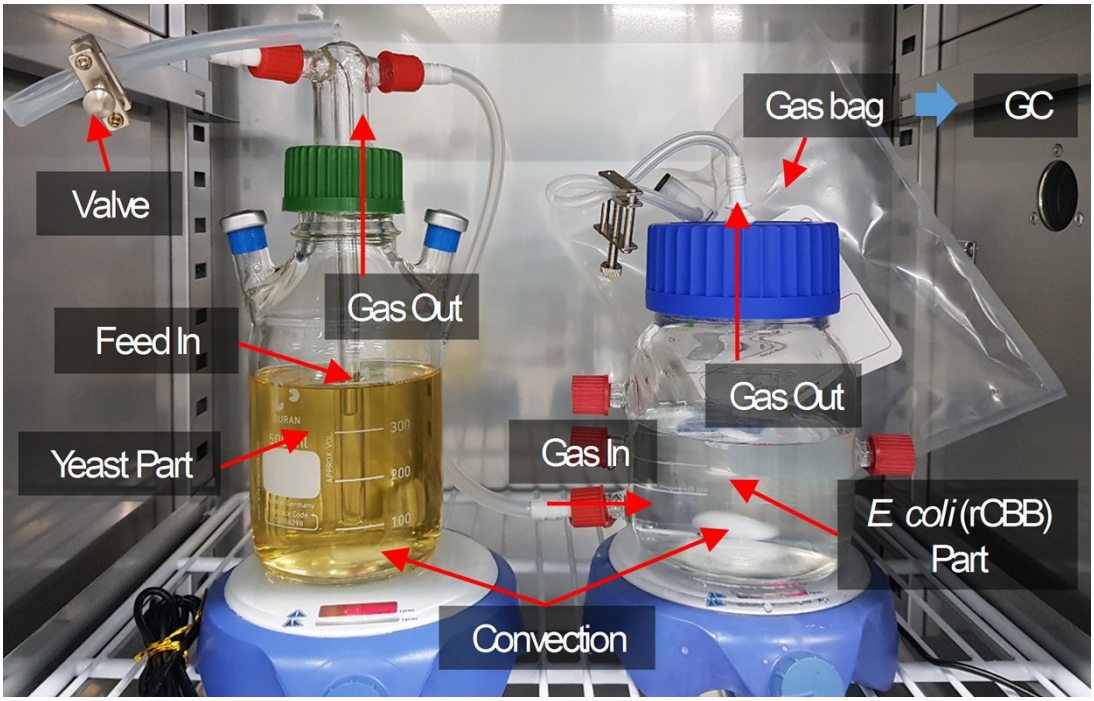

Fig. S7. Photograph of the CO_2 bio-mitigation process in this work. Double vessels were linked with gas-line for CO_2 recycling. Left, culture vessel for yeast fermentation; right, culture vessel for *E. coli* expressing whole rCBB ($CBB_{I/II}$) growth.