Electronic Supplementary Material (ESI) for Green Chemistry.
This journal is © The Royal Society of Chemistry 2014

Supplementary information

Table S1 Homologs of OGDC and SSADH in cyanobacteria (only identities ≥60% were listed).

Cyanobacteria	2-Oxoglutarate decarboxylase（OGDC）		Succinic semialdehyde dehydrogenase（SSADH）	
	Accession number	Identity (%)	Accession number	Identity (%)
Synechococcus sp. PCC 7002	*SYNPCC7002-A2770*	100	*SYNPCC7002-A2771*	100
Cyanothece sp. ATCC 51142	*cce-4227*	81.8	*cce-4228*	64.8
Cyanothece sp. PCC 8801	*PCC8801-4168*	81.5	*PCC 8801-4169*	64.6
Synechocystis sp. PCC 6803	*sll1981*	80	*slr0370*	61.8
Cyanothece sp. PCC 7424	*PCC7424-0688*	80	*PCC7424-2737*	66.5
Arthrospira platensis NIES-39	*NIES39-L06130*	79	*NIES39-L06120*	66.2
Trichodesmium erythraeum IMS101	*Tery-1928*	78.2	*Tery-1926*	64.4
Anabaena sp. PCC 7120	*all3555*	77.3	*all3556*	65.3
Anabaena variabilis ATCC 29413	*Ava-3533*	77.3	*Ava3534*	65.3
Nostoc punctiforme ATCC 29133	*Npun-R4630*	77.2	*Npun-R4631*	65.9
Microcystis aeruginosa NIES-843	*MAE06010*	77.2	*MAE25340*	62.3
Cyanothece sp. PCC 7425	*Cyan7425-2710*	76.1	*Cyan7425-2711*	61.8
Acaryochloris marina MBIC11017	*AM1-1865*	75.7	*AM1-1864*	65.2
Gloeobacter violaceus PCC7421	*gll2804*	72.4	*gll2805*	60

Figure Legends

Fig. S1 The sequence of *efe* from *Pseudomonas syringae sesami* with the hot spot (CGATG) silenced and the optimized codons for *Synechocystis* sp. PCC 6803.

Fig. S2 Ethylene calibration curve and the GC-MS identification of the ethylene products of *efe* transformants. (A) Calibration curve plotting the ethylene peak area based on the ethylene amount injected into the GC-FID. A serial dilution of vaporized pure ethylene standards of known concentrations was prepared. A 200 μL sample of each standard was injected and analyzed by the GC-FID. The calibration curve was based on the amount of ethylene (nL) in the 200 μL sample relative to the ethylene peak area from the chromatogram. (B) MS identification of the ethylene standard and headspace gas of the cultures of the *efe* transformants.

Fig. S3 Ethylene production of *Synechocystis* derivants (XX57), with *efe* controlled by P_{psbA2} of *Synechocystis* sp. PCC 6803. (A) The cell growth curve of *Synechocystis* sp. PCC 6803 wild type and XX57 under aerated cultivation with 250-mL flasks. (B) Stable ethylene production of XX57.

Fig. S4 SDS-PAGE of the purified thioredoxin (TrxA)-Sll1981 fusion protein and TrxA-Slr0370 fusion protein. (A) The gel of the purified TrxA-Sll1981. Lane 1: soluble protein; lane 2-5: the target protein eluted by Tris buffer containing 250 mM iminazole. (B) The gel of the purified TrxA-Slr0370. Lane 1: soluble protein; lane 1-5: the target protein eluted by Tris buffer containing 250 mM iminazole.

Fig. S5 The schematic diagram of construction of the recombinant *Synechocystis* strains harboring one, two or three copies of *efe* and *kgtP*.

Fig. S6 Cultivation and resuspension of XX109 recombinants in fresh 5×BG11* medium with 2-OG added or not. (A) Total ethylene production rate; (B) specific ethylene production rate; (C) growth curve of XX109 in 5×BG11* medium in the semi-continuous cultivation. Data presented are means and standard deviation of two

independent experiments, each with three replicates.

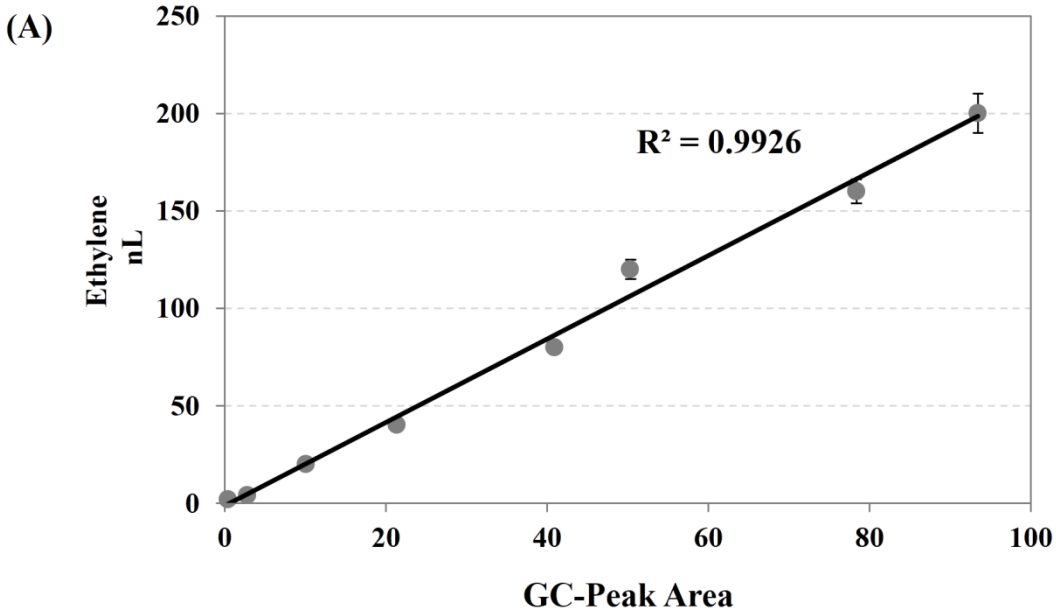
Fig. S7 A modified CO_2 diffusion-based gaseous/aqueous two-phase photobioreactor. The modified gaseous/aqueous two-phase photobioreactor was developed according to Bentley and Melis[1] in order to harvest ethylene continuously. The new gas collection section of the instrument was based on the water gas-displacing principle. *Synechocystis* cells of the stationary phase were spun down and resuspended in 5×BG11* medium to OD_{730}~2.5, and 800 mL culture was then grown in the photobioreactor while being constantly bubbled with 100% CO_2 at a rate slower than 2 mL min^{-1} and a high light intensity of 200 μmol photons m^{-2} s^{-1}. An experimental period of 16 days was set up, wherein a fed-bath was performed every 4 days to restore the cell density to OD_{730}~2.5, and approximately 500 mL of the gas mixture was collected every 8 hours from the gas collection section.

Fig. S8 Continuous cultivation of XX109 and XX110 and ethylene production in the modified gaseous/aqueous two-phase bioreactor. XX109 and XX110 of OD_{730}~2.5 were cultured in the bioreactor under constant irradiance (~200 μmol photons m^{-2} s^{-1}) with continual sparging with 100% CO_2 at a rate slower than 2 mL min^{-1}. Cultures were diluted with fresh 5×BG11* medium (2-OG of 1 mM was contained for XX110) every 4 days to recover OD_{730}~2.5 in order to maintain stable and efficient ethylene production. The assay was conducted for 16 days. The daily ethylene productivity was shown as the sum of the ethylene amount in the 3 bottles collected every 8 hours from the water gas displacing gas collection section. (A) Cell growth of each 4-day dilution during the continuous cultivation in the bioreactor. (B) Ethylene accumulated over the time course of the continuous cultivation.

Figure S1

ATGACTAACTTACAAACCTTCGAACTGCCAACCGAAGTAACTGGCTGTGCCGCTGACAT
TTCCCTGGGCCGCGCGCTCATTCAAGCCTGGCAAAAAGACGGTATTTTTCAAATTAAAA
CTGATAGCGAACAAGATCGGAAAACTCAGGAGGCCATGGCAGCCAGCAAACAATTCT
GTAAAGAGCCGCTAACCTTTAAAAGTTCCTGTGTGTCCGACCTGACCTATTCTGGTTAC
GTCGCCAGTGGCGAGGAAGTTACGGCCGGCAAACCCGATTTTCCTGAGATTTTTACAG
TGTGCAAGGATCTAAGCGTCGGTGATCAACGCGTGAAAGCTGGCTGGCCGTGTCATGG
ACCGGTGCCCTGGCCGAACAACACCTATCAAAAAAGCATGAAAACTTTTATGGAAGAG
TTAGGGTTAGCTGGAGAAAGGTTGCTCAAGTTGACAGCCTTGGGATTCGAGTTACCCA
TCAATACTTTTACCGATTTAACCAGGGATGGCTGGCATCACATGCGAGTGCTACGGTTC
CCTCCACAAACCAGTACCTTGTCTCGGGGAATCGGCGCCCATACCGATTATGGTTTGCT
CGTGATTGCTGCGCAGGATGACGTGGGCGGGCTGTACATTCGTCCCCCTGTTGAAGGG
GAAAAGCGTAATCGTAACTGGTTGCCCGGAGAAAGTTCTGCTGGGATGTTTGAACATG
ATGAACCCTGGACATTTGTTACCCCTACTCCCGGTGTATGGACCGTTTTTCCCGGTGAC
ATCTTACAGTTTATGACCGGGGGGCAGTTGCTGAGTACGCCCCATAAGGTCAAACTGA
ATACGCGGGAACGATTTGCATGCGCGTACTTCCACGAACCAAATTTTGAAGCCTCTGCG
TATCCTCTCTTTGAACCCTCCGCAAATGAACGCATCCATTATGGCGAACACTTTACAAAT
ATGTTTATGCGGTGCTACCCCGACCGCATTACCACTCAGTCCATTAATAAAGAAAATCGT
CTAGCCCACTTGGAAGATTTAAAAAAGTATAGTGATACGCGCGCCACCGGTTCCCACCA
CCACCACCACCACTAA

Figure S2


(A)
250
200
150
100
50
0
Ethylene nL
R² = 0.9926
0
20
40
60
80
100
GC-Peak Area

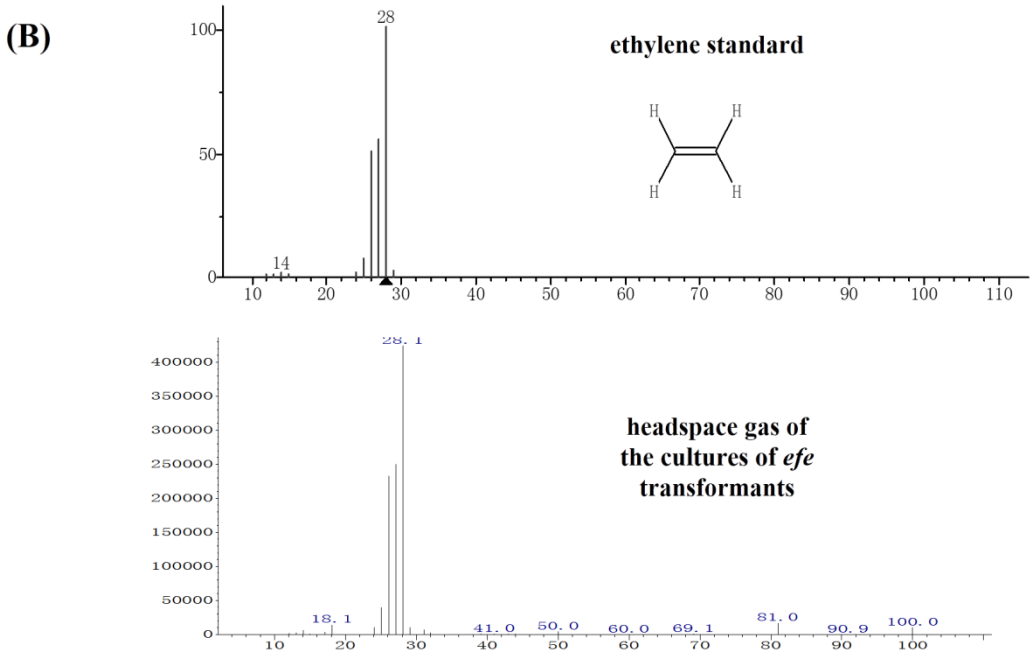

(B)
100
50
0
28
14
10
20
30
40
50
60
70
80
90
100
110
ethylene standard
H
H
H
H
400000
350000
300000
250000
200000
150000
100000
50000
0
18.1
41.0
50.0
60.0
69.1
81.0
90.9
100.0
10
20
30
40
50
60
70
80
90
100
headspace gas of the cultures of *efe* transformants

Figure S3

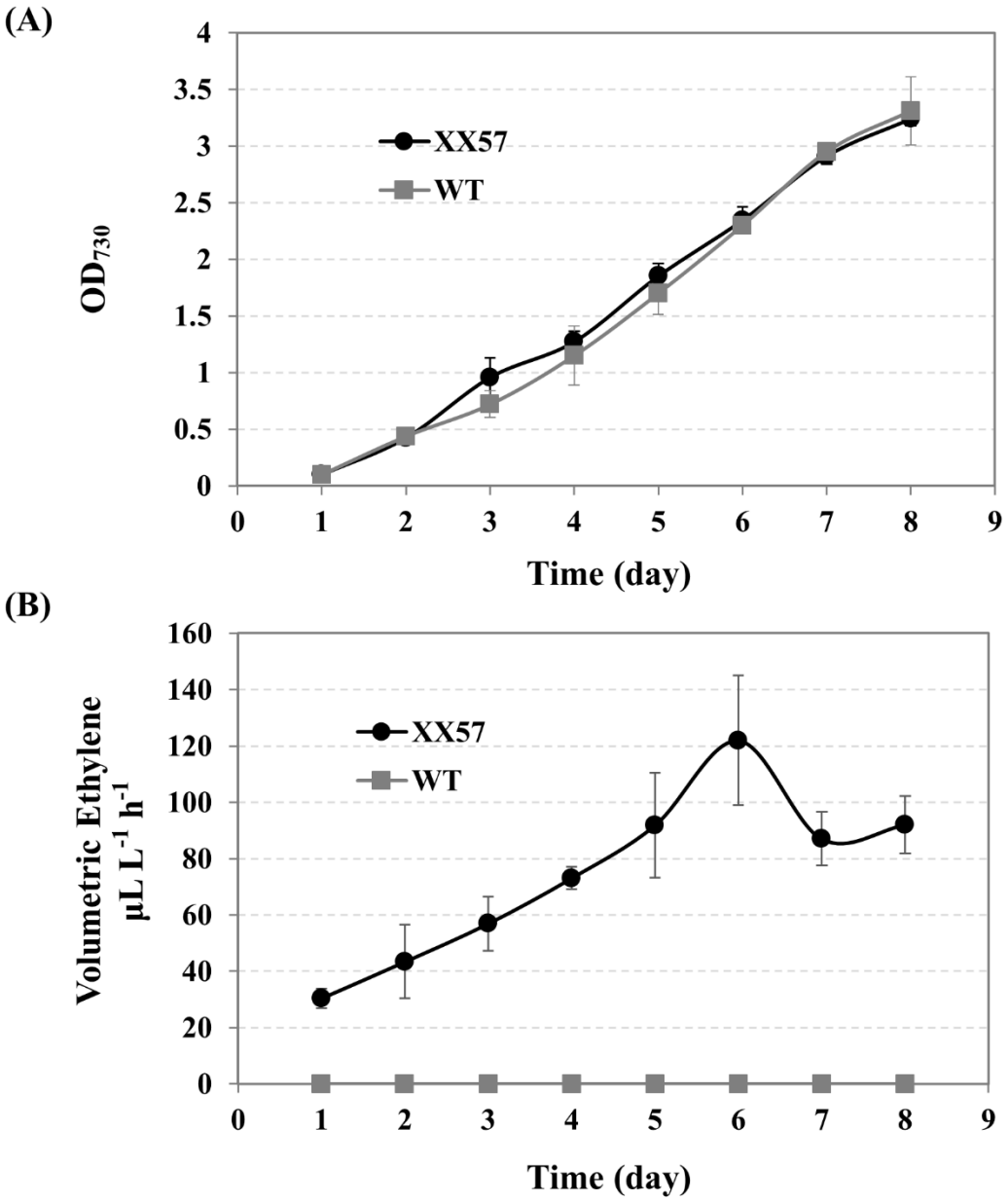

(A)
OD730
XX57
WT
Time (day)
(B)
Volumetric Ethylene
μL L-1 h-1
XX57
WT
Time (day)

Figure S4

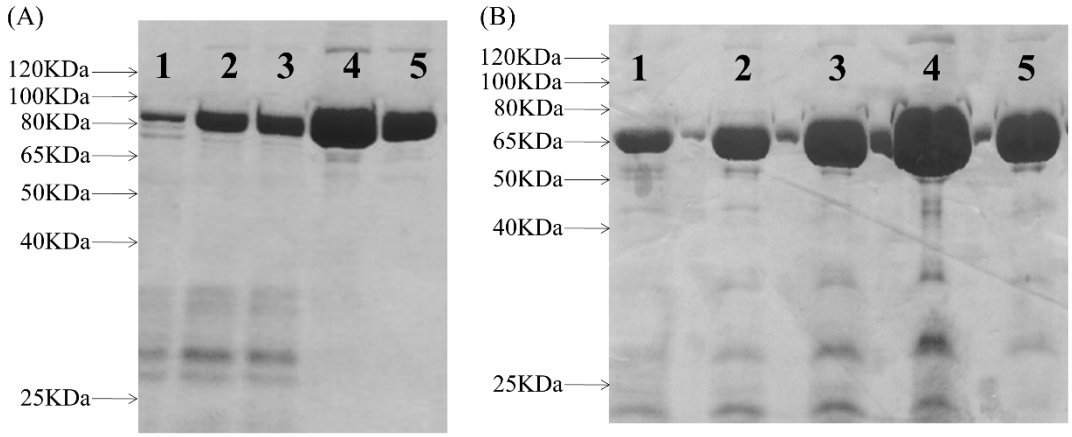

(A)
120KDa
100KDa
80KDa
65KDa
50KDa
40KDa
25KDa
1
2
3
4
5
(B)
120KDa
100KDa
80KDa
65KDa
50KDa
40KDa
25KDa
1
2
3
4
5

Figure S5

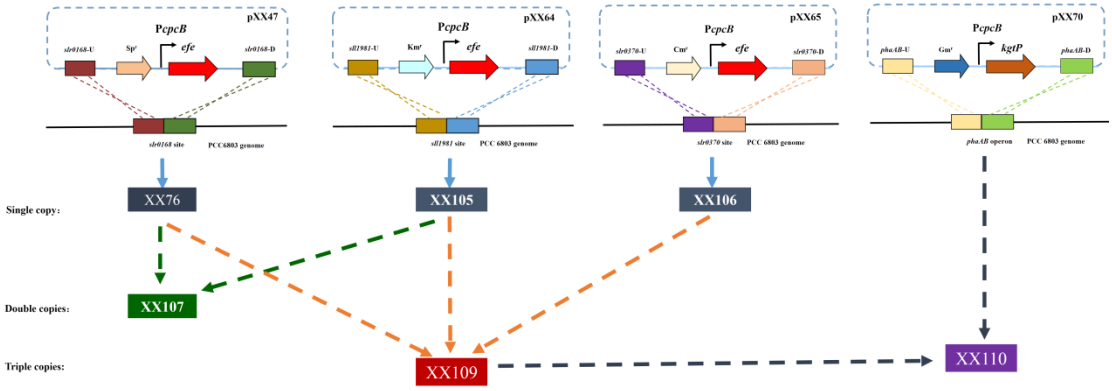

pXX47
PcpcB
slr0168-U
Sp^r
efe
slr0168-D
slr0168 site
PCC 6803 genome
pXX64
PcpcB
sll1981-U
Km^r
efe
sll1981-D
sll1981 site
PCC 6803 genome
pXX65
PcpcB
slr0370-U
Cm^r
efe
slr0370-D
slr0370 site
PCC 6803 genome
pXX70
PcpcB
pha.AB-U
Gm^r
kgtP
pha.AB-D
pha.AB operon
PCC 6803 genome
Single copy:
XX76
XX105
XX106
Double copies:
XX107
Triple copies:
XX109
XX110

Figure S6

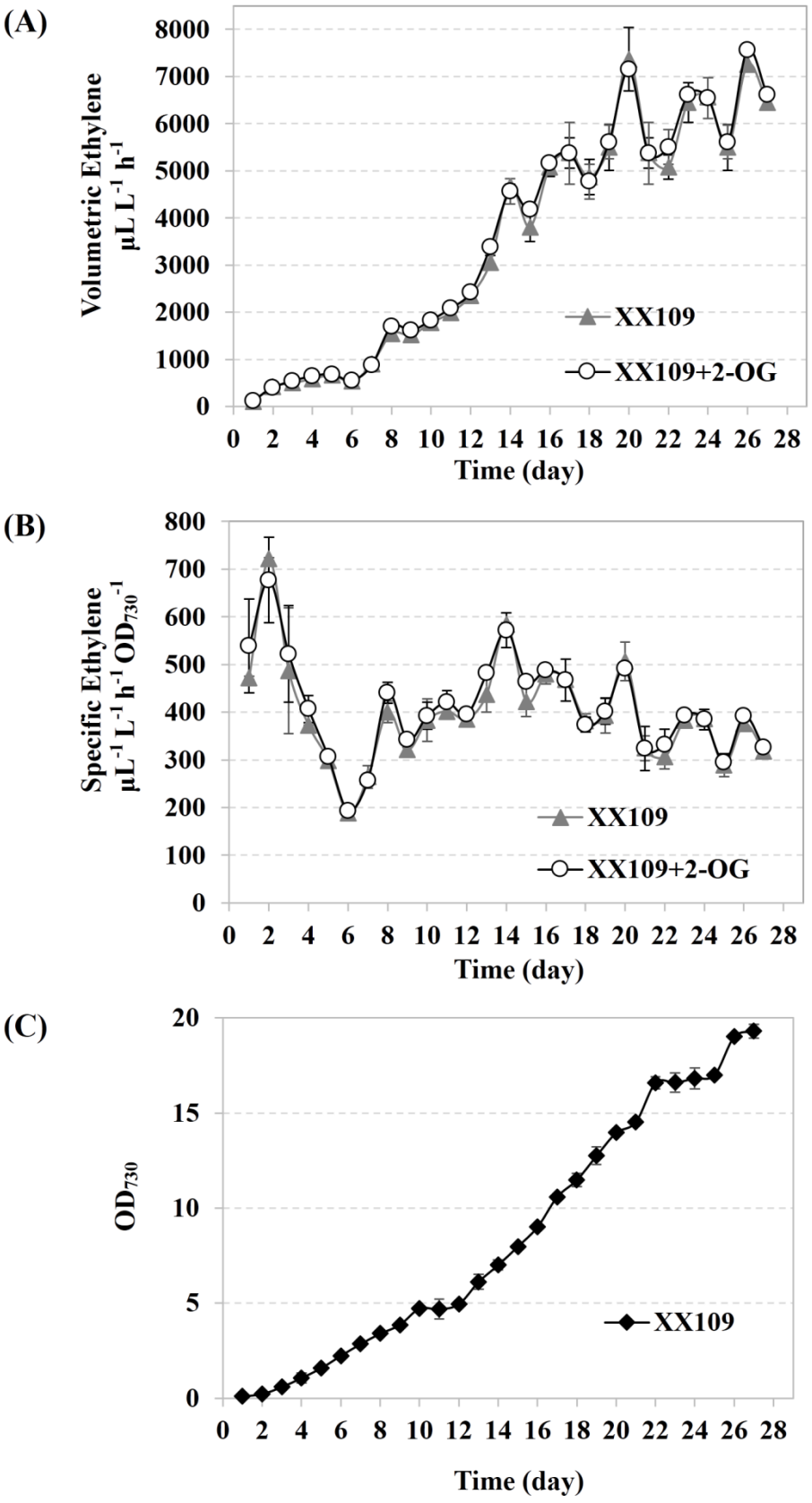

(A)
Volumetric Ethylene
μL L-1 h-1
8000
7000
6000
5000
4000
3000
2000
1000
0
XX109
XX109+2-OG
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28
Time (day)
(B)
Specific Ethylene
μL-1 L-1 h-1 OD730-1
800
700
600
500
400
300
200
100
0
XX109
XX109+2-OG
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28
Time (day)
(C)
OD730
20
15
10
5
0
XX109
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28
Time (day)

Figure S7

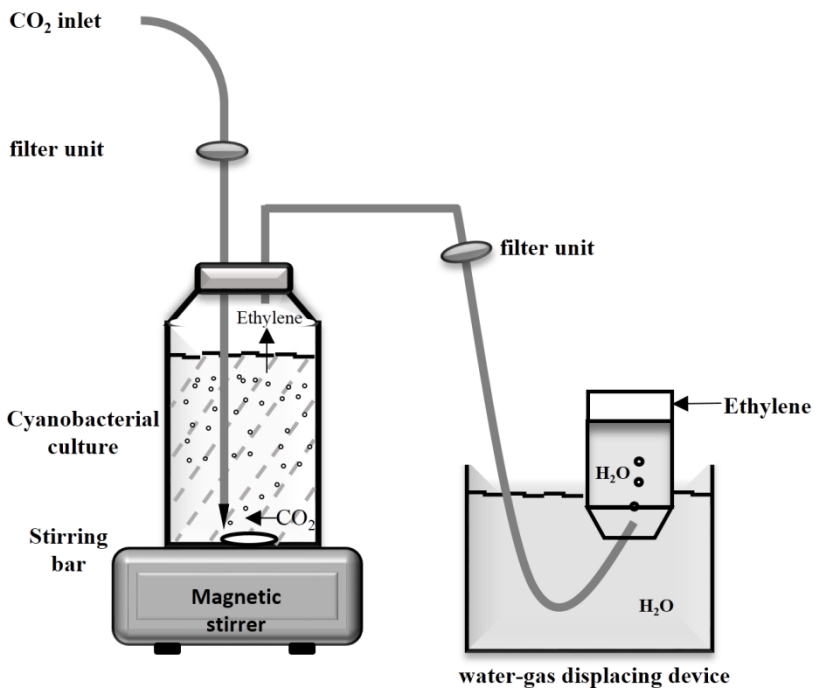
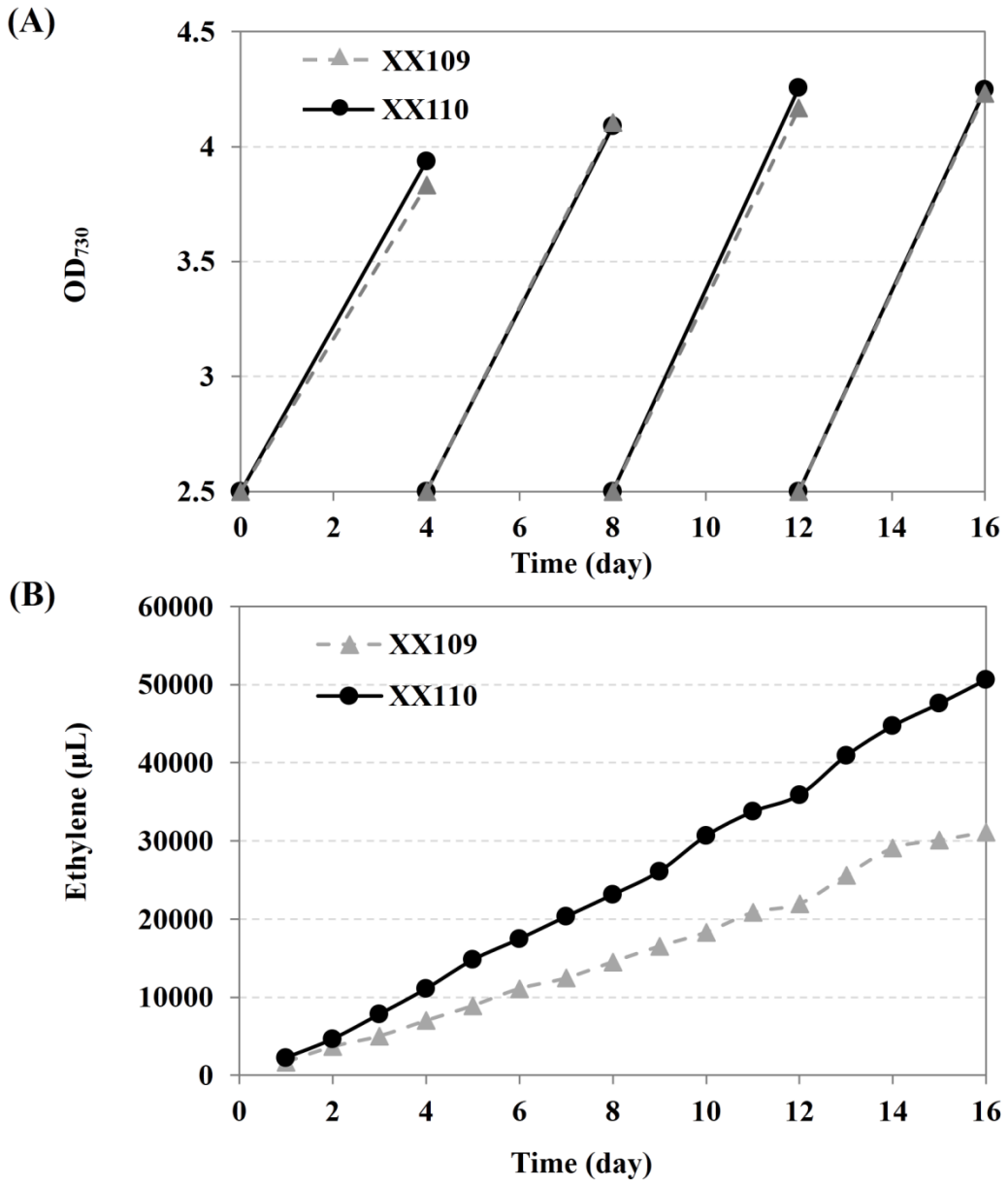

CO_2 inlet
filter unit
filter unit
Ethylene
Cyanobacterial culture
Stirring bar
CO_2
Magnetic stirrer
Ethylene
H_2O
H_2O
water-gas displacing device

Figure S8


(A)
XX109
XX110
OD_{730}
4.5
4
3.5
3
2.5
0
2
4
6
8
10
12
14
16
Time (day)
(B)
XX109
XX110
Ethylene (μL)
60000
50000
40000
30000
20000
10000
0
0
2
4
6
8
10
12
14
16
Time (day)

Reference

1. F. K. Bentley and A. Melis, *Biotechnol. Bioeng.*, 2012, **109**, 100-109.