



Scale-up cultivation of recombinant *Synechococcus* under natural light conditions for production of ethylene

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ABSTRACT

Cyanobacteria are globally considered as photosynthetic platforms for production of hydrocarbons, albeit at laboratory scales. However, to elevate their application at the commercial scale, scale up studies under real time cultivation conditions become a prerequisite. In the current study, attempts were made for cultivation of recombinant strain of *Synechococcus elongatus* PCC 7942 from 1L to 100L scale under natural light regime using closed tubular reactor systems. The strain was engineered for heterologous production of ethylene by overexpressing ethylene forming enzyme (*efe*) gene. Our studies successfully demonstrated cultivation of transformants with ethylene productivity of $1.57 \text{ mL L}^{-1} \text{ h}^{-1} A_{730}^{-1}$ using vertical air-lift photobioreactor under outdoor cultivation regime with natural dynamic light conditions (max. $1200 \pm 300 \mu\text{mol m}^{-2} \text{ s}^{-1}$). Further, inorganic carbon supplementation in the form of bicarbonate was found to improve the cell sustenance and biomass production at higher scales, surpassing typical inhibitions posed by physico-chemical attributes during scale-up. Overall, our investigation serves as the holistic foundation for future research in the field of scale-up cultivation of engineered cyanobacteria.

ASSESSING PROMOTER FUNCTIONALITY

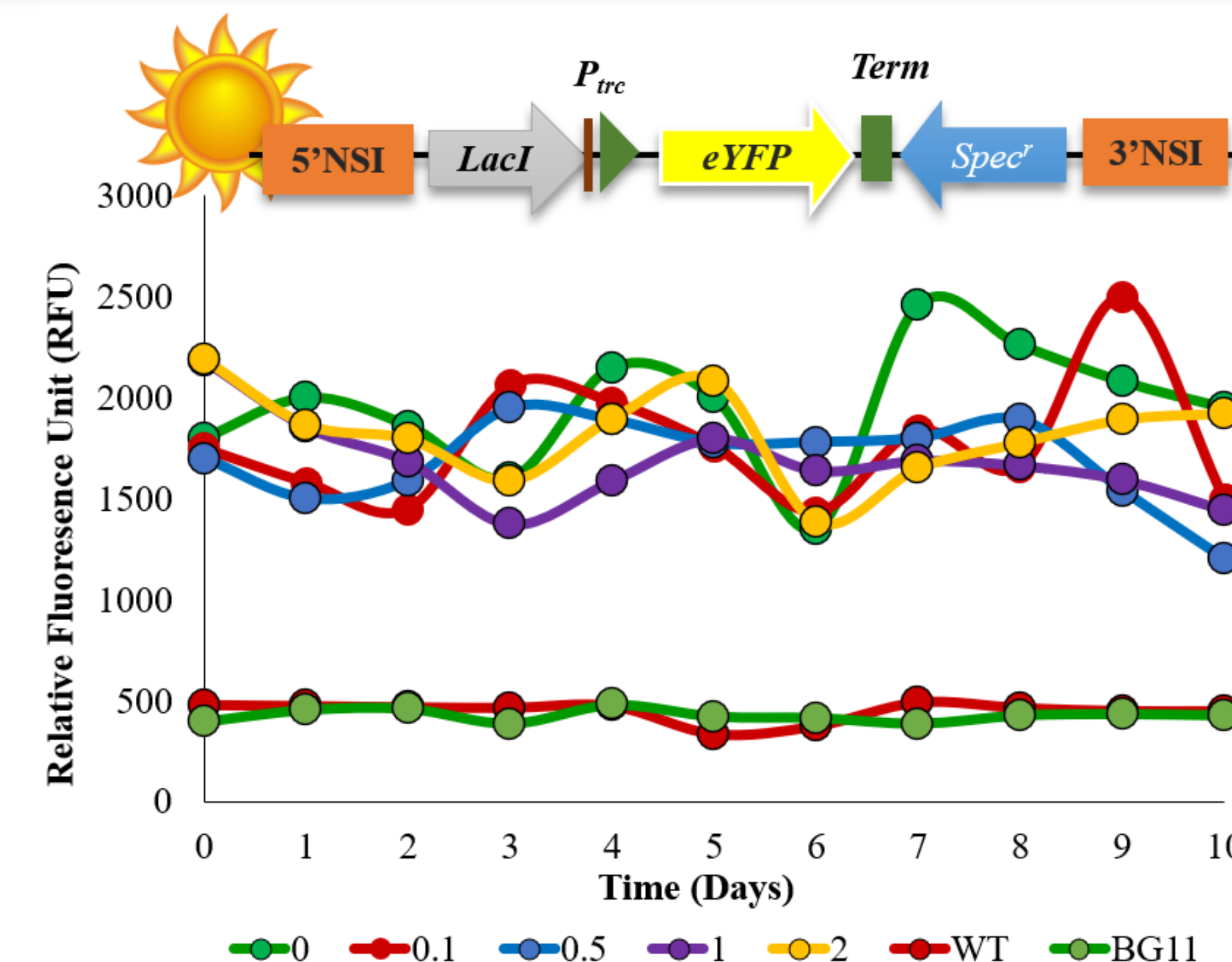
Host: *Synechococcus*

Strategy: Homologous recombination of *eyfp* at the NS

Variations: Strains were cultivated under different IPTG concentrations

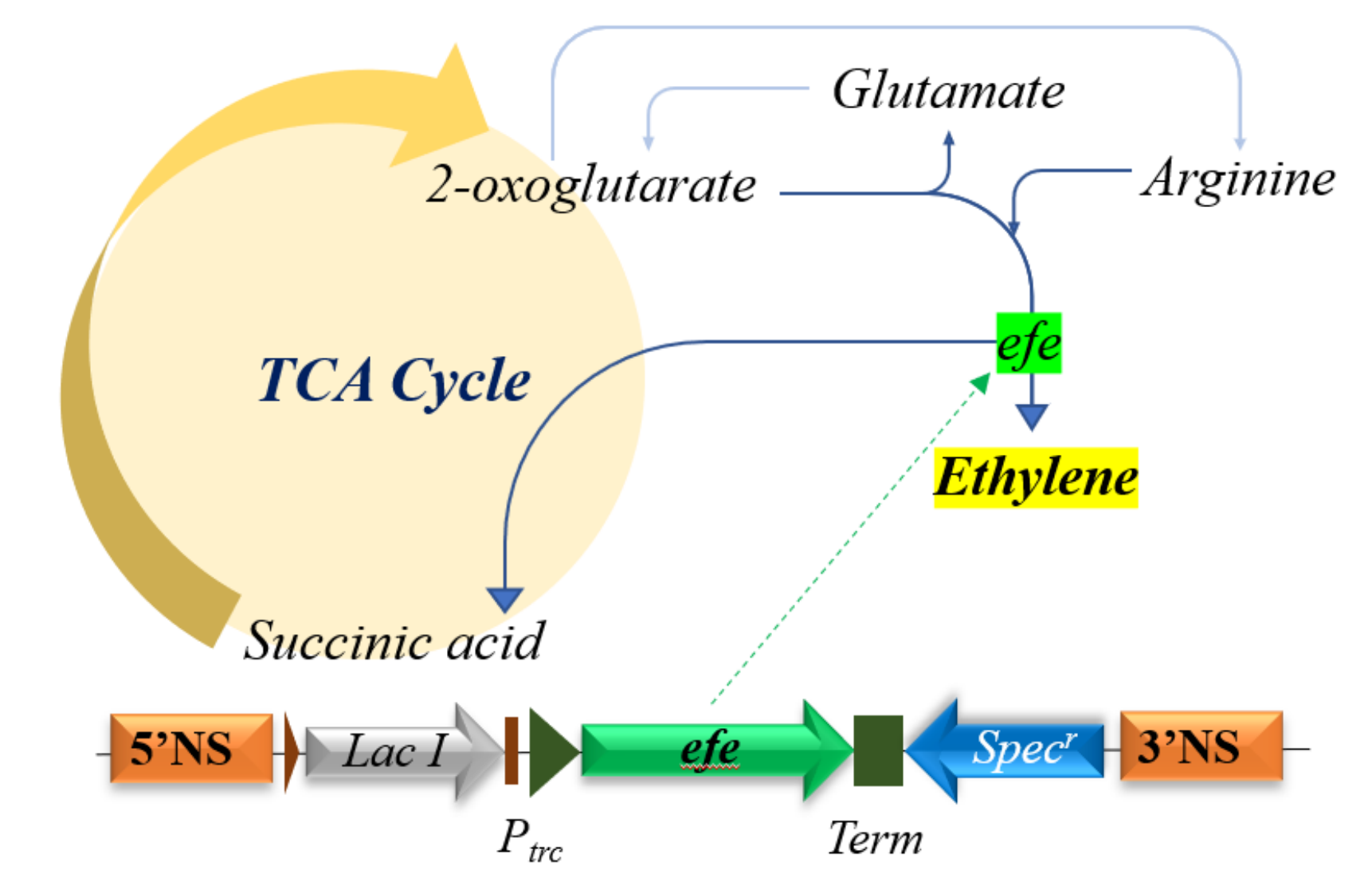
Inference: P_{trc} is near-constitutive under natural light conditions

Advantage: No requirement of inducer at higher scales

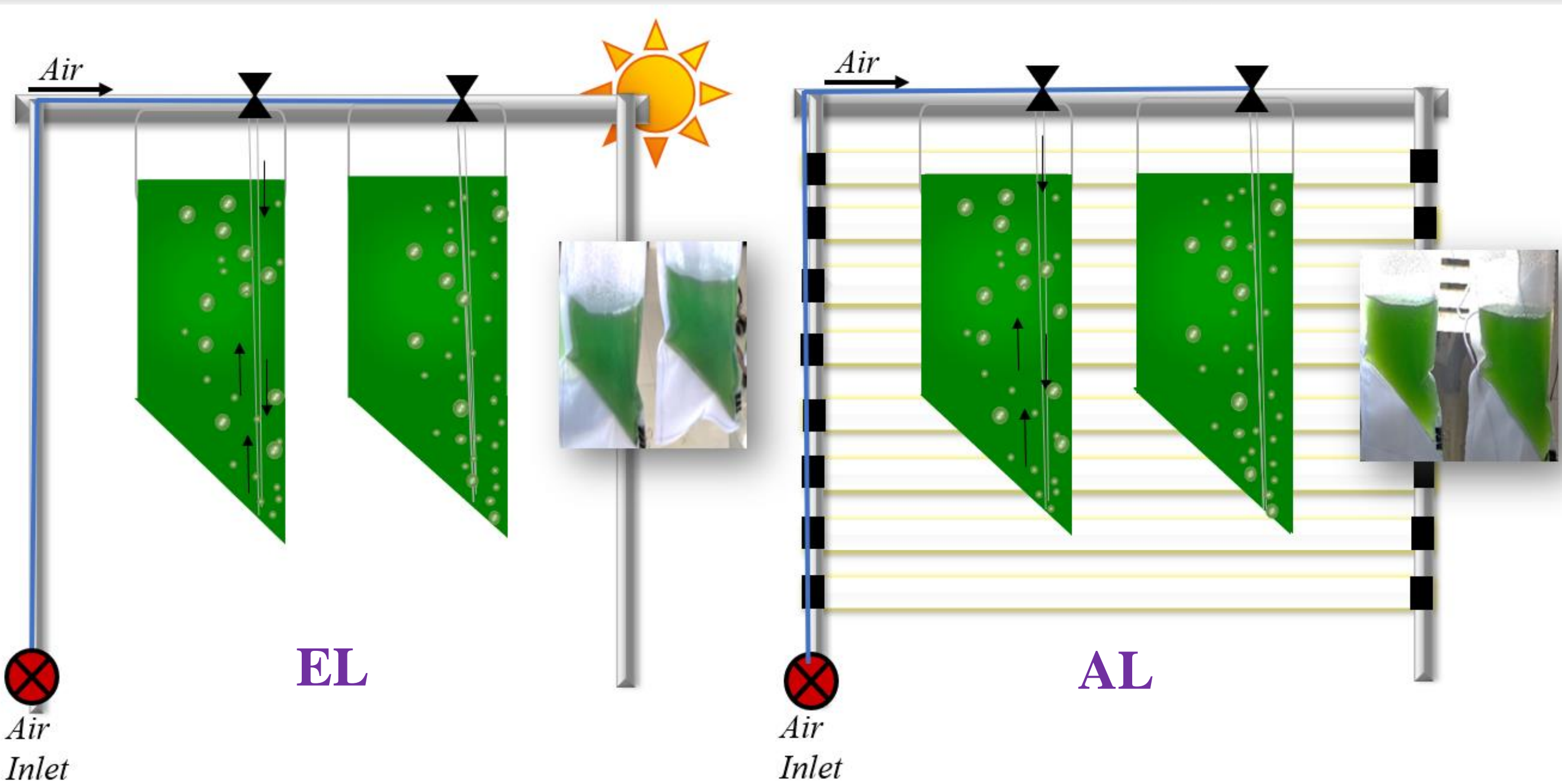


PATHWAY ENGINEERING

Cloning codon optimized *efe* gene in *Synechococcus*



PROTEIN SYNTHESIS AND ETHYLENE PRODUCTION



Host: *Synechococcus*

Cloned gene: *efe* under P_{trc} promoter

Variations:

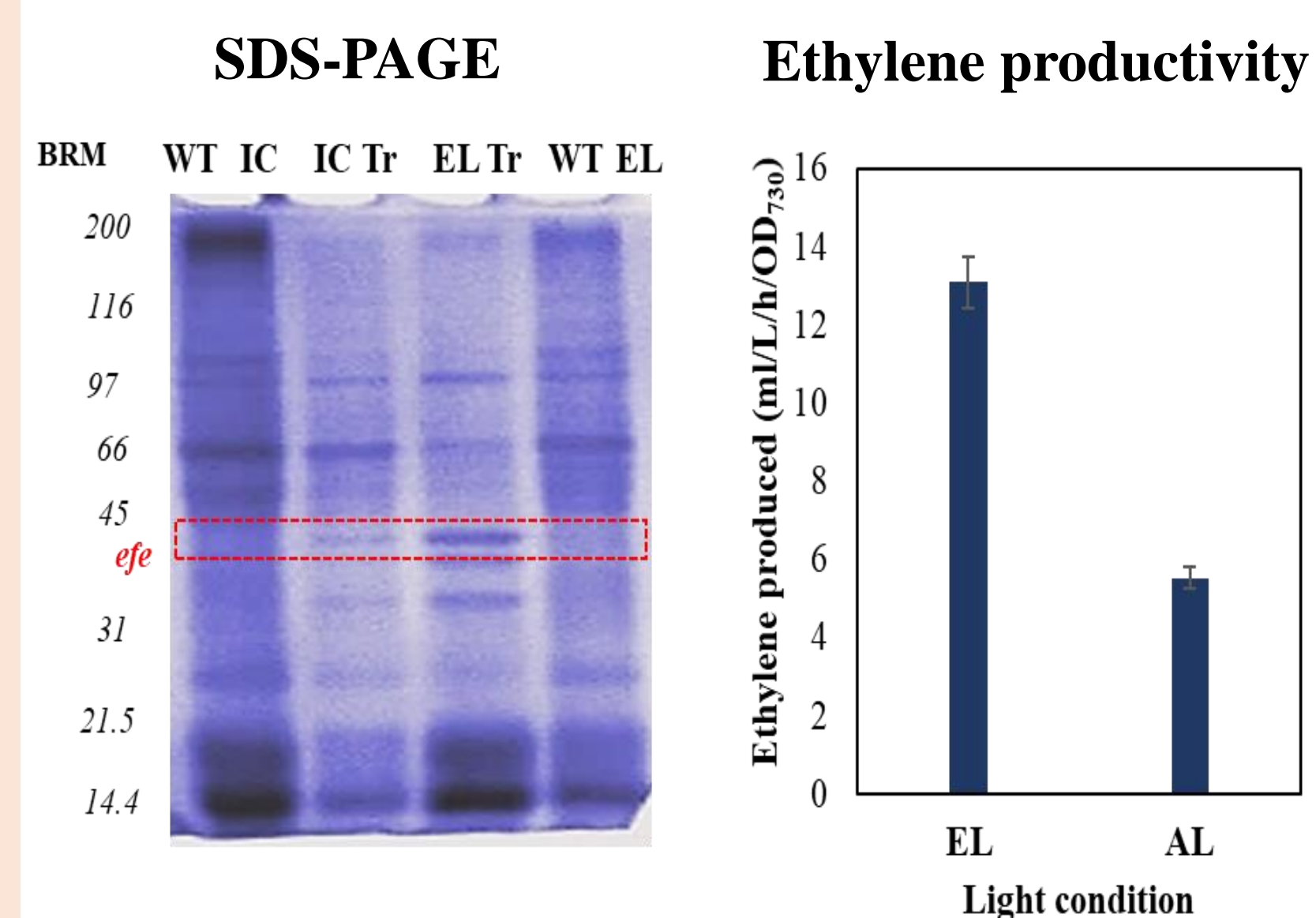
Light conditions:

EL: Natural light conditions (max. $1200 \pm 300 \mu\text{mol m}^{-2} \text{ s}^{-1}$)

AL: Diurnal light conditions using artificial lights

Inference: EL supports effective protein synthesis and ethylene productivity

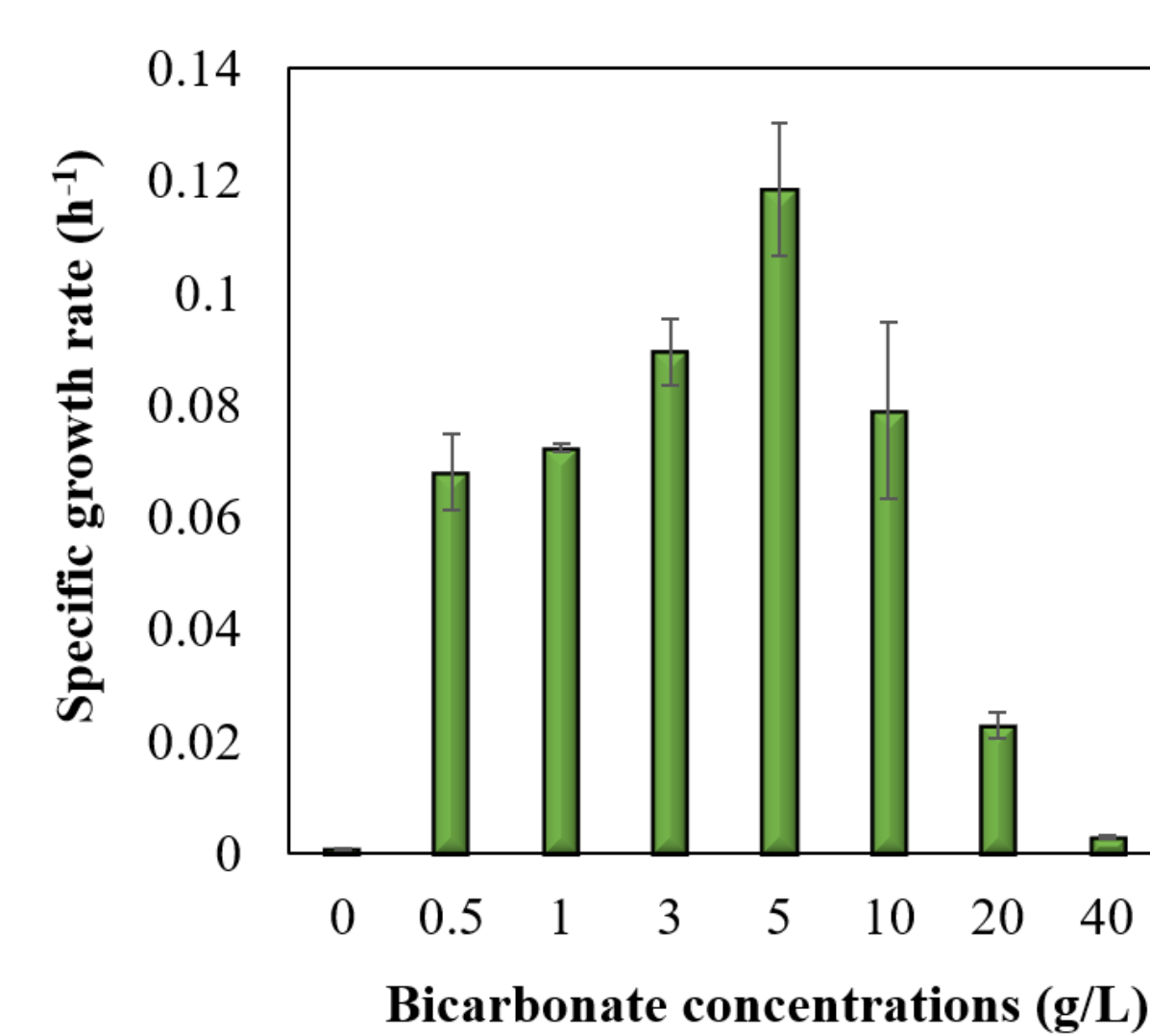
Comparative analysis



SCALE-UP CULTIVATION

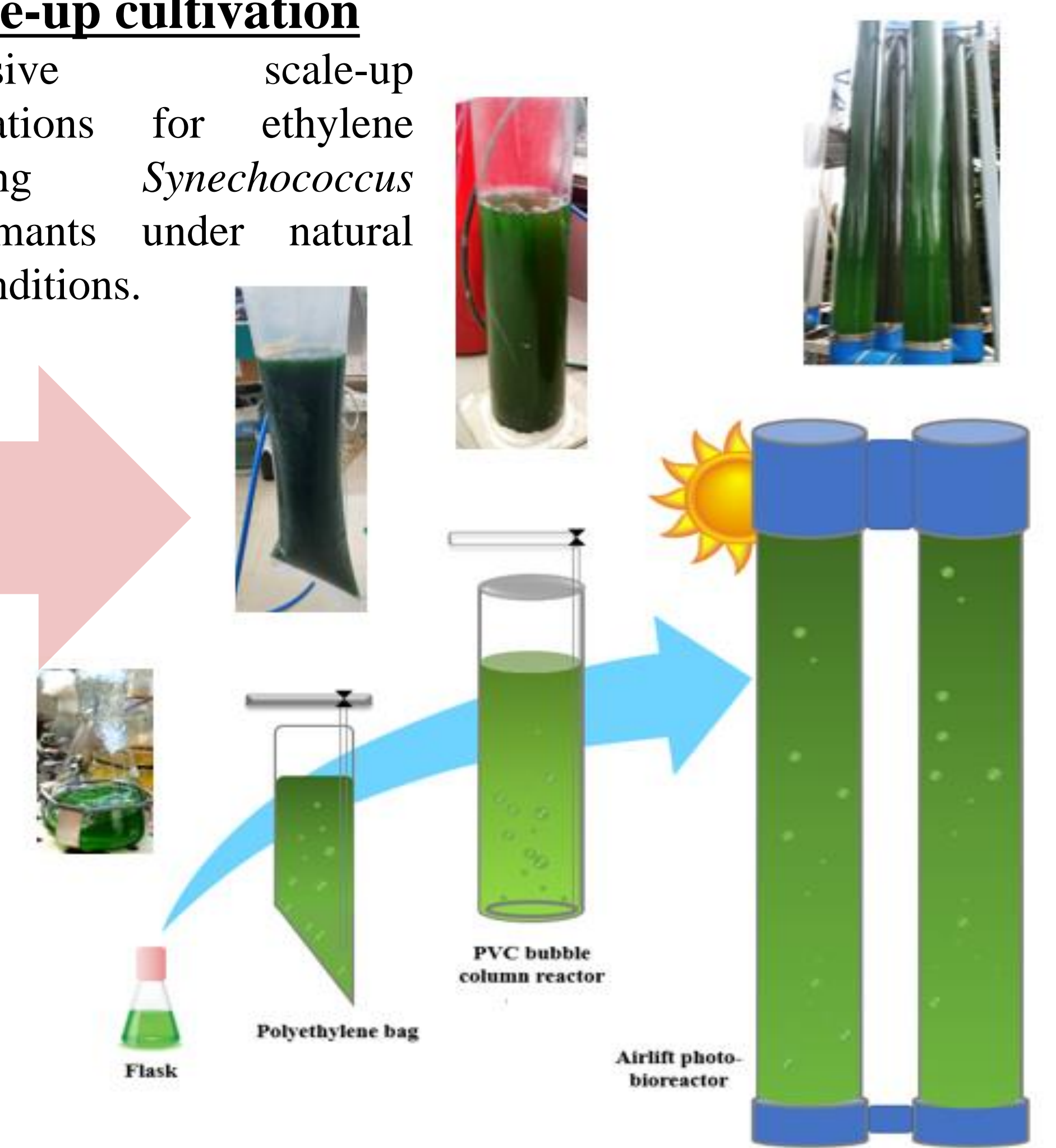
Growth optimization studies

5g/L bicarbonate supplementation was found to optimally support the cell growth under dynamic natural light conditions



Scale-up cultivation

Progressive scale-up optimizations for ethylene producing *Synechococcus* transformants under natural light conditions.



Host: *Synechococcus*

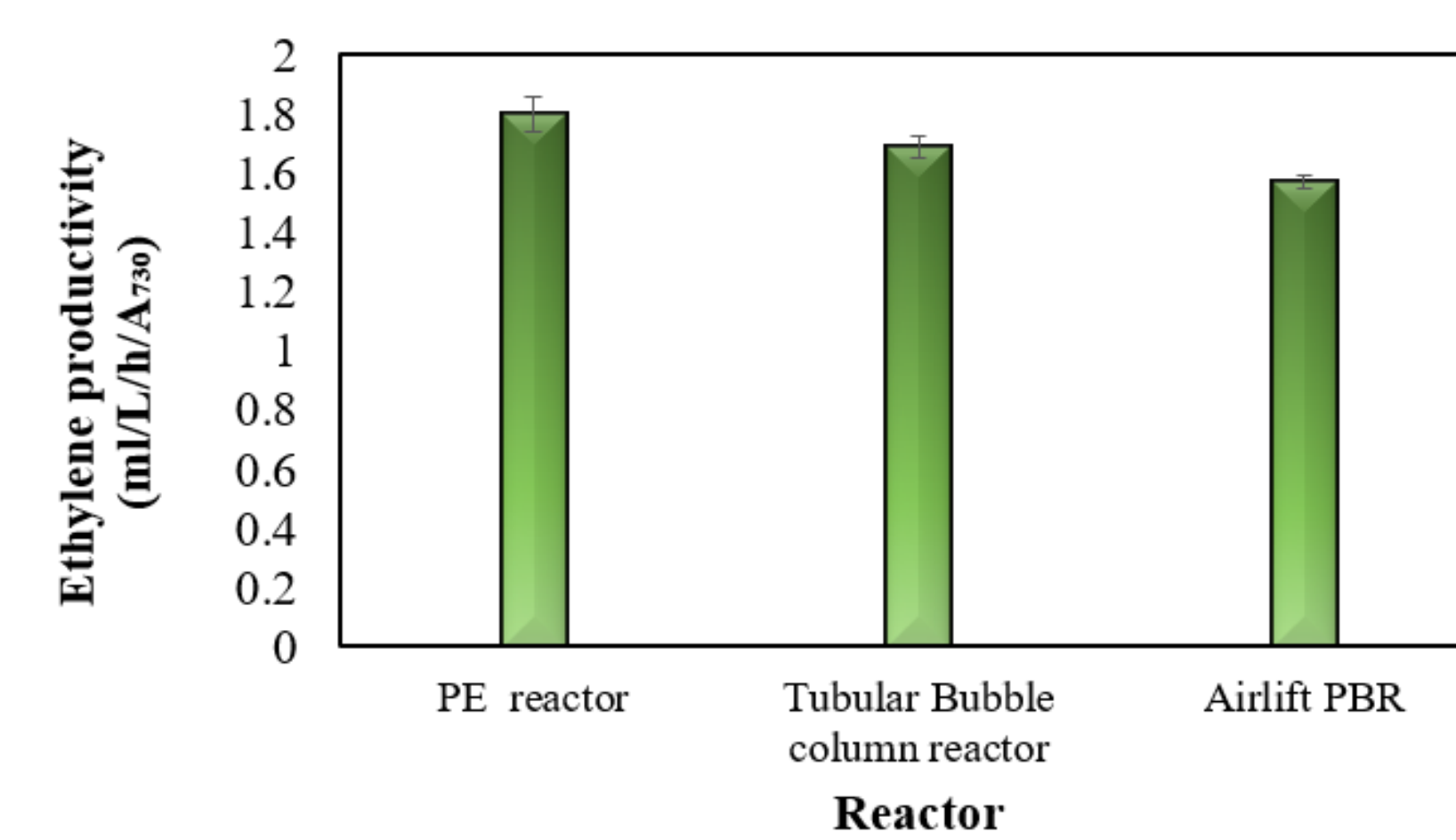
Cloned gene: *efe* under P_{trc} promoter

Variations:

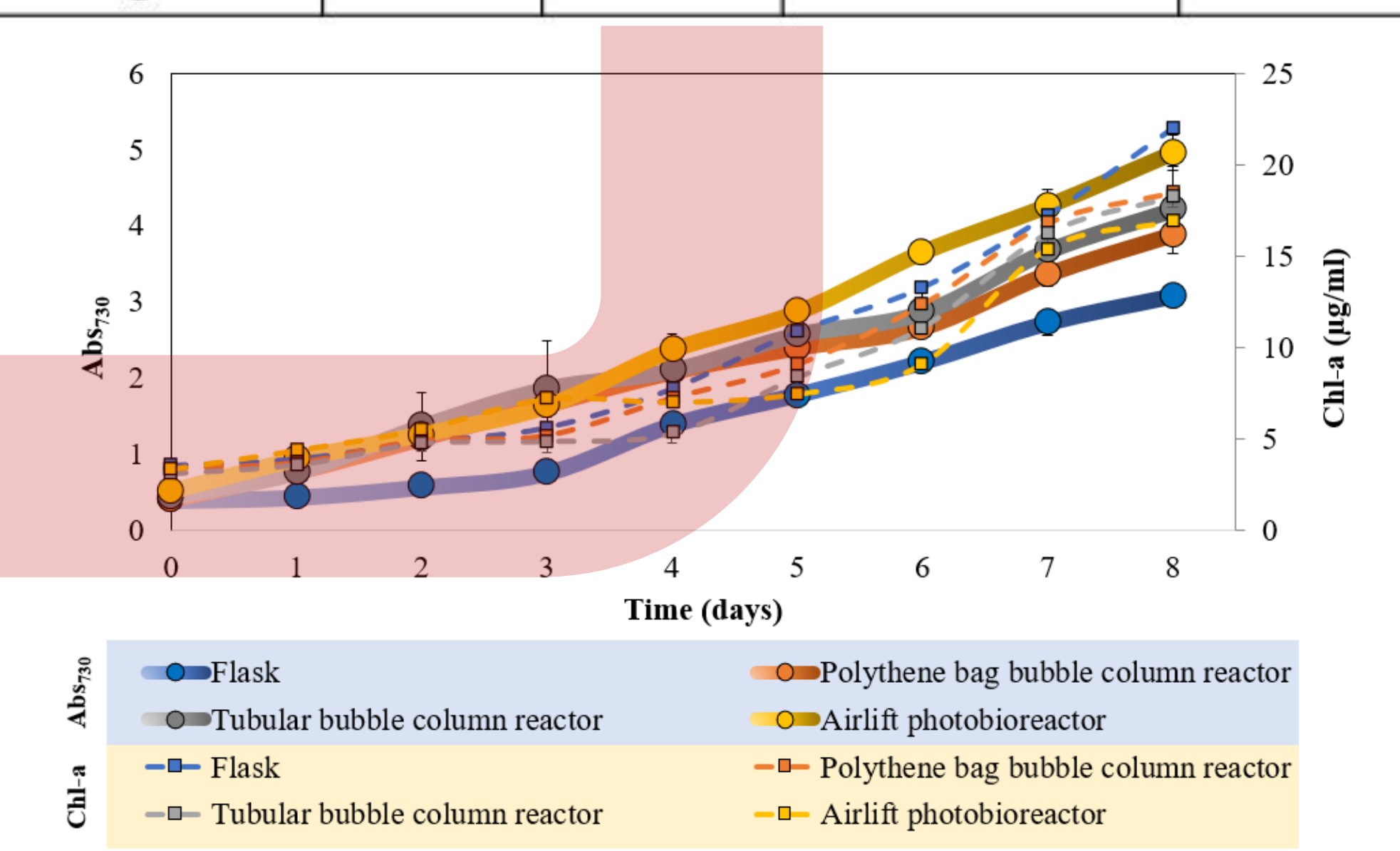
Light conditions:

EL: Natural light conditions (max. $1200 \pm 300 \mu\text{mol m}^{-2} \text{ s}^{-1}$)

Volumes: 1L (bubble column reactor) to 100L (Airlift Photobioreactor)



Container	Flask	PE bag	PVC bubble column	PBR
Total volume	1L	3L	12L	102L
Working volume	250ml	1L	10L	100L



CONCLUSION

The present study successfully demonstrated the progression of *Synechococcus* from genetic transformation to its volumetric scale-up, particularly under natural light. It was observed that the engineered strain exhibited improved biomass, with effective ethylene production under natural light conditions. The transformant sustained longer when medium was amended with sodium bicarbonate, owing to its buffering capacity and characteristic carbon concentration mechanism. Furthermore, the successful transition of the transformants from laboratory scale to polyethylene bags and subsequently to airlift photobioreactors was established for production of ethylene. Thus, our research sets a remarkable foundation for future scale-up cultivation of engineered cyanobacteria.

FUTURE ASPECTS

- Design and execute the ethylene capture system for continuous cultivation system
- Comparative analysis of ethylene production under the control of endogenous light promoters and P_{trc} in outdoor light cultivation
- Engineering and quantifying value-added molecules under P_{trc} promoter in outdoor condition at 100L Airlift photobioreactor

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OUR GROUP



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