Towards sustainable ethylene production with cyanobacterial artificial biofilms

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Supplementary information†

Section S1: Experimental section

Extraction, purification, and identification of photosynthetic pigments

Synechocystis WT and *efe* mutant cells were harvested from the pre-culture growth conditions (see growth conditions section). The cells containing 15 µg of total Chl were collected by standard Chl determination method (see Chl determination section). The pigments were extracted from centrifuged cells with 100% methanol for 30 min at 4°C in the dark. The extracted pigment samples were centrifuged at 20000 rpm for 5 min to eliminate the cell debris. The supernatants were filtered through a syringe filter (0.2 mm PTFE 0.2-µm pore size). The extracted photosynthetic pigments were separated by the Agilent 1100 Series HPLC (Agilent Technologies) with the C18 reverse-phase column (4×125 mm, 5µm, LiChroCART, Merck KGaA) and identified with a diode array detector. Two solvents systems were used in a row at a constant flow rate of 500 µl min^{-1}.[1] The solvent A buffer containing acetonitrile/methanol/0.1M Tris–HCl buffer at pH 8.0 (72: 8 : 3, v/v) was used for 4 min isocratic run, followed by a linear gradient with solvent B containing methanol/hexane (4: 1, v/v) for 15 min from 0-100%. After that, solvent B was used in the isocratic run for 26 min. In between samples, the re-equilibration of columns was applied for 10 min with solvent A buffer. The pigments were identified at 440 nm. Major carotenoids in the *Synechocystis* species (ie. *β*-carotene, xanthophylls) and Chl pigments were detected based on their absorption spectra and compared with standard pigments retention times.

Section S2: Supplementary figures

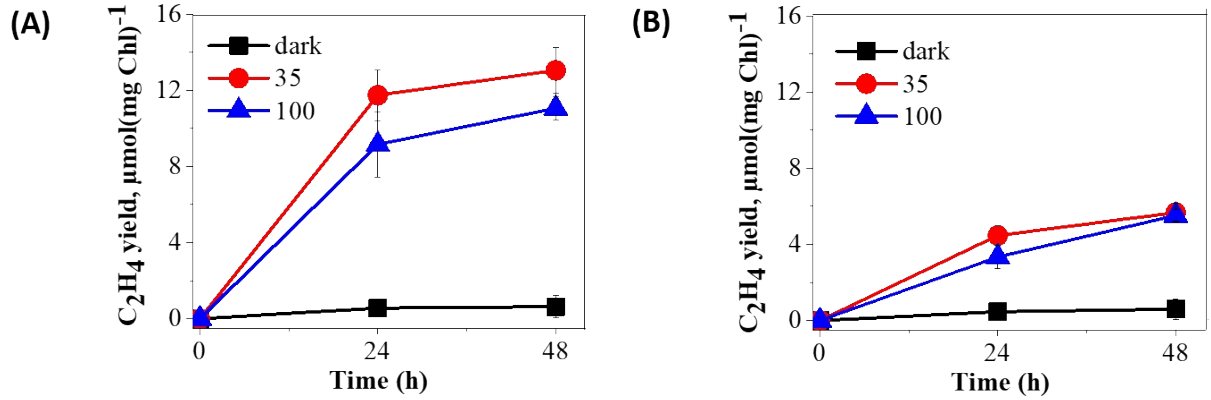

Figure S1. Effect of two different radiances on ethylene production from *Synechocystis efe* mutant. A) Cumulative ethylene yield (at 24 and 48 h) was normalized per initial total Chl content of Synechocystis *efe* strain under different radiances (35 and 100 µmol photons m^{-2} s^{-1}, respectively), compared to the dark regime. B) The yield of ethylene was calculated per total Chl content that was obtained every 24 h. The condition of ethylene production and induction was implemented as described in Fig. 1.

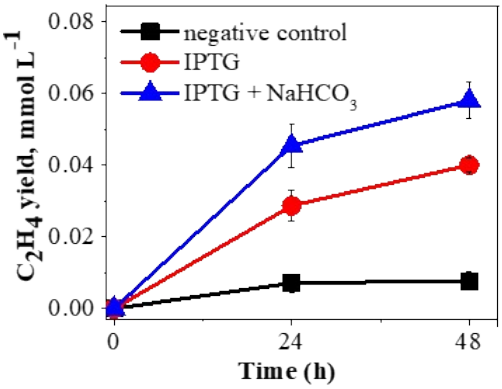

Figure S2. Effect of inducer and Ci source on the ethylene production in *Synechocystis efe* mutant under continuous illumination (35 µmol photons m^{-2} s^{-1}). Ethylene production was monitored for 48 h in the absence of IPTG and $NaHCO_3$ (negative control), in the presence of 1 mM IPTG alone and combined with 20 mM $NaHCO_3$. The yield of ethylene was calculated per liter of suspension cultures.

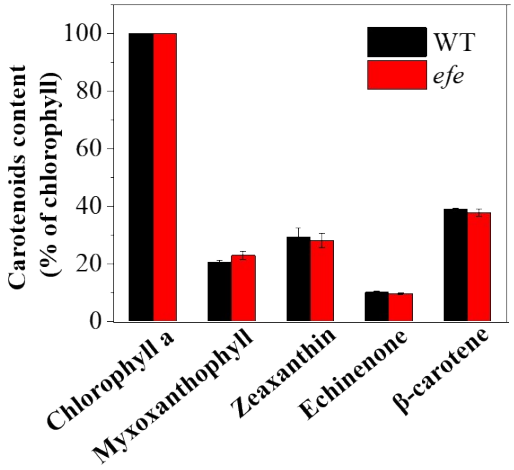

Figure S3. HPLC analysis of the photosynthetic pigment composition of *Synechocystis* WT and *efe* mutant cells. Prior to the pigments extraction, the Chl content of both cultures was adjusted to 15 µg. Values are given as the percentage of carotenoid molecules relative to its Chl.

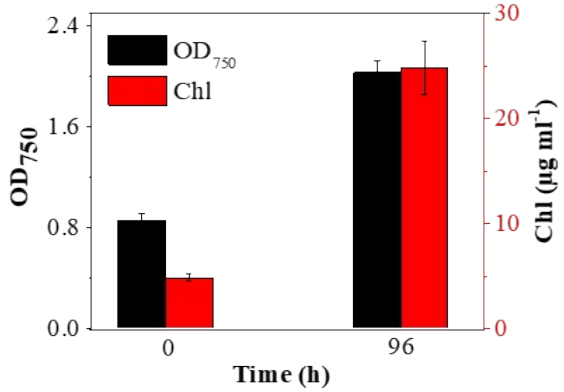

Figure S4. The growth of *Synechocystis efe* cultures in the presence of 1 mM IPTG and 20 mM $NaHCO_3$ in the flasks allowing efficient gas exchange. OD_{750} and Chl content of the cells was monitored at 0 and 96 h of the start of incubation.

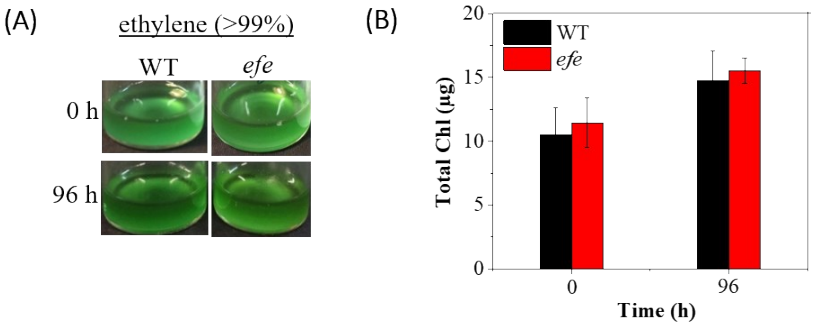

Figure S5. Effect of exogenously added >99% ethylene on the *Synechocystis efe* mutant and its corresponding WT. A) the growth phenotype and B) Chl content of cells was observed at 0 and 96 h of the start of the incubation.

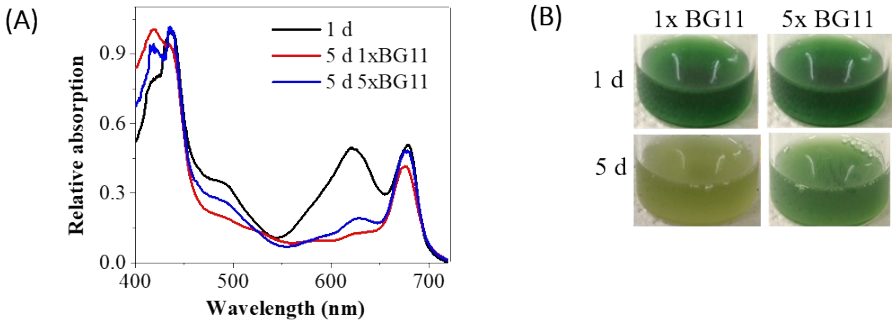

Figure S6. Effect of 1x and 5x BG11 medium on the photosynthetic pigments of the ethylene producing *Synechocystis efe* mutant. The suspension cultures were incubated with 200 mM $NaHCO_3$ and 1 mM IPTG under ethylene induction conditions. A) the whole-cell absorption spectra were obtained from cells possessing the same OD_{750}. The spectra were normalized to the Chl absorption measured at 438 nm. B) phenotypic changes of cells were picturized under supplementation of 1x and 5x BG11 medium.

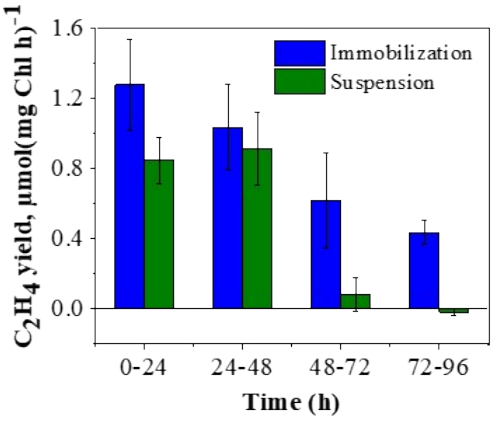

Figure S7. The rate of ethylene production was compared between immobilized and suspension batch cultures of *Synechocystis efe* over 96 h. Total yield and rate of ethylene productivity between suspension and immobilized cells were normalized on their initial total Chl content.

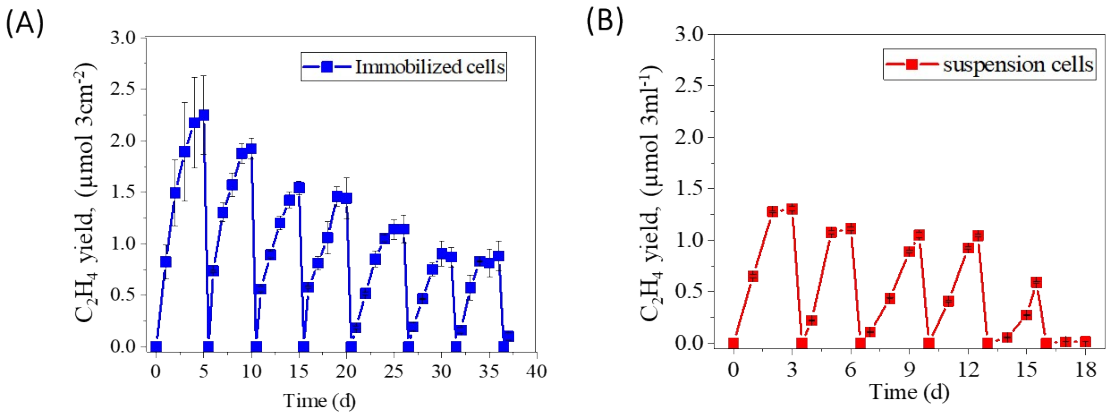

Figure S8. The yield of absolute production of ethylene between immobilized (A) and suspension cells (B) without Chl normalization. The experimental cultures were started with the similar biomass load for a direct comparison of ethylene yield from two different production systems. The cells were incubated with 200 mM $NaHCO_3$ and 1 mM IPTG under ethylene induction conditions. Every 5 d immobilized cells and 3 d suspension cells were periodically replenished with fresh BG11 medium (3 mL in 23.5 mL vial) containing inducer and Ci source.

Section S3: Supplementary table

Table S1. Relative changes of pH under various concentrations of $NaHCO_3$ supplementation of *Synechocystis efe* cells.

Time	$NaHCO_3$ (mM)				
	0	20	100	200	300
0-5 min	7.5	7.9	8.0	8.0	8.0
24-96 h	7.5-8.0	10.2-10.5	10-10.5	9.8-10.4	10.1-10.2

References

1 A. M. Gilmore and H. Y. Yamamoto, *J. Chromatogr.,* 1991**, 543,** 137–145