

Photosynthetic chemical production: Applications of synthetic biology

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Issues for space travel

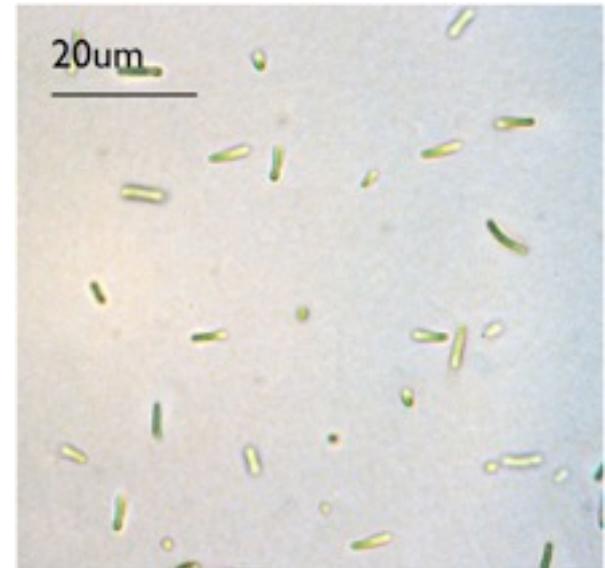
- Efficient production of nutrients
- Palatability and sensory stimulation
- Light distribution for photosynthetic production

Technology Platform #1: Cyanobacteria

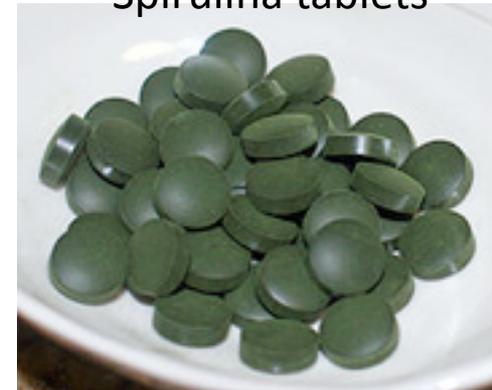
- *Synechococcus elongatus* PCC7942
 - Transformable, good genetic engineering (Knockouts, foreign genes at multiple sites).
 - Doubles every 8 hours. Grows to high density. Medium contains only inorganic salts, no carbohydrate.
 - Engineering steps portable to other cyanobacteria (e.g. filamentous, nitrogen-fixing).

- *Arthrospira* species (Spirulina)
 - Complete nutritional source
 - Palatable (at least somewhat)

Synechococcus elongatus
PCC7942

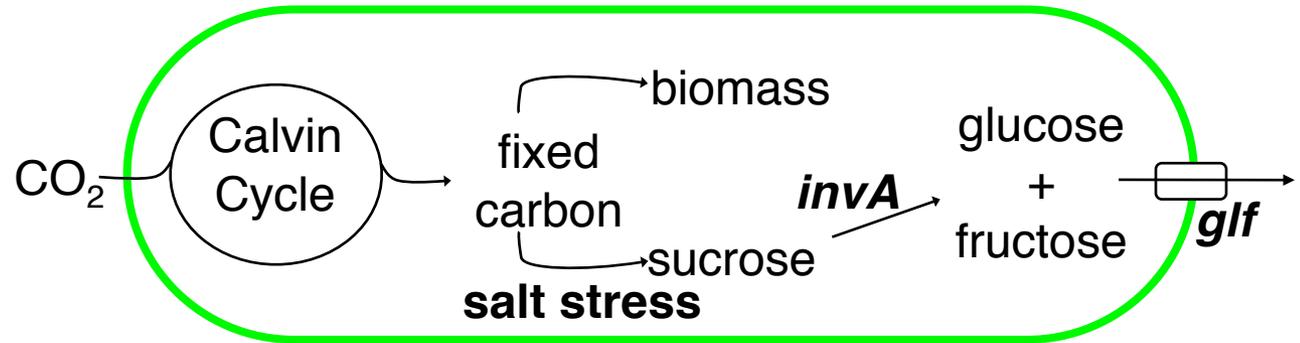


Spirulina tablets

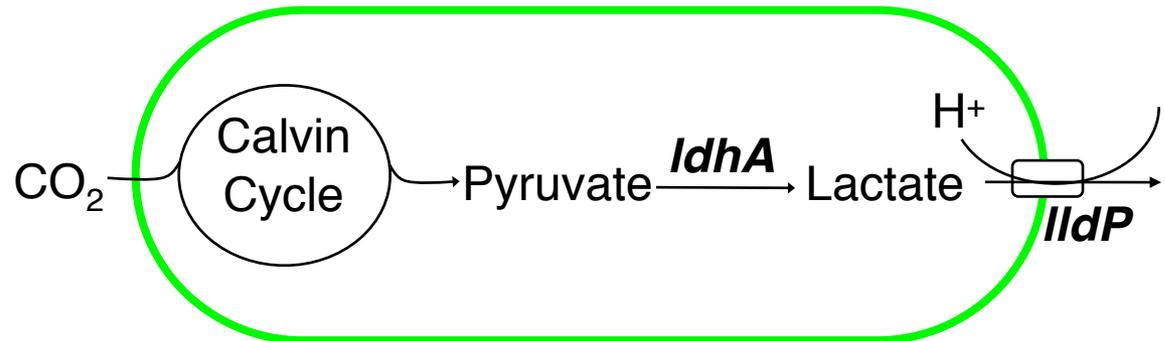


Engineering cyanobacterial production of hexose sugars and lactic acid.

Sugar production

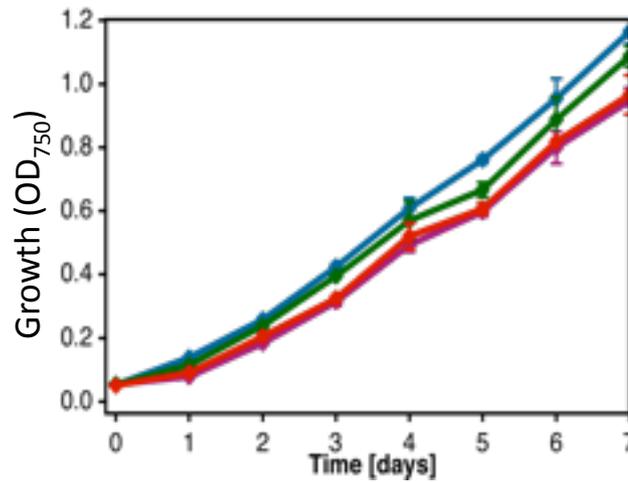
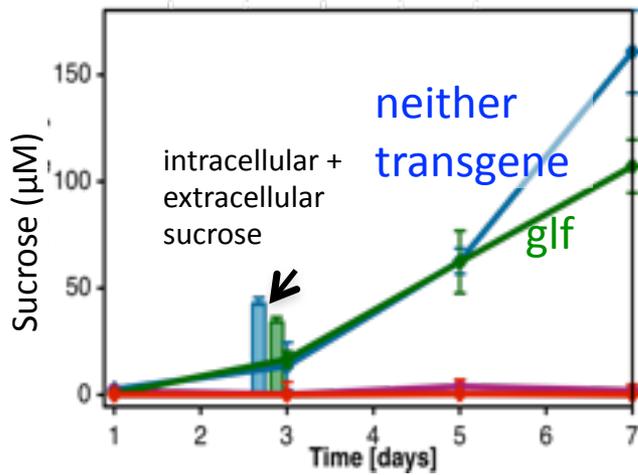
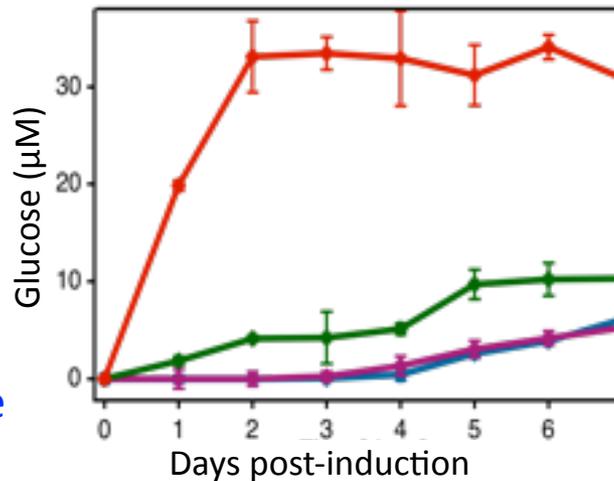
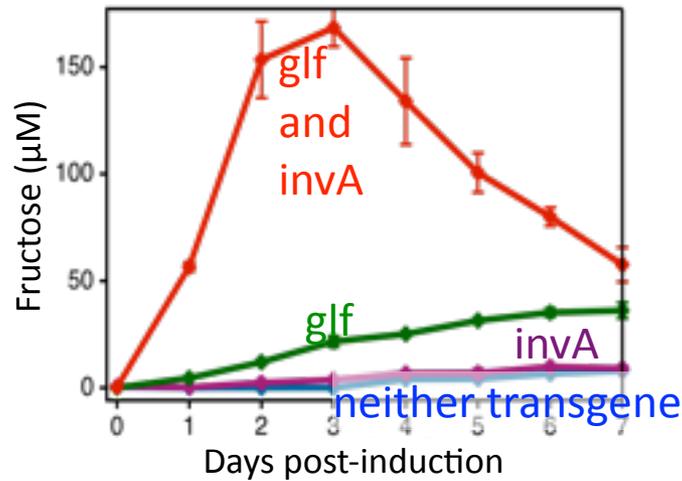


Lactic acid production



Niederholtmeyer et al., (2010) Applied and Environmental Microbiology 76(11):3462

Sugar production and growth of *Synechococcus* strains expressing invertase and a hexose transporter



Interpretations:
 1. Metabolism of the sugars by the bacteria results in significant loss of yield.

2. The impact on cell growth is low → flux into sugars could be further optimized.

Initial conclusions about cyanobacterial production

- Secretion into medium requires transporters
 - Absolute product levels are low
 - Product levels correspond to much of net loss in biomass production (i.e. efficient conversion)
 - Induction of transgenes has little effect on cell growth, consistent with low flux into desired pathway
- Significant room for improvement by traditional metabolic engineering

Expected Value for Cyanobacterial Production

Molecule	Production (g/m ² /day)	Nominal Market value (\$/kg)	Market size	Productivity (relative \$)
Diesel	6.2	\$0.77	\$190 B	1
Sugar	20	\$0.77	\$60 B	3.2
Lactate	20	\$1.5	\$500M	6.4
Lysine	16	\$4	\$500M	13.6

Sustainable, photosynthetic production of fuels may evolve through more expensive commodity chemicals

Engineering sensory stimulation

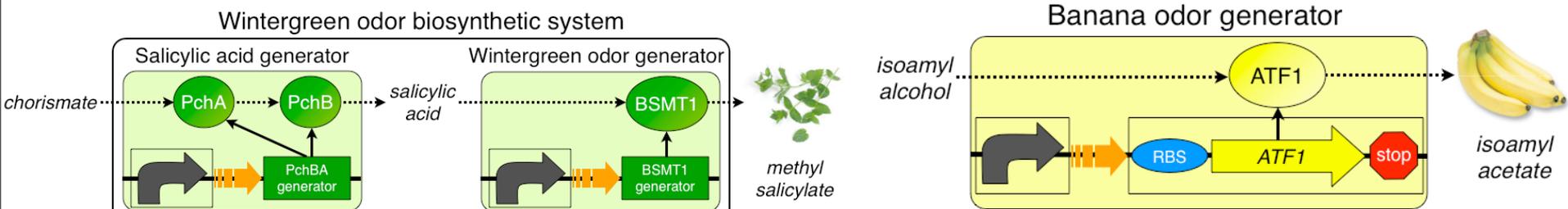
- Harvard iGEM 2010, previous iGEM teams
- Goal: use genetic engineering to create plants and bacteria with ‘designer’ flavors, odors, and colors



Engineering taste & smell



- **Miraculin**
 - Miraculin is a 'flavor inverting' protein, found naturally in the fruit of the plant *Synsepalum dulcificum*. Not sweet by itself, miraculin binds to taste receptors on the tongue, altering receptors and causing sour flavors to be perceived as sweet.
- **Brazzein**
 - Brazzein is a sweet-tasting protein that is found in the Western African fruit, the Oubli. (54 amino acids.) It is sweeter than sugar and is used as an alternative low calorie sweetener.
- **Valencene**
 - Valencene is a gene that codes for a molecule that gives Valencia oranges their citrus flavor and aroma.
- Odors: Wintergreen, Banana
 - The MIT 2006 iGEM team created a system to express methyl salicylate, a mint smelling compound, and isoamyl acetate (banana scent)





Engineering color

- Pigments: **Lycopene** and **beta-carotene**
 - Intermediates in carotenoid synthesis pathway
- By interfering with the pathway, accumulation of these intermediates should produce color.
 - Inhibiting the enzymes lycopene epsilon cyclase (LUT2), carotene beta-ring hydroxylase (BETA-OHASE 1), and lycopene beta cyclase (LYC) via artificial microRNA interference.

Platform #2: Physical-biological hybrids

- Assignment:
Create organisms that absorb e- from electrodes, synthesize biofuels
- Funding initiated July 1, 2010

“RECOVERY ACT”

FINANCIAL ASSISTANCE
FUNDING OPPORTUNITY ANNOUNCEMENT

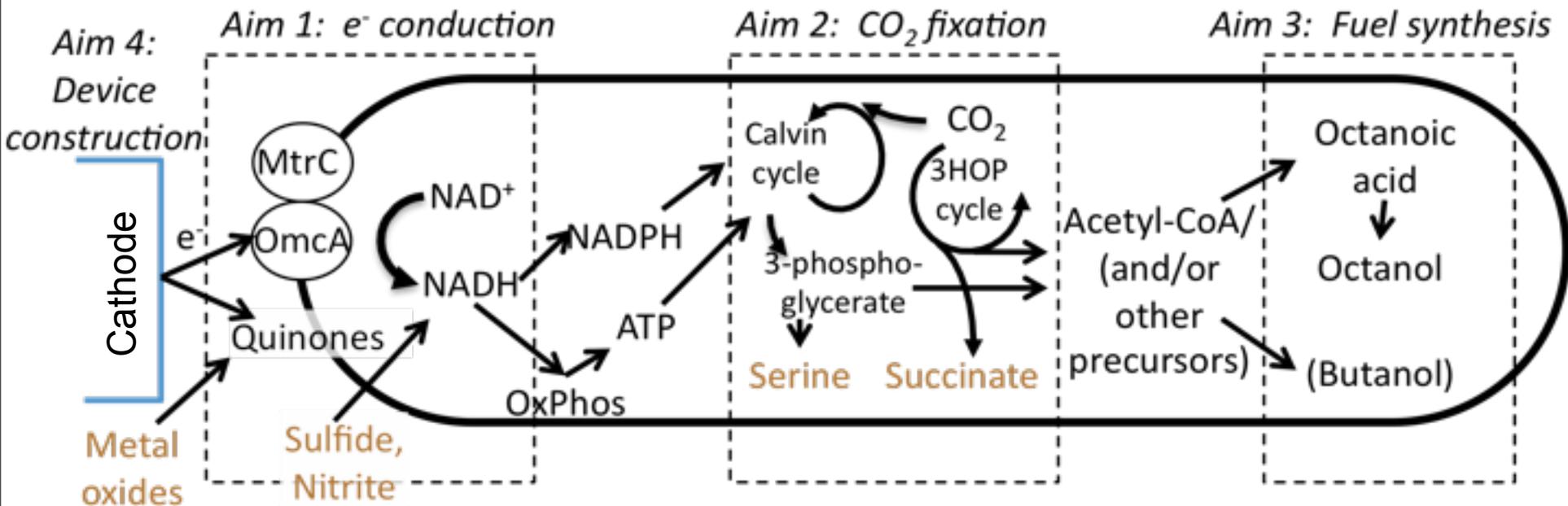
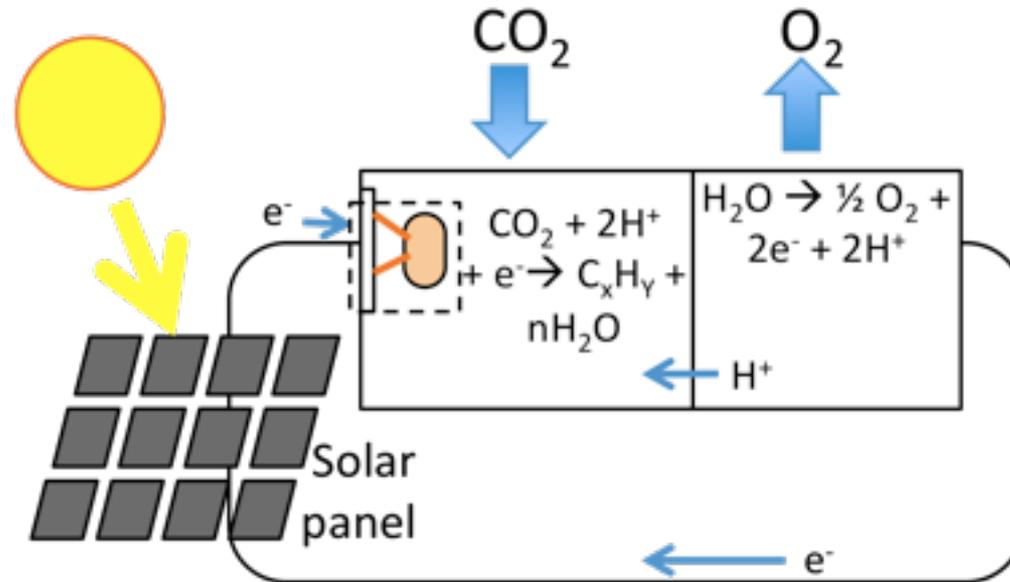


U. S. Department of Energy - Headquarters
Advanced Research Projects Agency – Energy (ARPA-E)

ELECTROFUELS

Funding Opportunity Number: DE-FOA-0000206

Platform #2

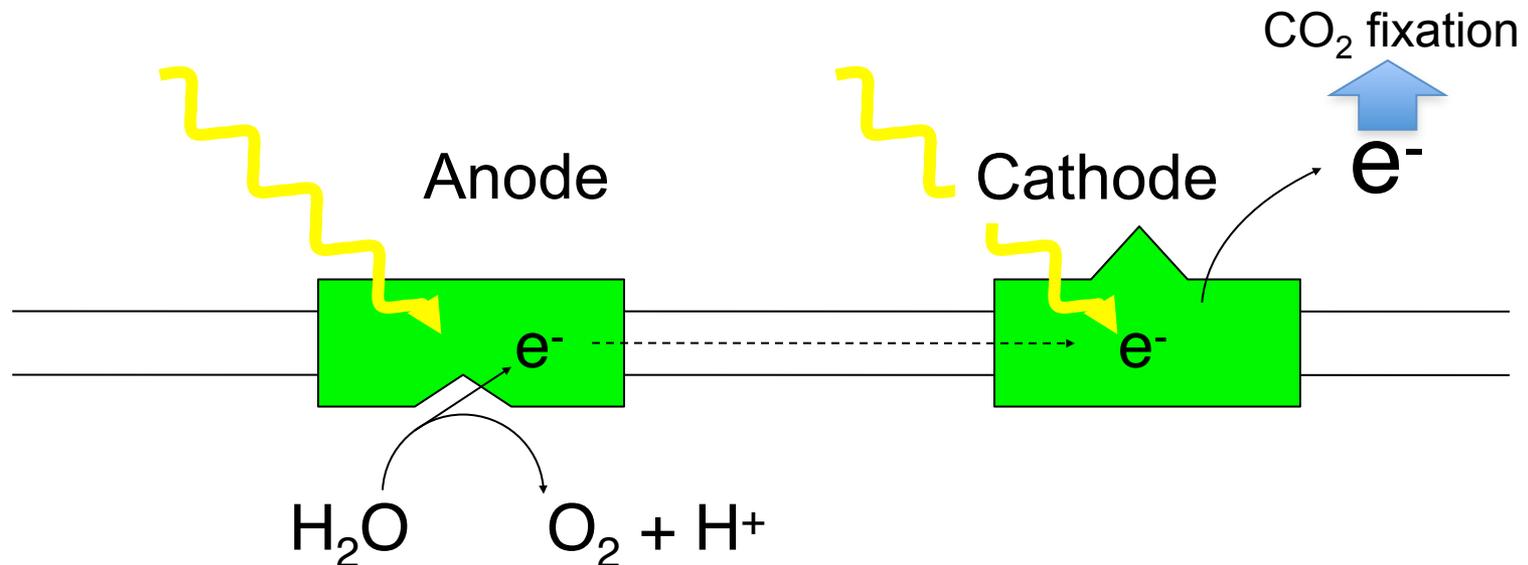


Photosynthesis vs. Electrosynthesis

- Initial photon is captured in Photosystem II, used to split H_2O , produce O_2

- Electrons are transferred to Photosystem I

- Another photon is absorbed, electron is energized to enter cell metabolism



Why “Electrosynthesis”?

- Potentially more efficient than photosynthesis
- Better control of heat loss
 - Heat loss occurs at solar panels, separate from organisms
 - Heat distribution is a major problem in photobioreactors
- Note: electrosynthesis has not been reduced to practice

Platform 3: Photosynthetic animals

- Zebrafish with injected cyanobacteria



What research should NASA support?

- Dual-use space/terrestrial life-support technologies
 - Solar-driven production of nutritional chemicals and other useful commodity chemicals
 - Not fuel (but complementary to DOE efforts)
- Possible goal: space-compatible technology that is commercially viable on earth
 - E.g. palatable bacterial food

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