-BioSolarH_2→

Autofermentative biological hydrogen production by cyanobacteria

G.C. Dismukes

Rutgers University

Waksman Institute

and

Department of Chemistry & Chemical Biology

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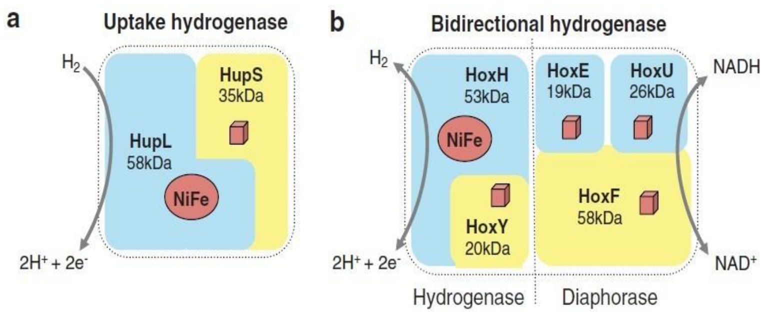
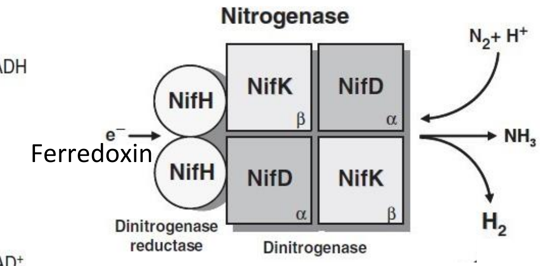
in cyanobacteria:



Ghirardi et al., 2007

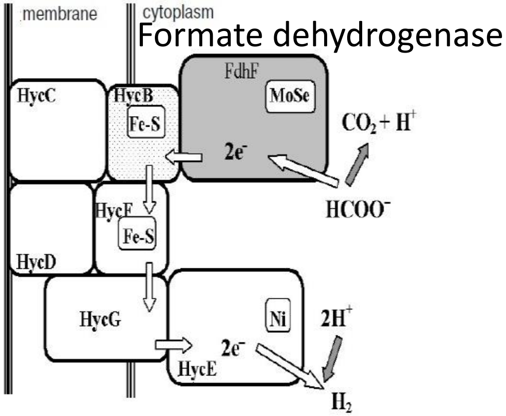
Km (MV) = 16.1µM
Kcat (MV) = 1242 s^{-1}
(Francis et al., 1990)

K_i (O_2) = 1%
(McIntosh et al., 2011)



Tamagnini et al., 2007

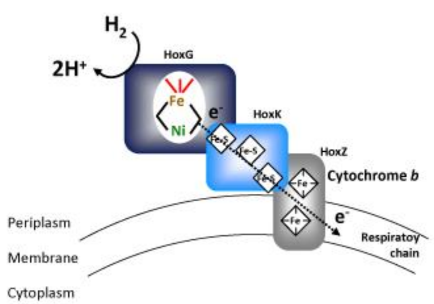
Km (C_2H_2) = 1.8*10^{-3} atms
(**Hallenbeck et al. 1979**)



Hydrogenase

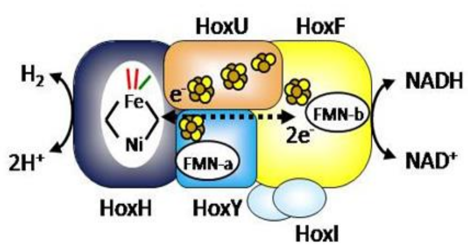
Bagramyan et al., 2003

in *Ralstonia eutropha*:



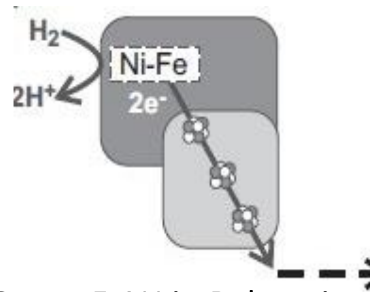
The membrane-bound hydrogenase (MBH)

Km (H_2) =6.1µM
Kcat (H_2) = 238 s^{-1} (**Schäfer et al., 2013)**
K_i (O_2) = 47.5% (**Lenz et al., 2010**)



Soluble NiFe hydrogenase (SH)

Km (H_2) =11µM
Kcat (H_2) = 384 s^{-1} (**Schäfer et al., 2013)**
80% activity at 60% O_2 (**Lauterbach et al., 2013**)

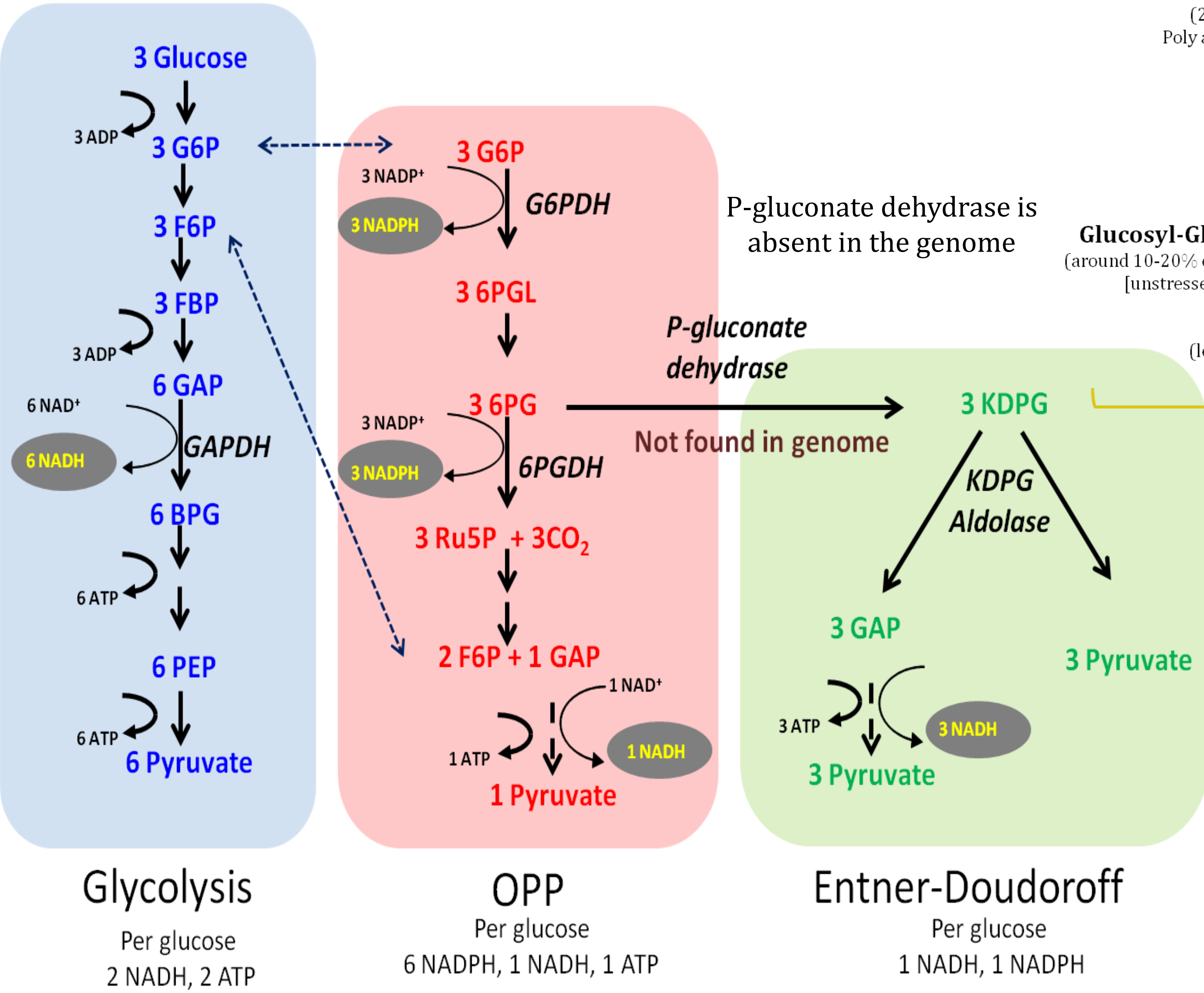


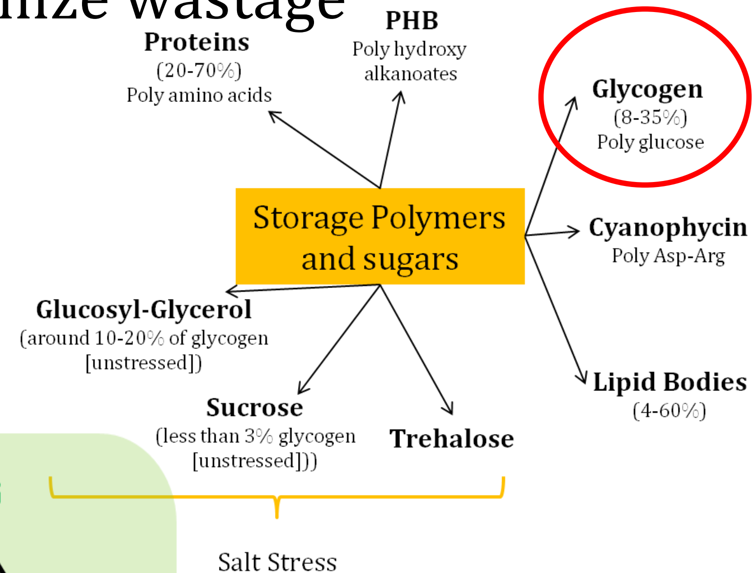
Group 5 AH in Ralstonia eutropha H16

Schäfer et al., 2013

Km (H_2) =3.5µM
Kcat (H_2) = 0.5 s^{-1}
Oxygen insensitive (**Schäfer et al., 2013**)

Carbohydrate Catabolism in Cyanobacteria: maximize reductant minimize wastage



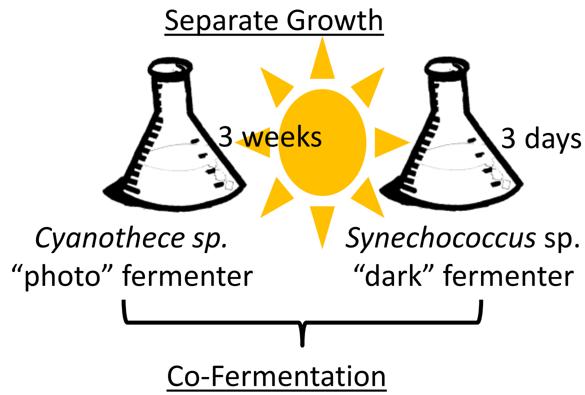


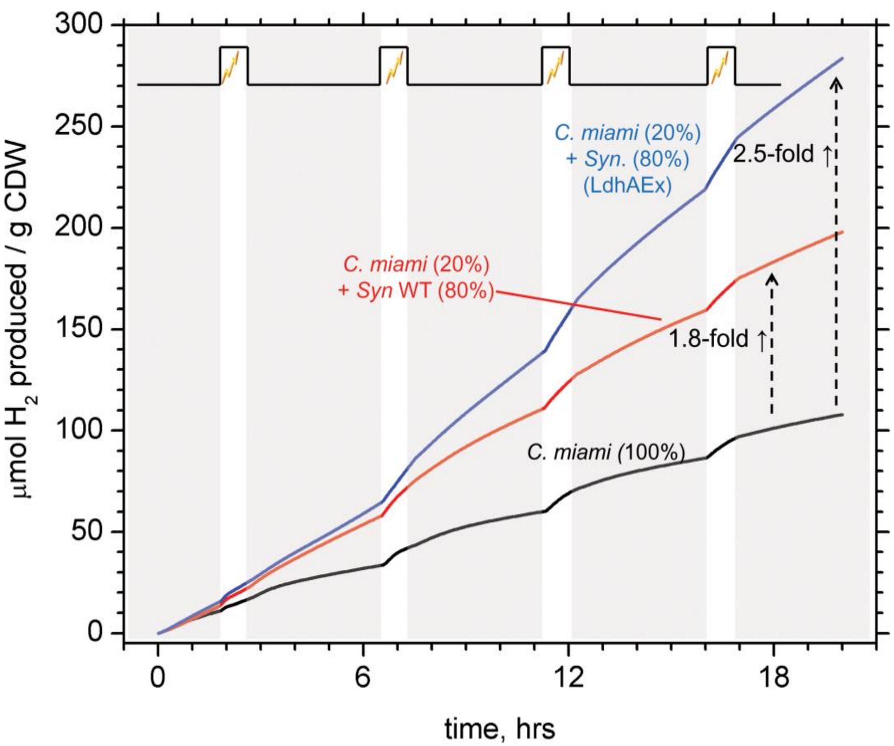
- Glycogen is the preferred carbon source for fermentative H_2 in cyanos
- Glycolysis is the preferred catabolism pathway under fermentative conditions

(Tang, 2011) (Guerra, 2013) (Hasunuma, 2013) (Liberton, 2011) (Quintana, 2011)

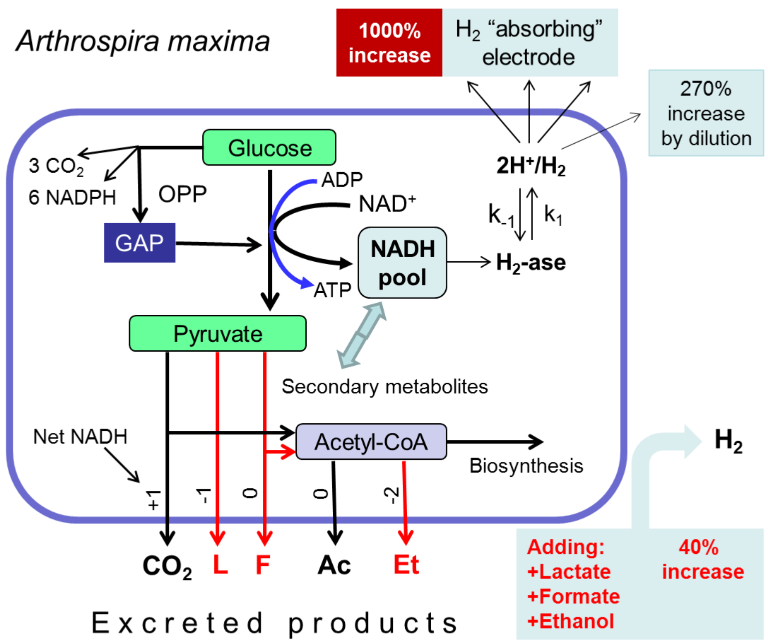
-BioSolarH_2→ **Dismukes lab: H_2 Milking**

Milking H_2 by Co-fermentation





Arthrospira maxima wins the gold medal for autofermentation H_2 yield



- Max. 39% of all catabolized carbohydrate is converted to H_2 (4.8 H_2/ glucose eq.) under electrochemical "milking" of H_2
- 600mL H_2/L culture in five days w/o lysing
- Another 40% increase in H2 is produced by adding the "magic mix" (formate, lactate and ethanol)

Ananyev et al., J Biotech. 2012

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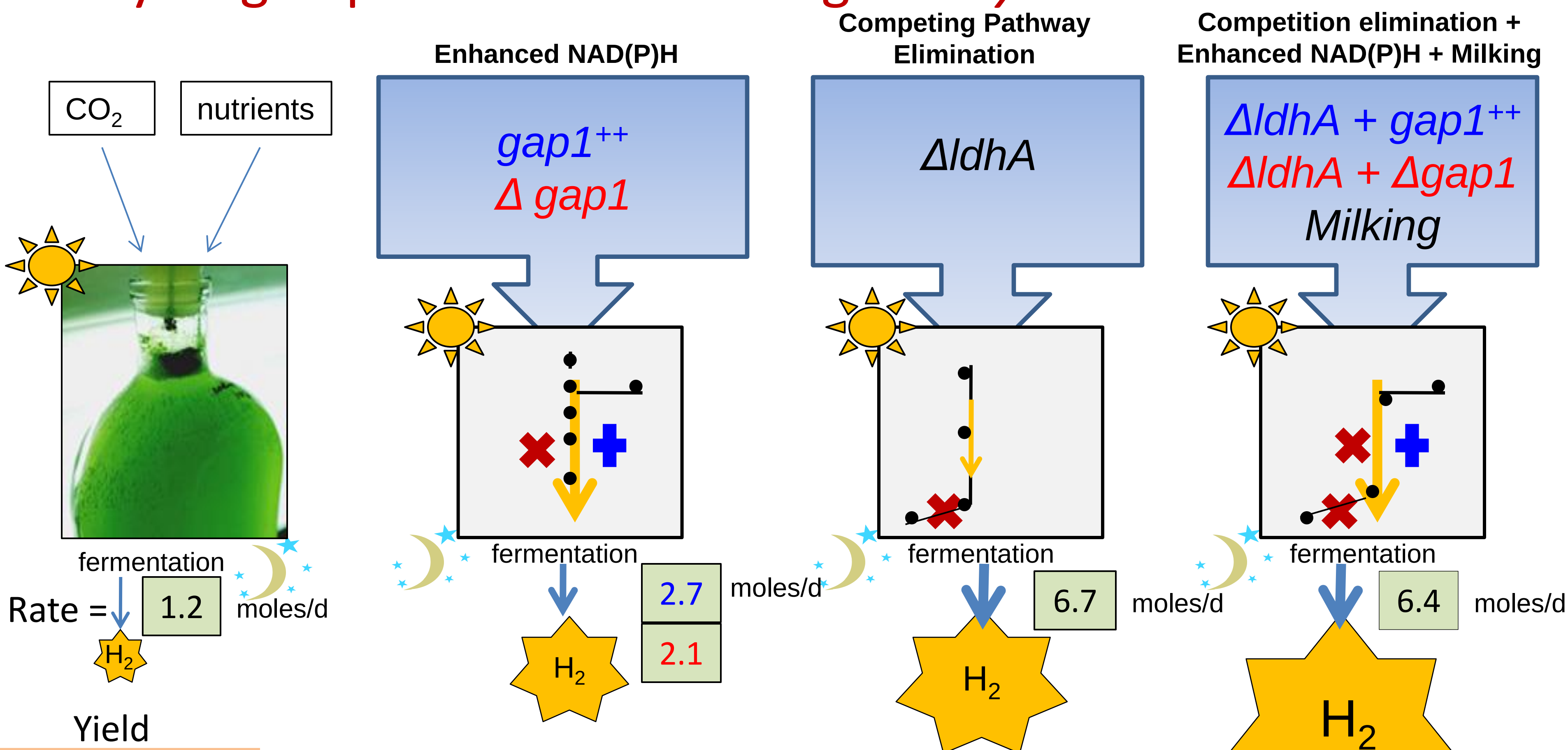
Hydrogen production in transgenic *Synechococcus* 7002



Yield

moles/10^17 cells	WT	GAPDH	LDH	GAPDH + LDH
No milking/ 4 d	5.0	15.5 :: 13.0	26.9	25.5
milking/ 1d	55.2	nd	92.6	255.0
mol/mol glucose	0.6	1.1 :: 1.4		
ECE	5 %	9.2 :: 11.7 %		

Kumaraswamy et al. ***EES***, in press.

Status and Future of Autofermentative H_2 Production in Cyanobacteria

Limitations	**Possible Solutions**	Y/N
1) Bidirectional [NiFe]-H_2ase		
-O_2 sensitivity	Group V: actinobacterial H_2ase	√
-H_2↑ unfavorable ΔG° is +	↑[NAD(P)H] , ↑[H^+]	√
-ATP allosteric down-regulation ?	ATP tolerant H_2ase →high ATP homeostasis	?
-H_2↓ uptake	Milking: active removal of H_2	√
2) PS Storage of Carbohydrates		
-only glycogen, not osmolytes	Gluconeogenesis: Overexpress GAPDH1 starch accumulation	√ ?
3) Carbo Catabolic Flux →NAD(P)H		
-glycolytic flux too slow	EMP-glycolysis: Overexpress GAPDH1 OPP pathway: Knockout GAPDH1	√ √
-stronger reductant needed	PFOR: Pyr→Ferredoxin → N_2ase photo-H_2↑ FNR: NAD(P)H →Fd → N_2ase photo-H_2↑	√, ? ?
-max H_2 yield 33% (acetogenic)	Reroute 100% glycogen catabolism via OPP	?