Conversion of CO_2 into chemicals by engineered cyanobacteria

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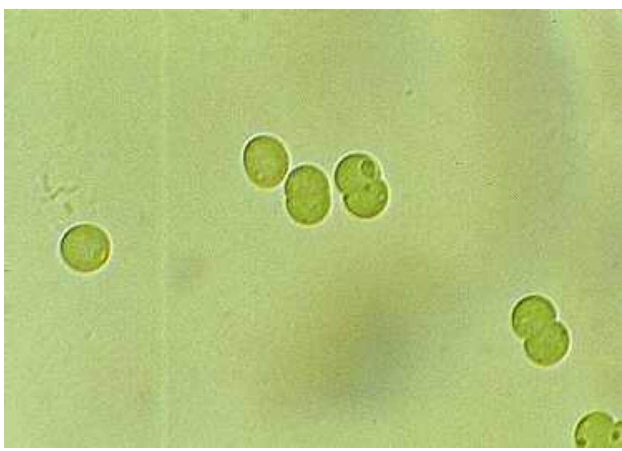
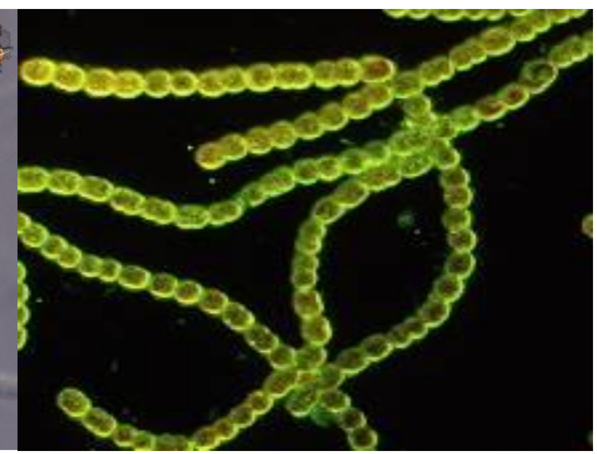
Cyanobacteria

- Photoautotrophic prokaryotes, evolutionary precursor of chloroplasts
- 20-30% organic carbon derived from cyanobacteria
- Photosynthesis efficiency 10-fold that higher plants
- Fastest doubling time 2 hours (*Synechococcus elongatus* UTEX 2973)
- Genetic manipulation easier than higher plants/eukaryotic algae



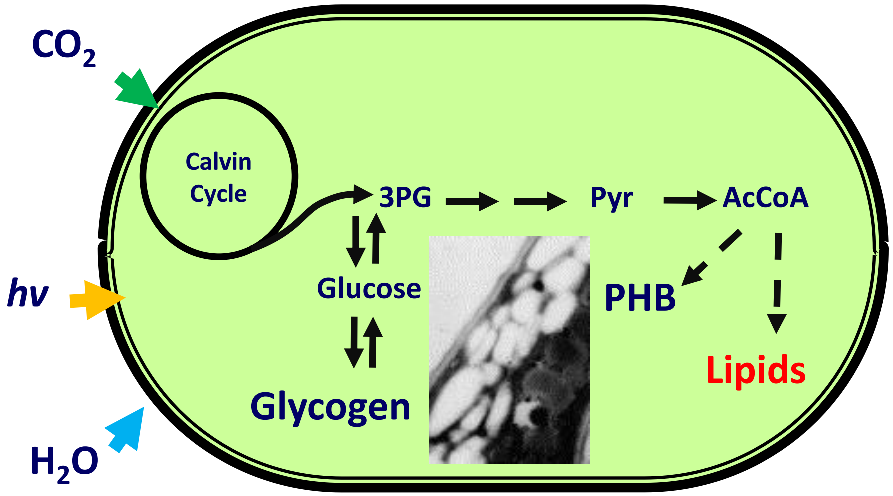
Unicellular Form

Aggregates

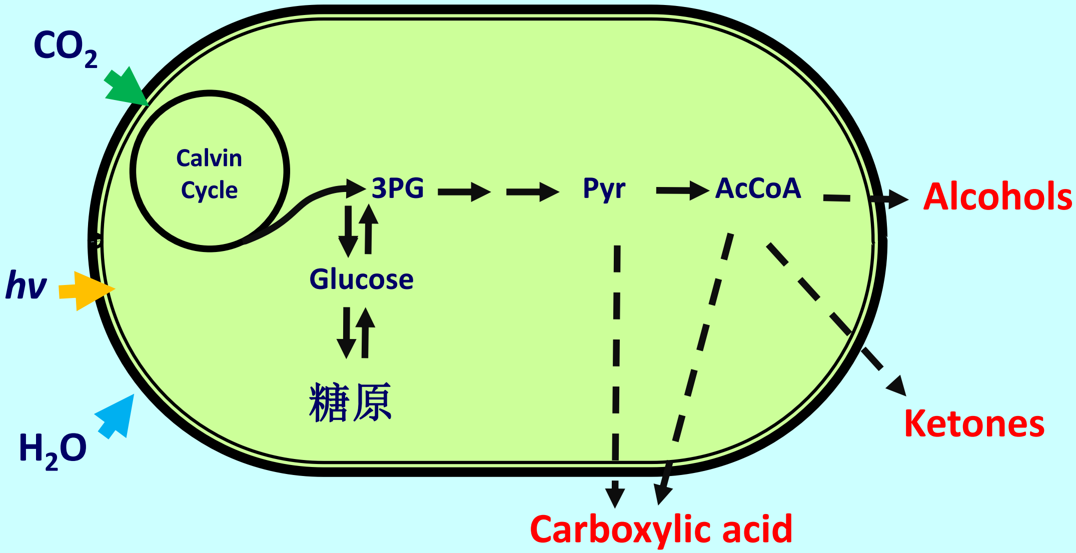


Filamentous form

CO_2 to fuels / chemicals

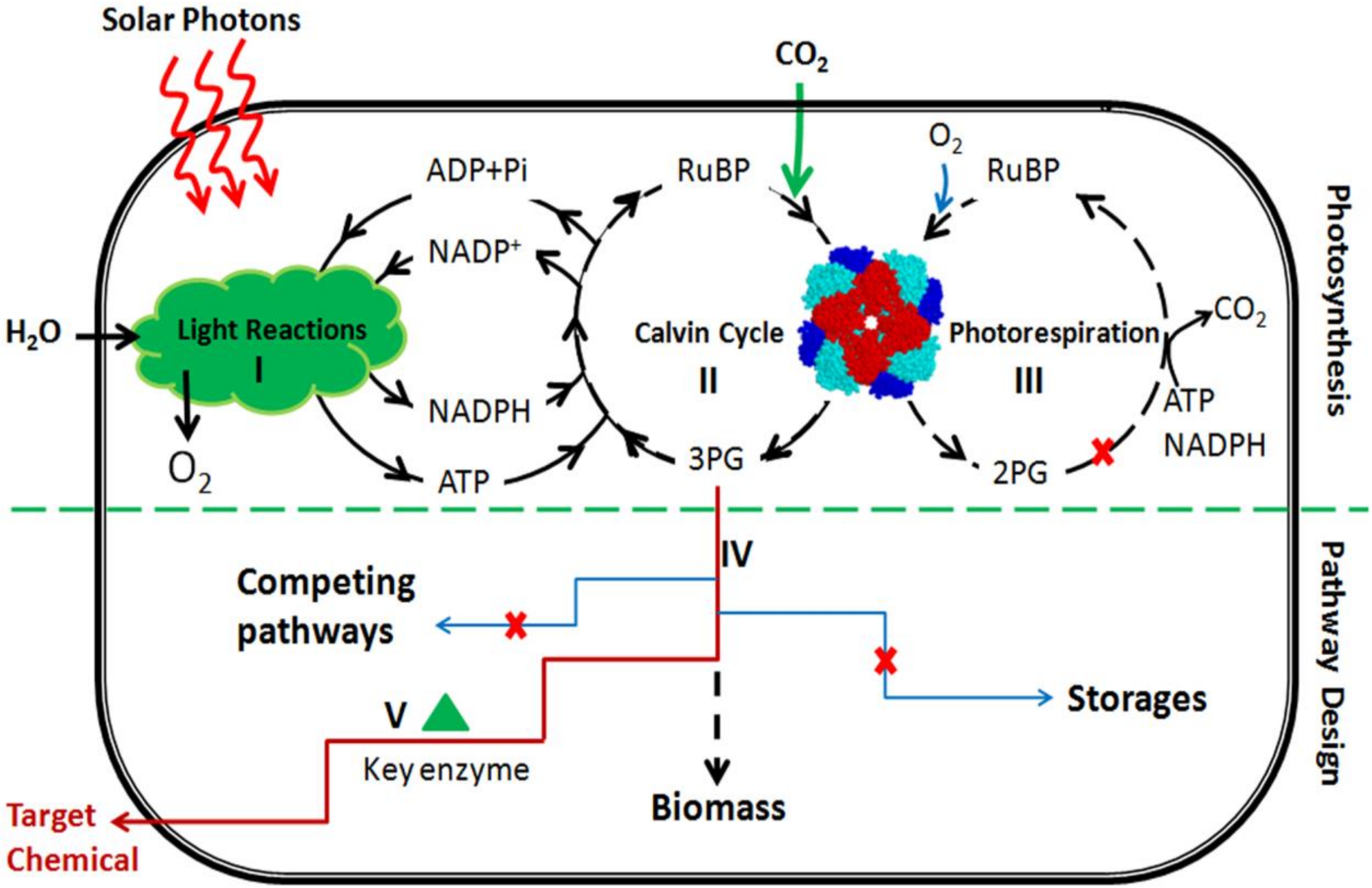


- Biomass concentration limitation



- Extracellular small molecules
- Photobiocatalysts

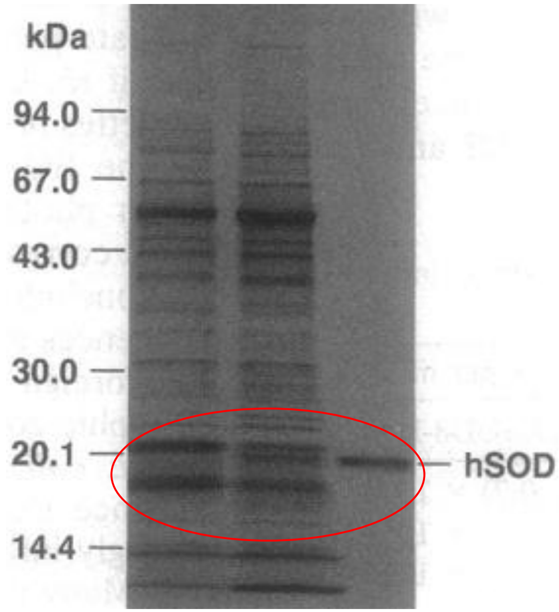
Strategies for cyanochemicals



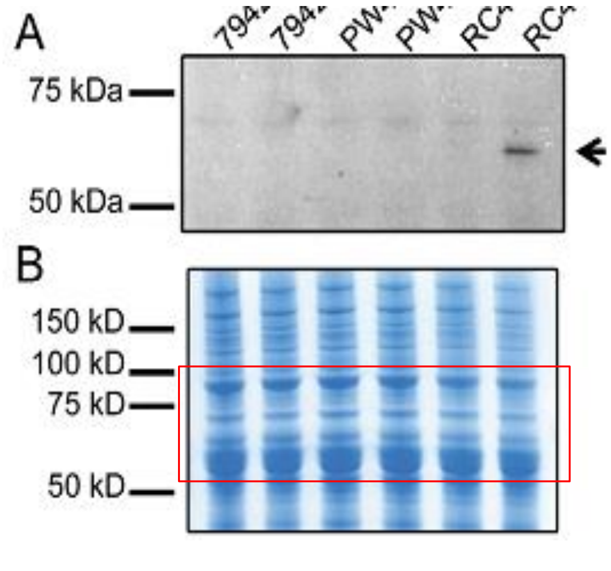
Common challenges

- **Efficiency for genetic operation**
- **Efficiency for redirecting carbon flux**

Cyano-gene expression



Takeshima, et al., *PNAS*. 1994, 91: 9685-9689



Expression of [NiFe] Hydrogenases in Cyanobacteria

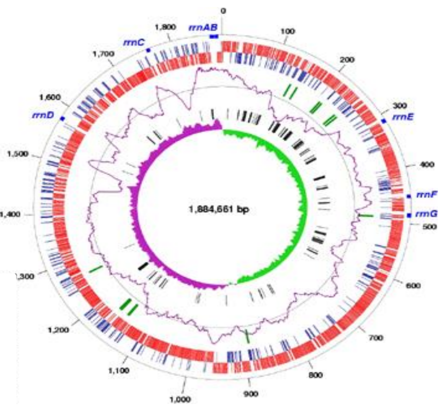
Weyman, et al., *PLoS One*. 2011, 5:1-6

- Native PrbcL promoter and optimized SD sequence
- High level expression SOD in cyanobacteria (3%)

- Identification of gene expression by western blot
- Target protein bands are invisible in the gel

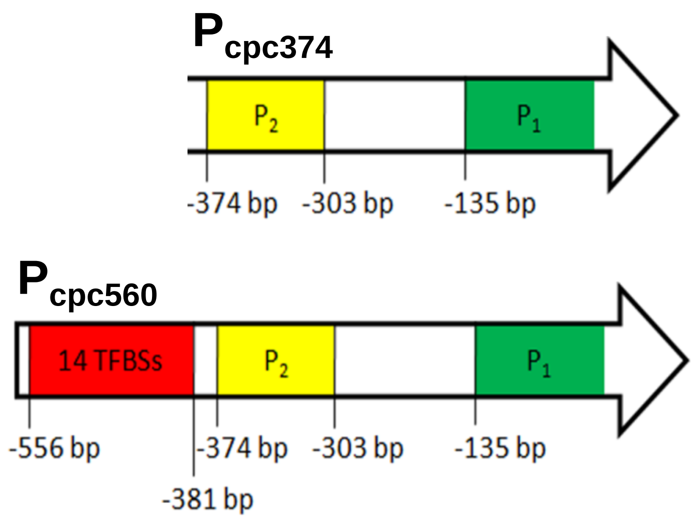
Strong promoter Pcpc560

Database search



Regulatory Elements

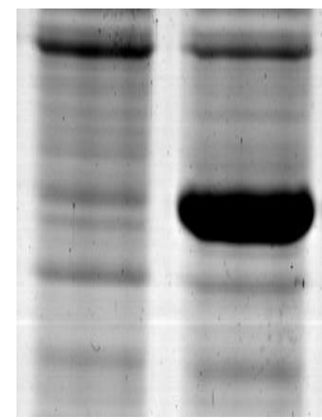
Promoter design



TFBSs

Expression levels

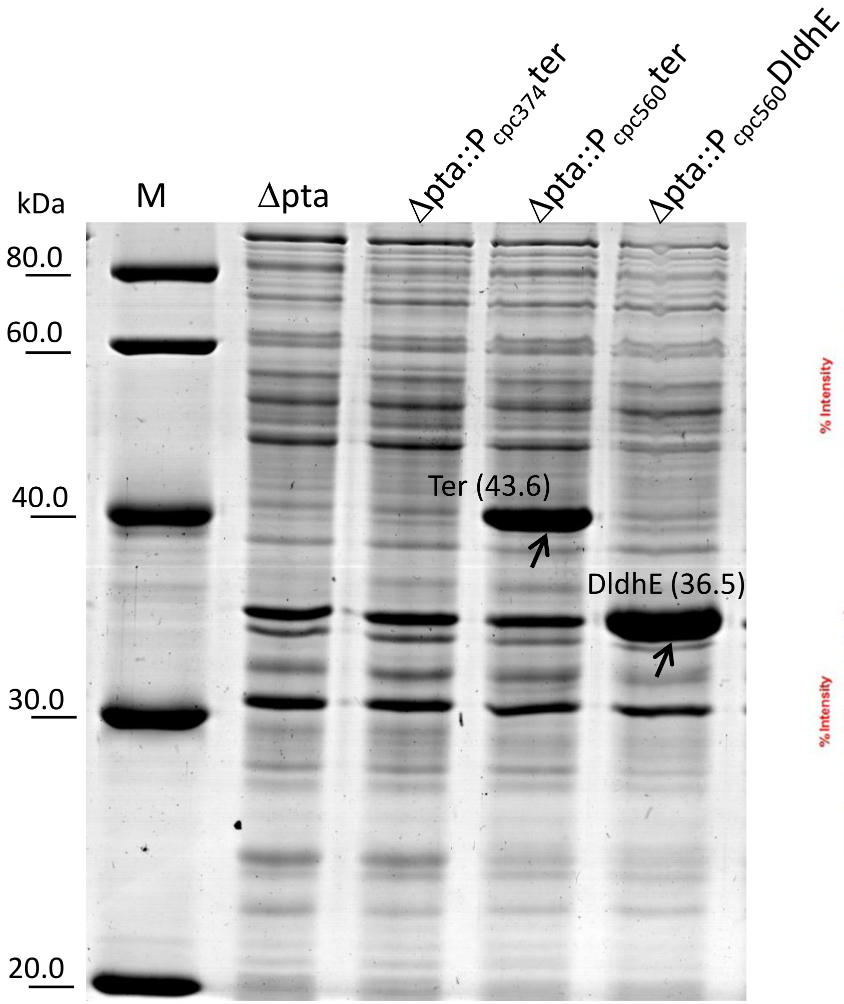
P_{cpc374} P_{cpc560}

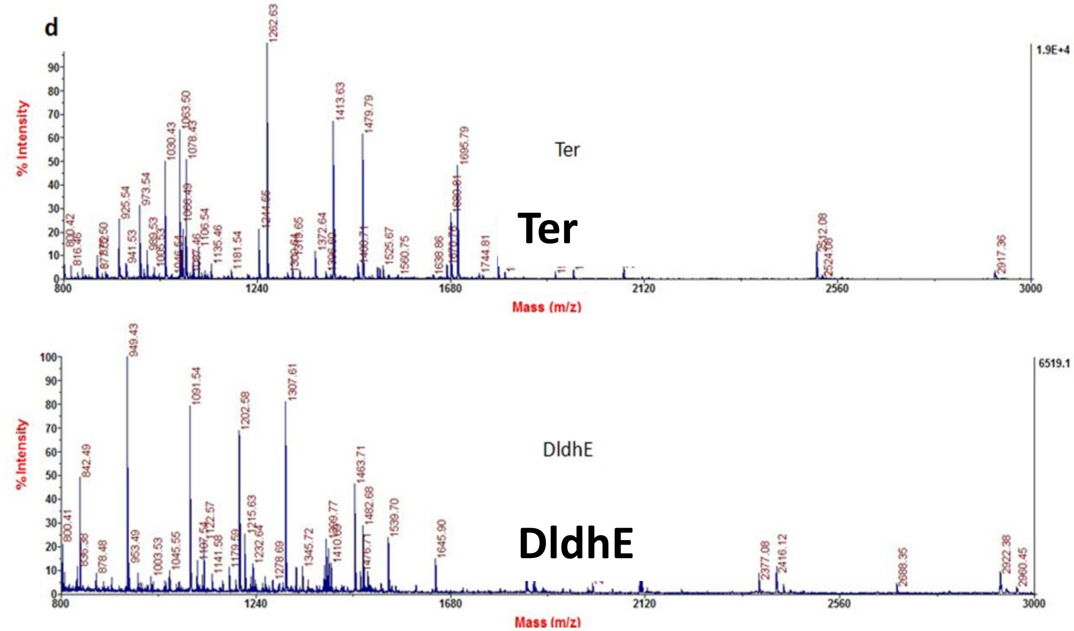


15% of total protein

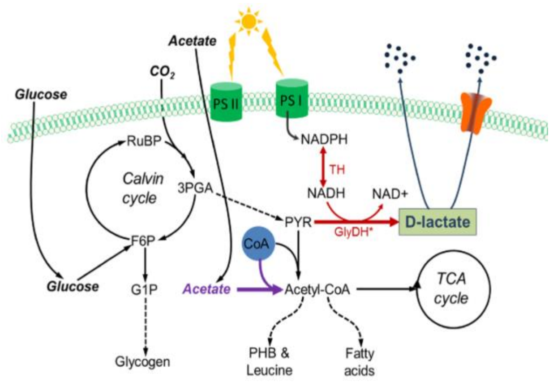
Zhou et al., *Sci. Rep.* 2014, 4:4500

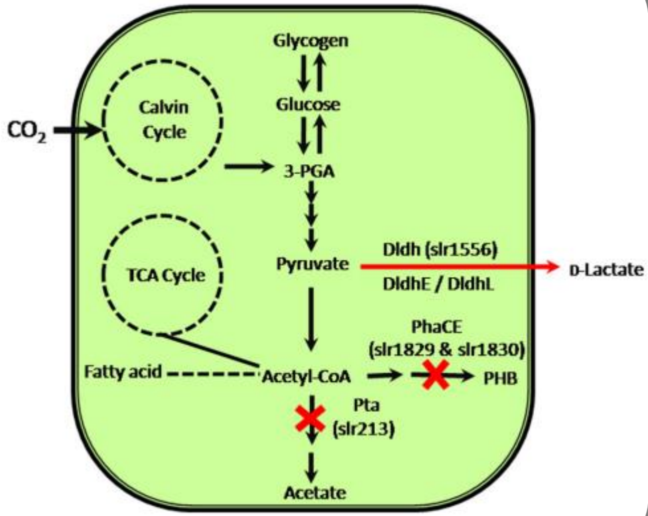
Verification of Pcpc560





Cyano-D-lactate





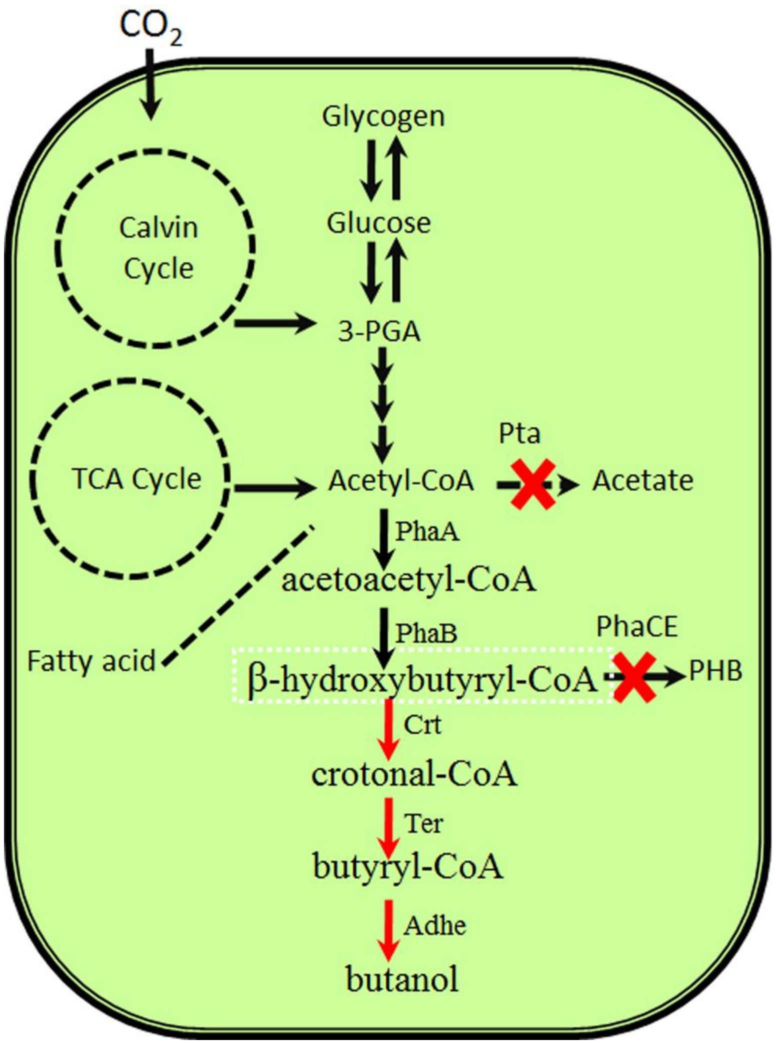
>1 g/L

Strains	Genome modification	Metabolic production		
		PHB (% (w/w) of DW)	Acetate (g/L)	Lactate (g/L)
WT	*phaE* up, *phaE phaC*, *phaC* down; *pta* up, *pta*, *pta* down	5.59 ± 0.57	1.67 ± 0.30	0.04 ± 0.02
ΔphaCE	*phaE* up, *Cm*^{r}, *phaC* down	0.00 ± 0.05	0.88 ± 0.13	0.06 ± 0.01
Δpta	*pta* up, *Km*^{r}, *pta* down	9.85 ± 1.34	0.56 ± 0.05	0.08 ± 0.03
ΔphaCE Δpta	*phaE* up, *Cm*^{r}, *phaC* down; *pta* up, *Km*^{r}, *pta* down	0.00 ± 0.01	0.41 ± 0.08	0.36 ± 0.04
ΔphaCE Δpta::DldhE	*phaE* up, *Cm*^{r}, *phaC* down; *pta* up, *Km*^{r}, *DldhE*, *pta* down	0.00 ± 0.01	0.20 ± 0.06	0.68 ± 0.1
ΔphaCE Δpta::DldhL	*phaE* up, *Cm*^{r}, *phaC* down; *pta* up, *Km*^{r}, *DldhL*, *pta* down	0.00 ± 0.01	0.10 ± 0.1	1.06 ± 0.07

Step-wise optimization

Zhou et al., *Process Biochem.* 2014, 49: 2071-2077

Cyano-butanol

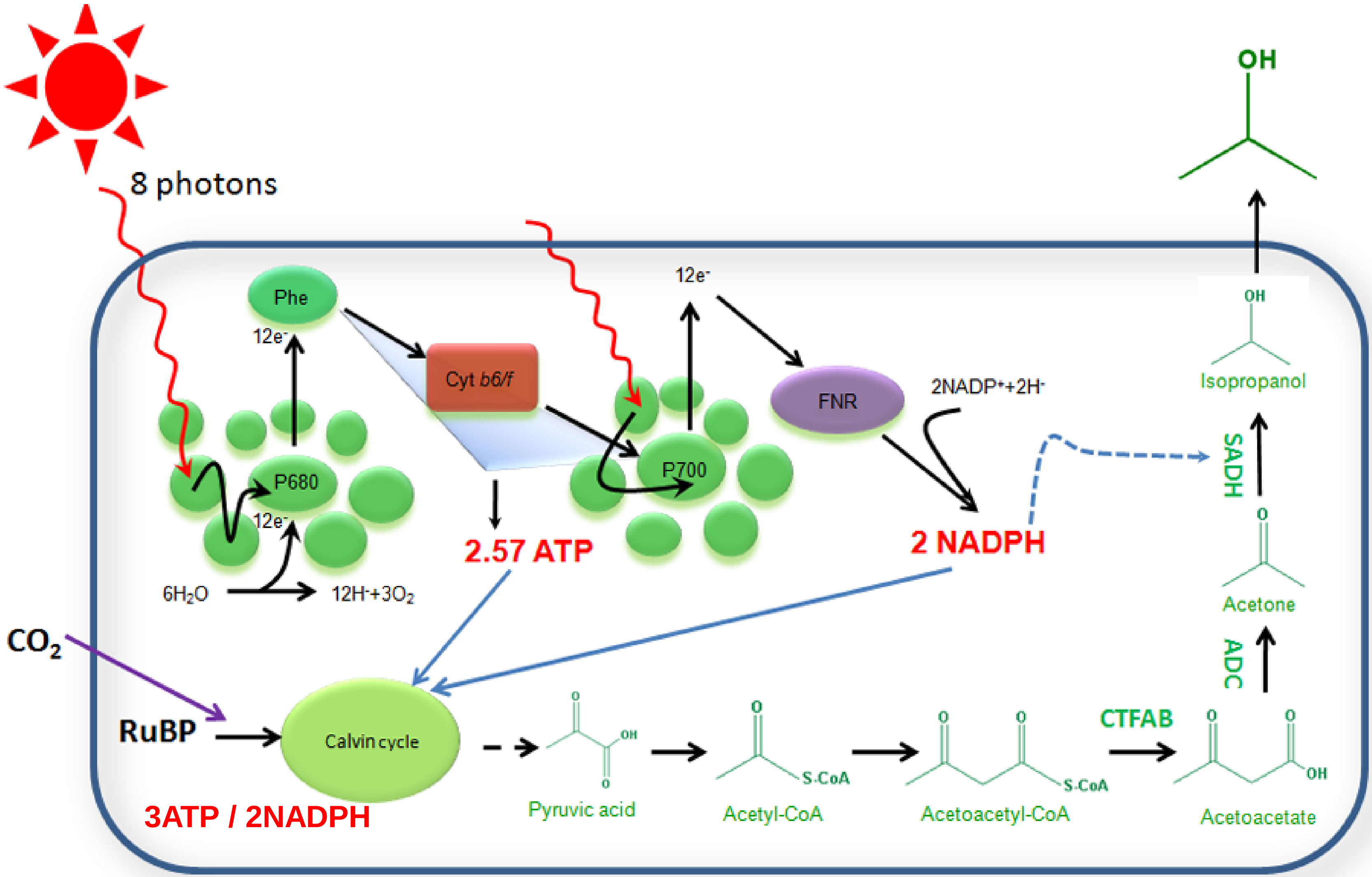


Light and aerobic conditions

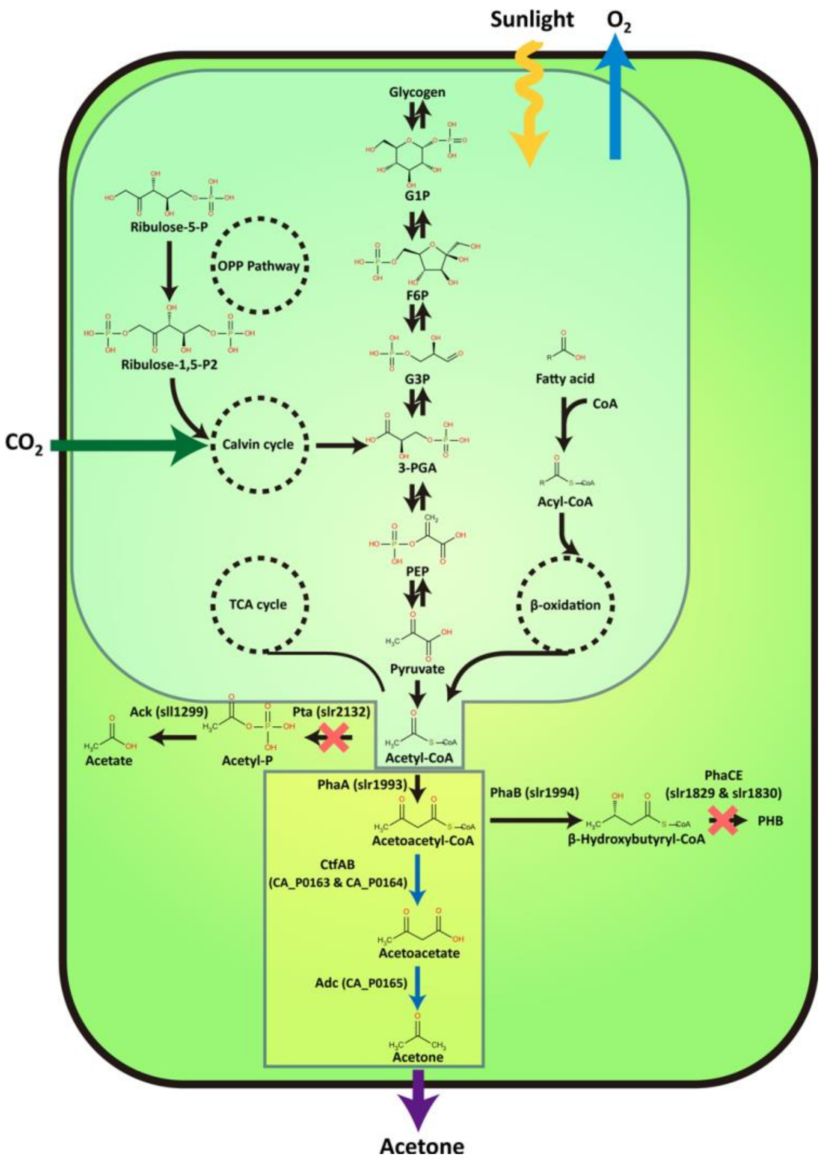
Strains	Genome modification	Metabolic production		
		PHB (% (w/w) of DW	Acetate (g/L)	Butanol (mg/L)
WT	*phaE* up, *phaE phaC*, *phaC* down; *pta* up, *pta*, *pta* down	5.59 ± 0.57	1.67 ± 0.30	0
△phaCE △pta	*phaE* up, *Cm^r*, *phaC* down; *pta* up, *Km^r*, *pta* down	0.00 ± 0.01	0.41 ± 0.08	0
△phaCE::adhe △pta::crt::ter	*phaE* up, *Cm^r adhe*, *phaC* down; *pta* up, *Km^r crt ter*, *pta* down	0.00 ± 0.01	0.37 ± 0.06	50 ± 1.5

Ter, trans-enoyl-CoA reductase from *Treponema denticola*, replaceing Bcd and EtfA/B from *Clostridium acetobutylicum*.

Imbalanced ATP/NADPH



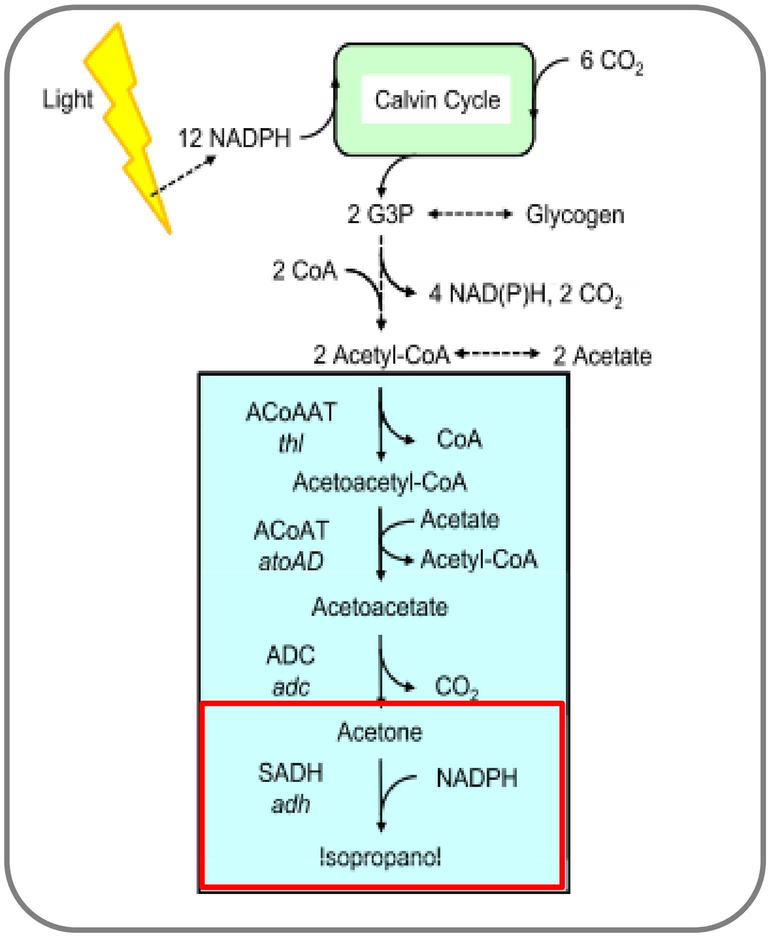
Cyano-acetone



Strain	Insertion	Acetone production (mg l^{-1})
SM1	*phaE* up – Cm^r – *phaC* down	0
SM2	*phaE* up – Cm^r – *phaC* down; *pta* up – Km^r – *pta* down	0
SM3	*phaE* up – PphaE – *adc* – *ctfAB* – PrbcL – Cm^r – *phaC* down	3.0 ± 0.4
SM4	*phaE* up – Pcpc – *adc* – *ctfAB* – PrbcL – Cm^r – *phaC* down	5.0±1.0
SM5	*pta* up – Km^r – *pta* down; *phaE* up – Pcpc – *adc* – *ctfAB* – PrbcL – Cm^r – *phaC* down	36.0±1.5

Zhou et al., *Metab. Eng.* 2012, 14:394-400

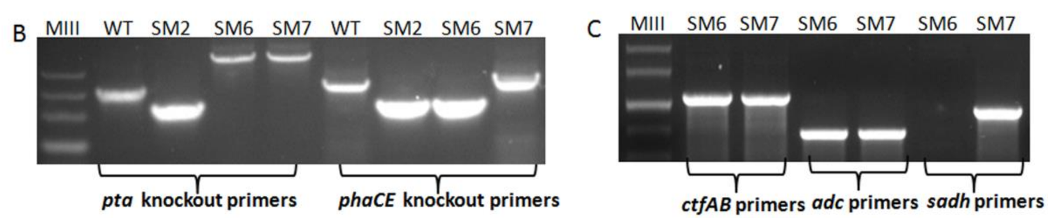
Cyano-isopropanol



Light and aerobic conditions

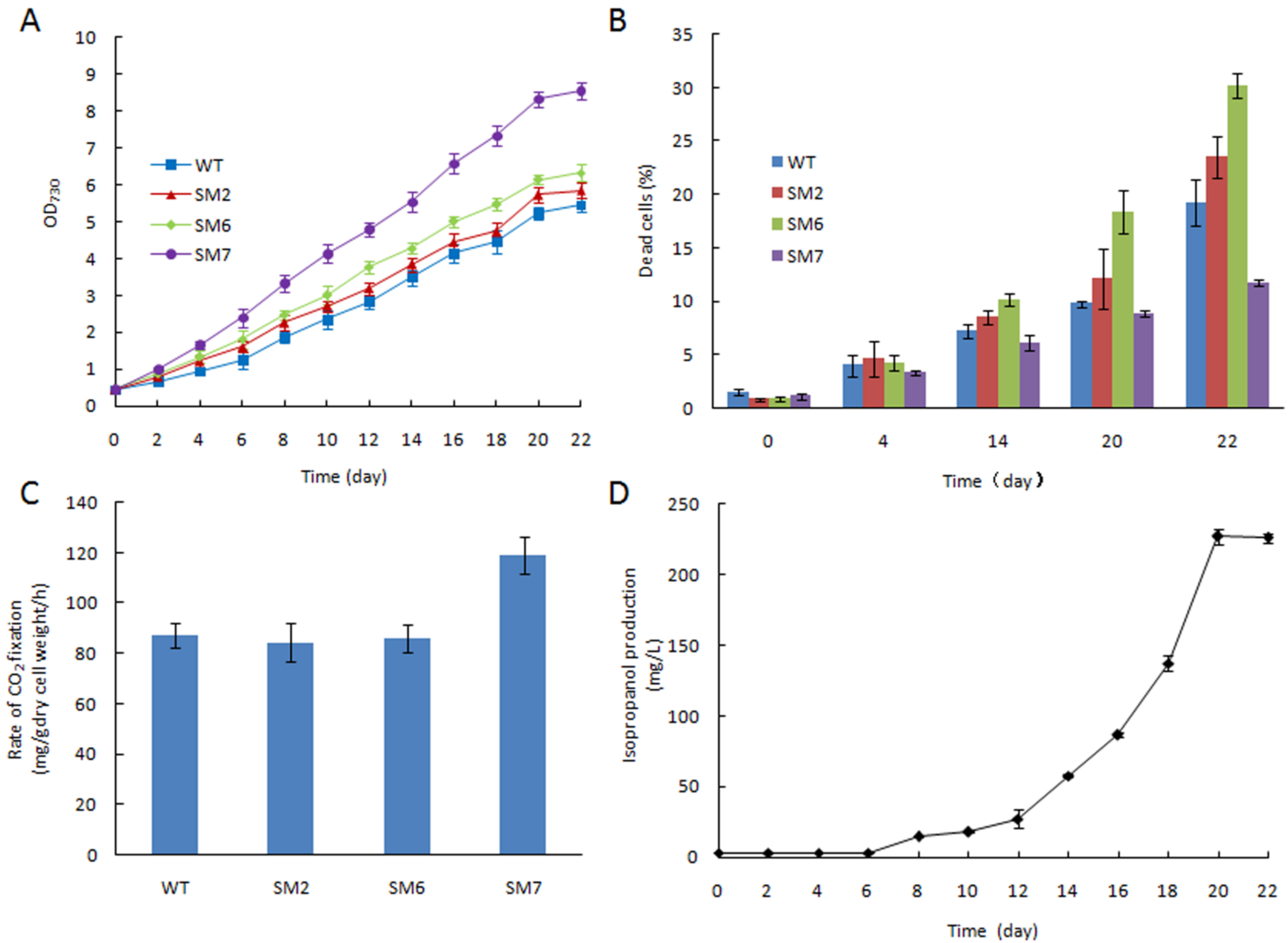
A

Strains	Genomic type	Acetone (mg/L)	Isopropanol (mg/L)
WT	*phaE* up, *phaEC*, *phaC* down; *pta* up, *pta*, *pta* down	0	0
SM2	*phaE* up, *Cm*^{r}, *phaC* down; *pta* up, *Km*^{r}, *pta* down	0	0
SM6	*phaE* up, *Cm*^{r}, *phaC* down; *pta* up, P_{rbcL}, *adcO*, *ctfABO*, P_{cpc560}, *Km*^{r}, *pta* down	108.5	0
SM7	*phaE* up, P_{cpc560}, *sadhO*, Cm^r, *phaC* down; *pta* up, P_{rbcL}, *adcO*, *ctfABO*, P_{cpc560}, *Km*^{r}, *pta* down	0	226.89 ± 5.43

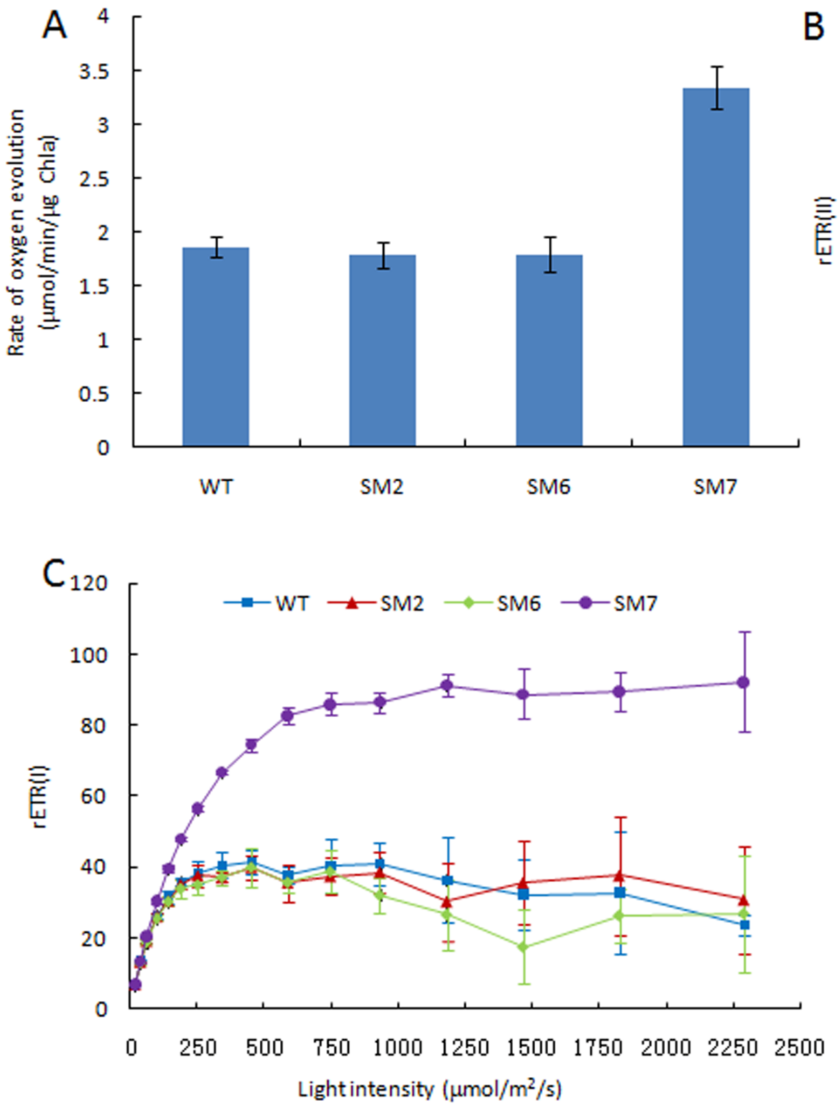


Zhou et al., *Metab. Eng.* 2016, 38: 217–227.

Physiological changes



Improved photosynthesis



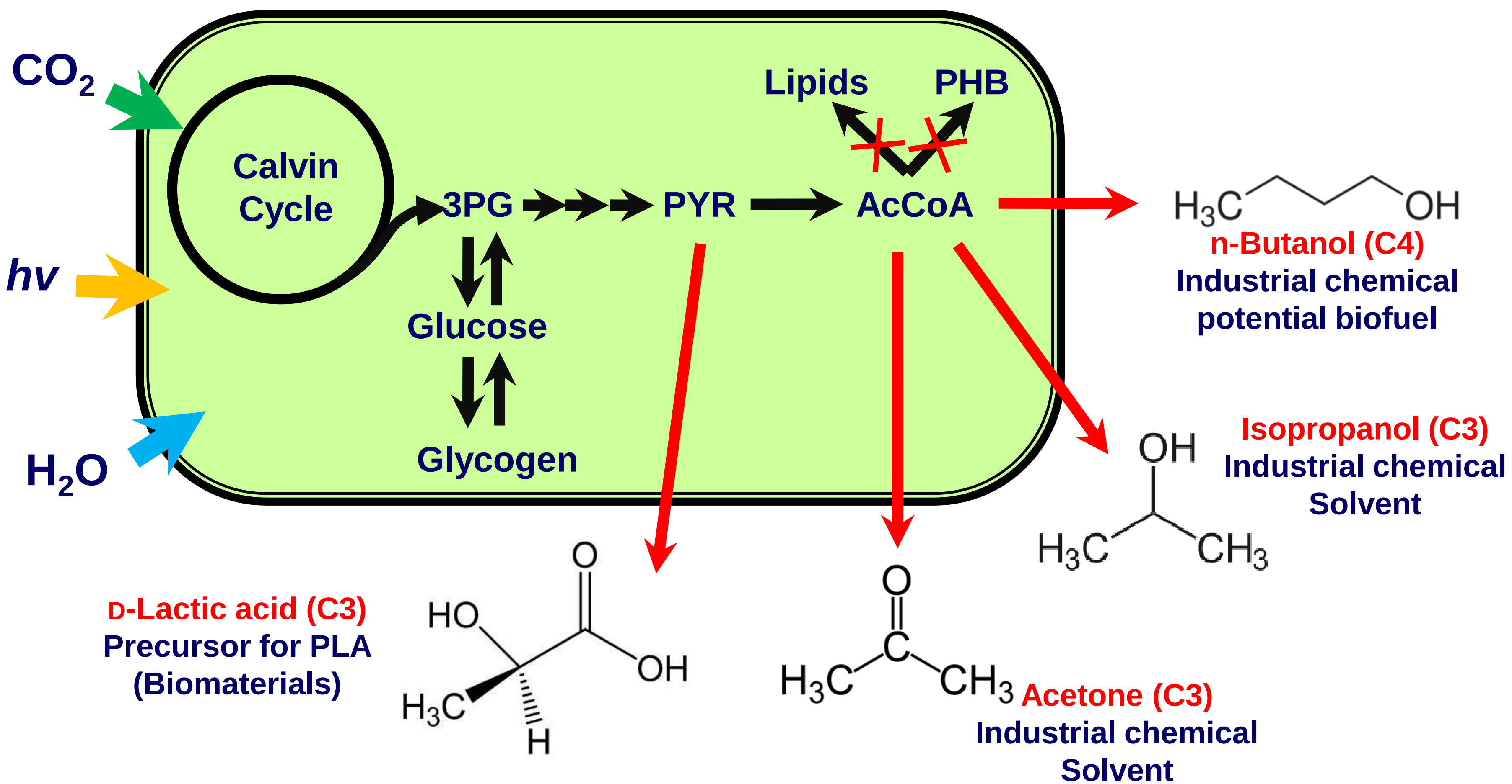
Pv/Pm, maximum quantum efficiency of PS I.

Y(I), effective quantum efficiency of PS I.

rETR(I), relative electron transport rate of PS I.

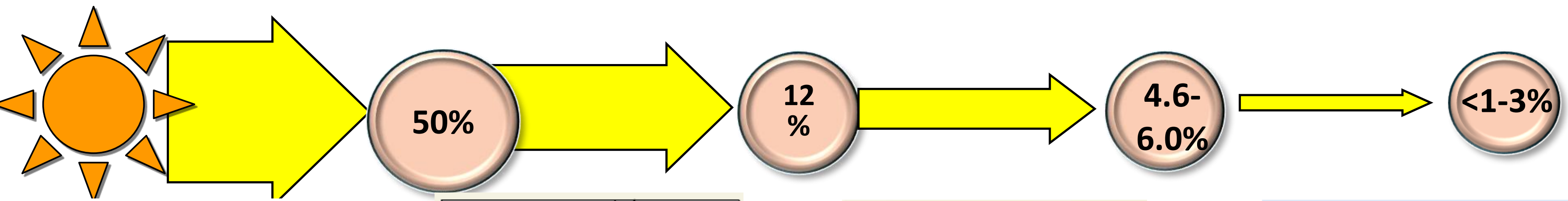
Enabling utilization of high-intensity lights

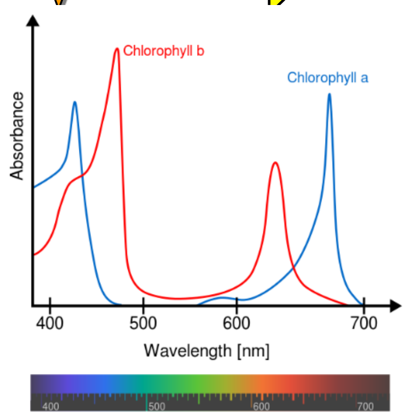
Cyanochemicals

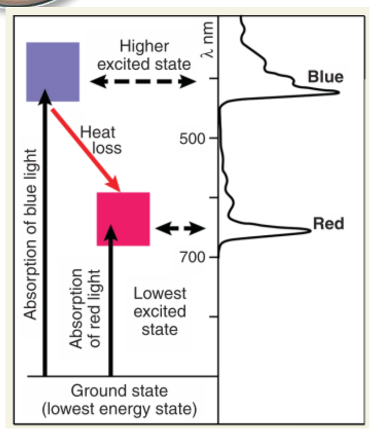


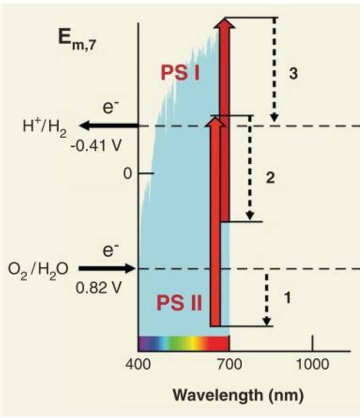
Energy conversion efficiency

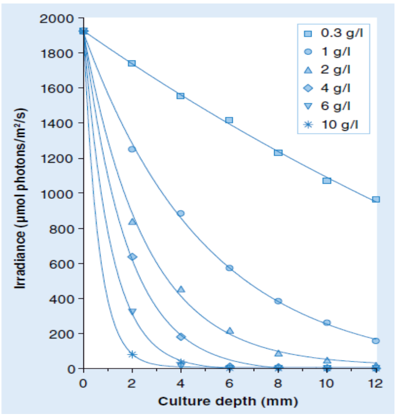
- **Visible region 400-700 nm**
- **Blue light heat loss**
- **Photorespiration**
- **Delivery of CO_2**











Data from *Science*, 2011, 332: 805

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Cyano-chemicals

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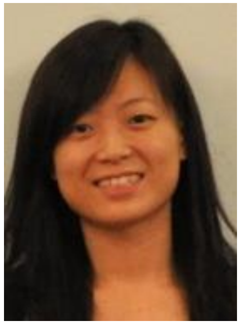

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Microbial Cell Factories

REVIEW

Open Access

From cyanochemicals to cyanofactories: a review and perspective

Jie Zhou, Taicheng Zhu, Zhen Cai and Yin Li*

Abstract

Engineering cyanobacteria for production of chemicals from solar energy, CO_2 and water is a potential approach to address global energy and environment issues such as greenhouse effect. To date, more than 20 chemicals have been synthesized by engineered cyanobacteria using CO_2 as raw materials, and these studies have been well reviewed. However, unlike heterotrophic microorganisms, the low CO_2 fixation rate makes it a long way to go from cyanochemicals to cyanofactories. Here we review recent progresses on improvement of carbon fixation and redistribution of intercellular carbon flux, and discuss the challenges for developing cyanofactories in the future.

Keywords: Cyanobacteria, Cyanochemicals, Cyanofactories, Rubisco, Metabolic Engineering, Rerouting carbon flux, Strong promoter, Cofactor balance

Thank you for your attention!

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