# Cocultivation of Algae and Bacteria for Improved Productivity and Metabolic Versatility

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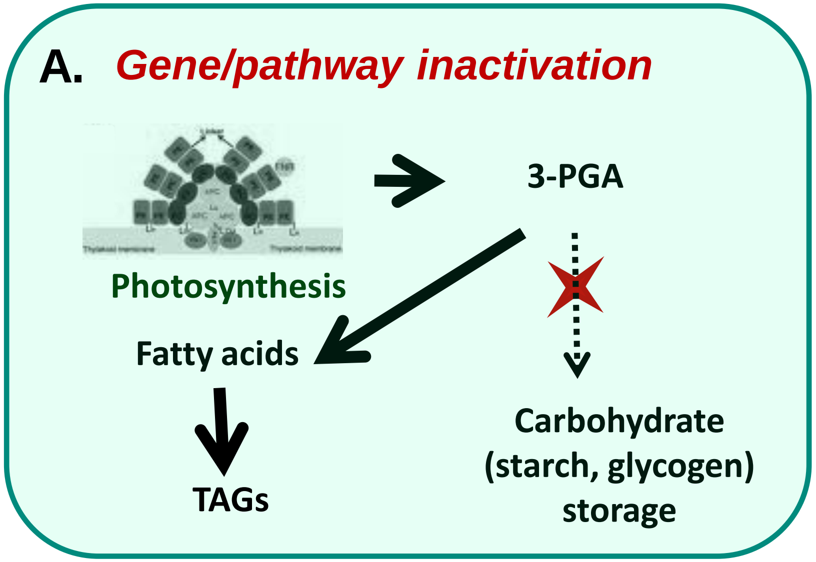
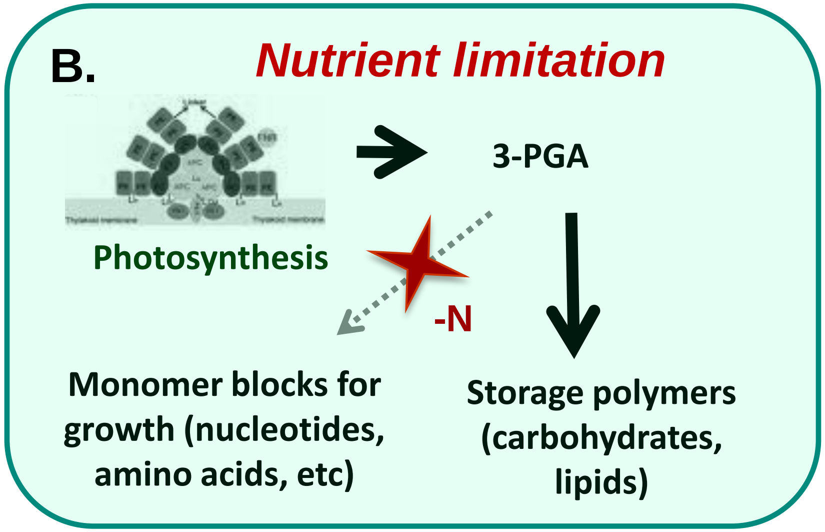
# Axenic Cultures in Algal Biotechnology




- Current approaches use axenic (pure) cultures of microalgae and/or cyanobacteria
- Productivity is manipulated by imposing environmental or genetic perturbations
- *Examples:*

  *A) Inactivation of competing pathways to redirect flux towards specific products*

  *B) Nutrient (N, S) limitation to inhibit growth and enhance storage product accumulation*

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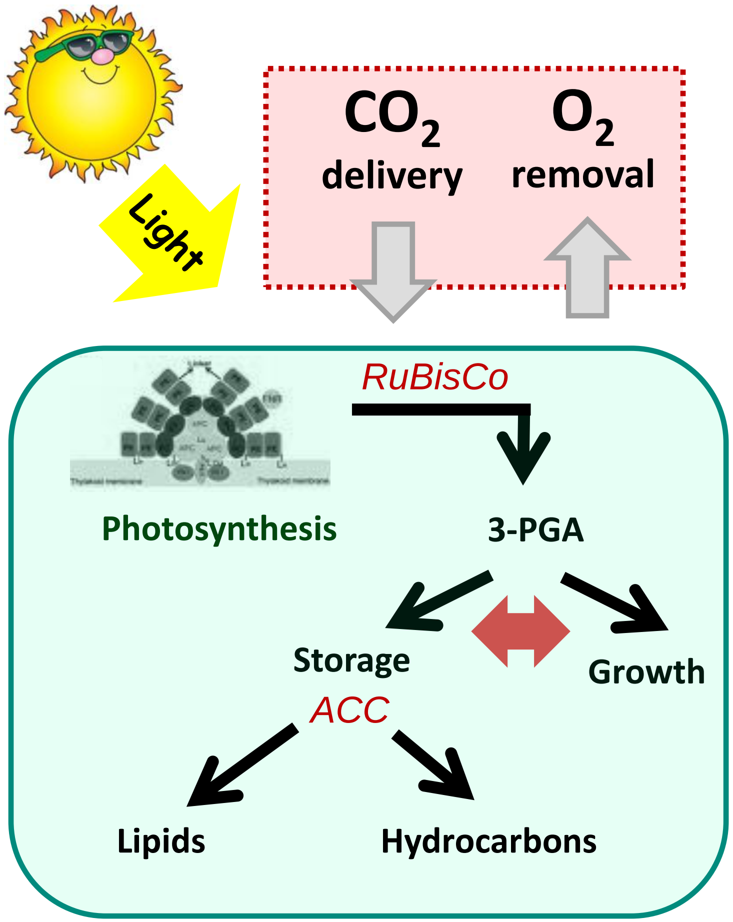
# Axenic Culture Challenges

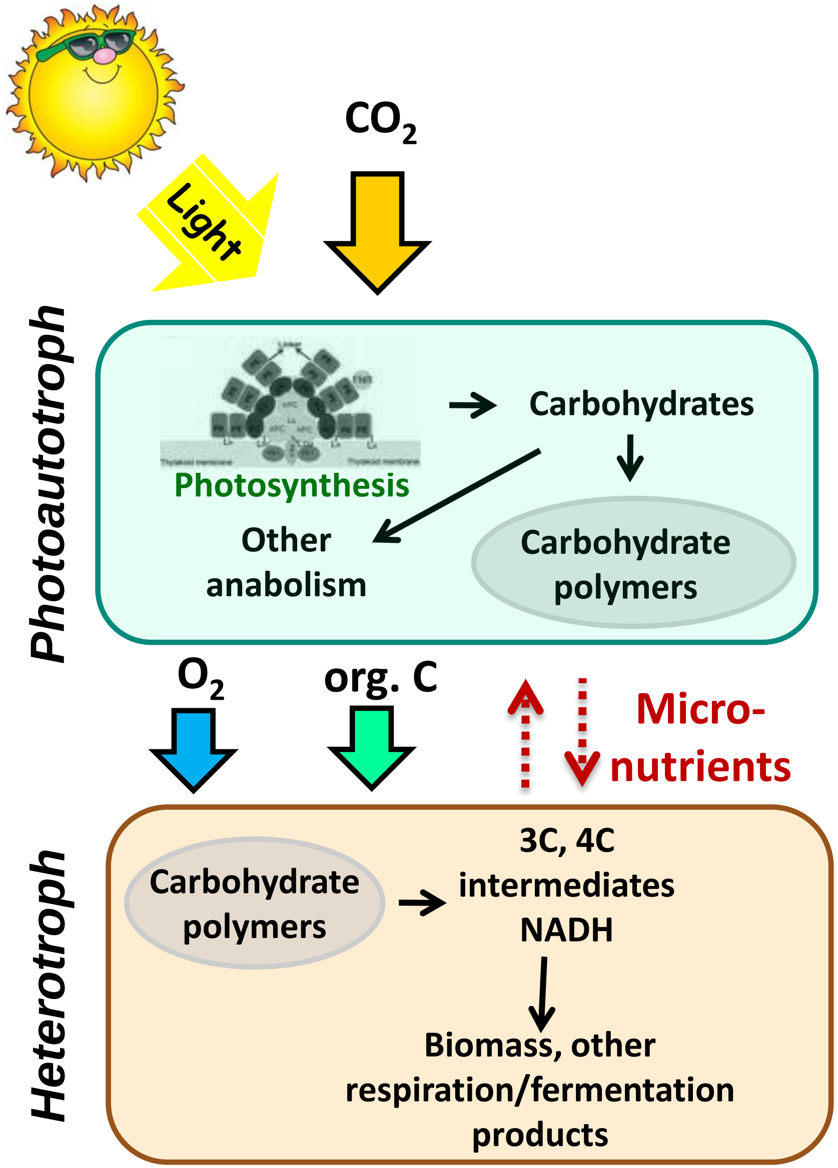



- ***Process engineering***: mass-transfer limitations involving gaseous substrate delivery ($CO_2$) and product removal ($O_2$)

- ***Growth physiology***: balance the energy input with the downstream biosynthetic processes (growth vs. storage compounds)

- ***Metabolic engineering:*** coordination of various pathways needed; changes in expression and/or activity levels may have unanticipated secondary consequences upon product yields. Some functions are subject to product inhibition or allosteric regulation (*e.g.*, RuBisCo photorespiration; acetyl-CoA carboxylase regulation by palmitoyl-CoA).

# Co-Existence of Algae & Bacteria in Nature




- **Algae and cyanobacteria use sunlight and $CO_2$ and produce $O_2$ and $C_{org}$ molecules that support growth of heterotrophic bacteria**
- **Heterotrophic bacteria provide intrinsic stability and support growth of phototrophs by removing excess $O_2$, increasing micro-nutrient availability, vitamin biosynthesis**
- **Algae-bacterial associations represent metabolically interactive, self-sustaining communities, which display adaptation to a range of harsh conditions**

# Phototroph-Heterotroph Co-Cultures

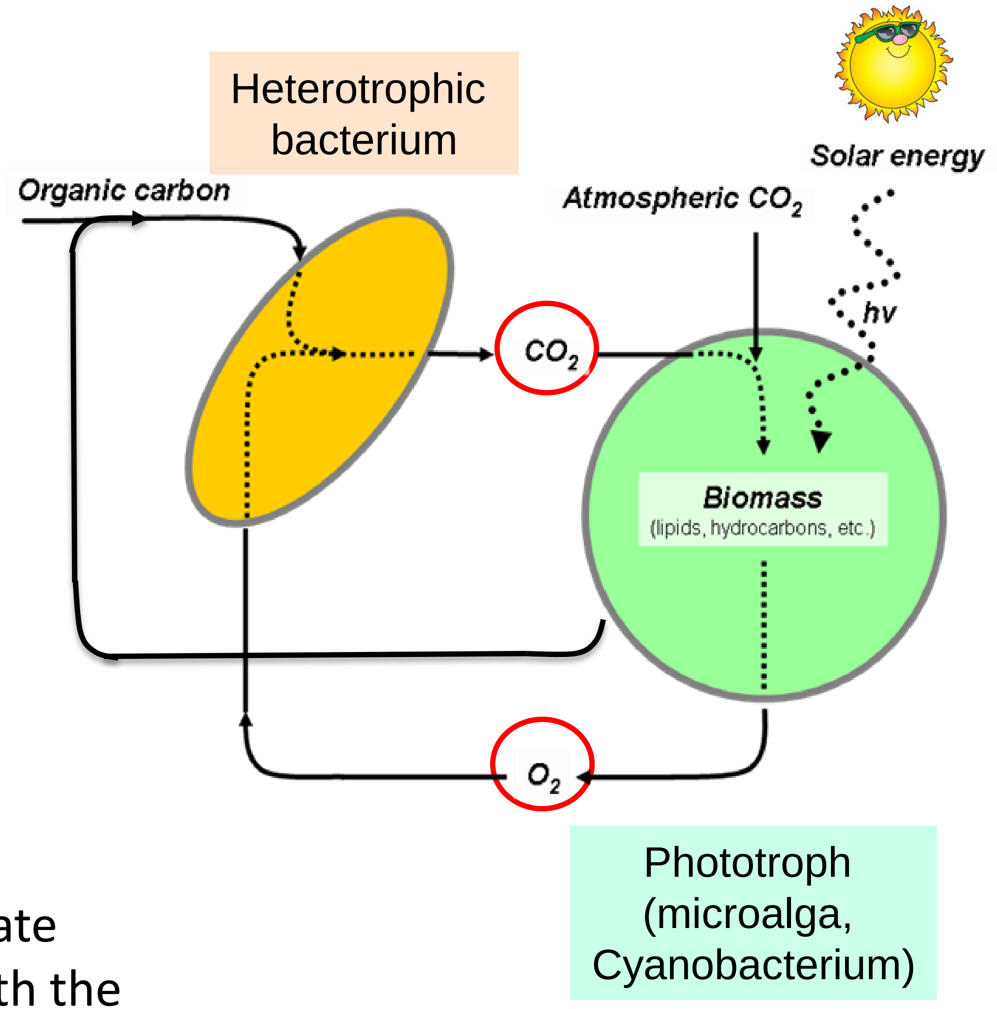
- ***Metabolic coupling***: $O_2$ produced by the algae is consumed by the heterotroph making stoichiometric amount of $CO_2$ through oxidation of (endogenous or exogenous) organic C. Stoichiometric constraints drastically increase the intrinsic stability.

- ***Advantages:***

-Improved mass transfer & productivity

- Increased range of carbon sources

- Modularity & ability to spatially separate the processes of light & $CO_2$ capture with the downstream photosynthate conversion

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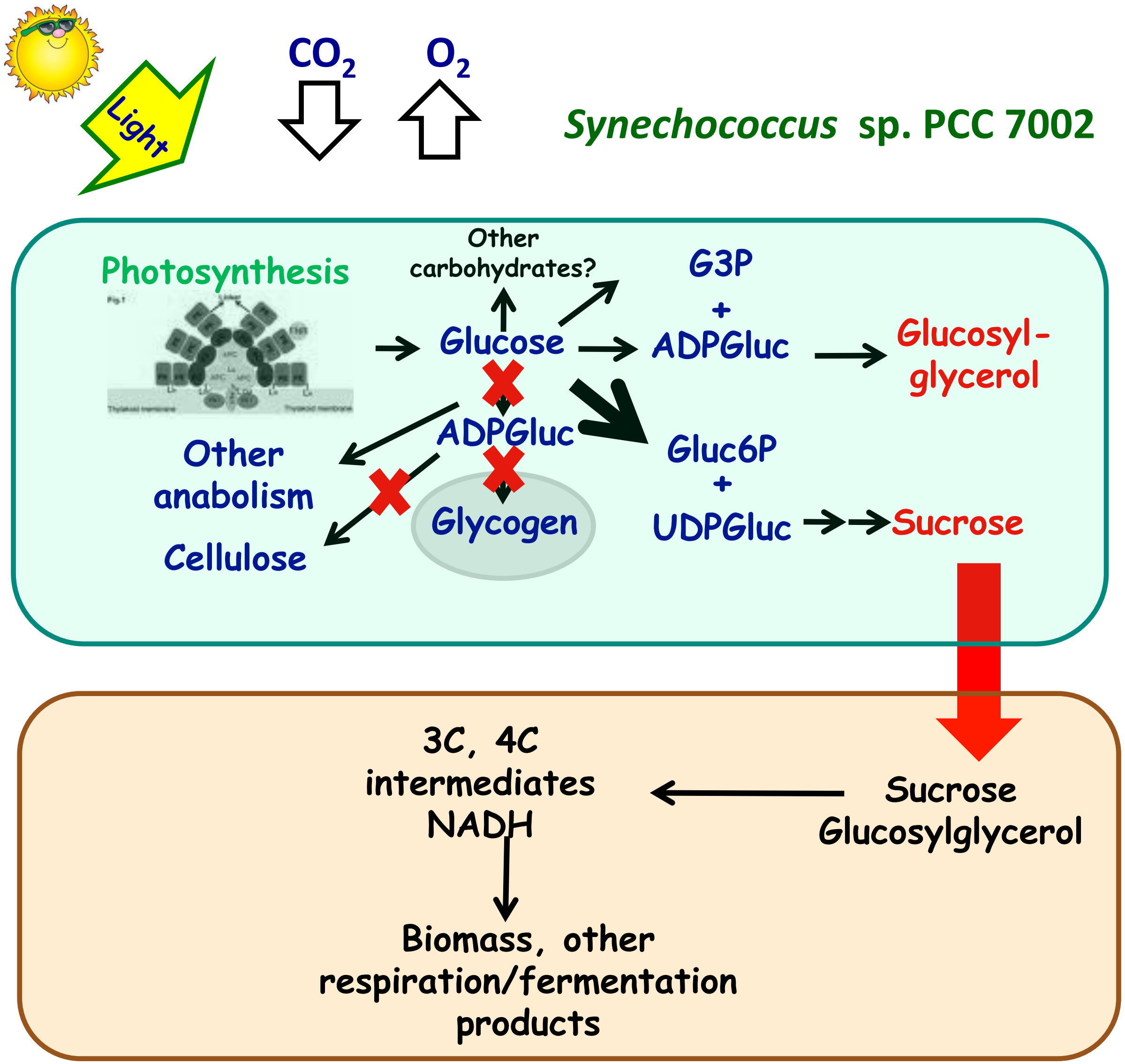
# Coupling through Photosynthate Secretion



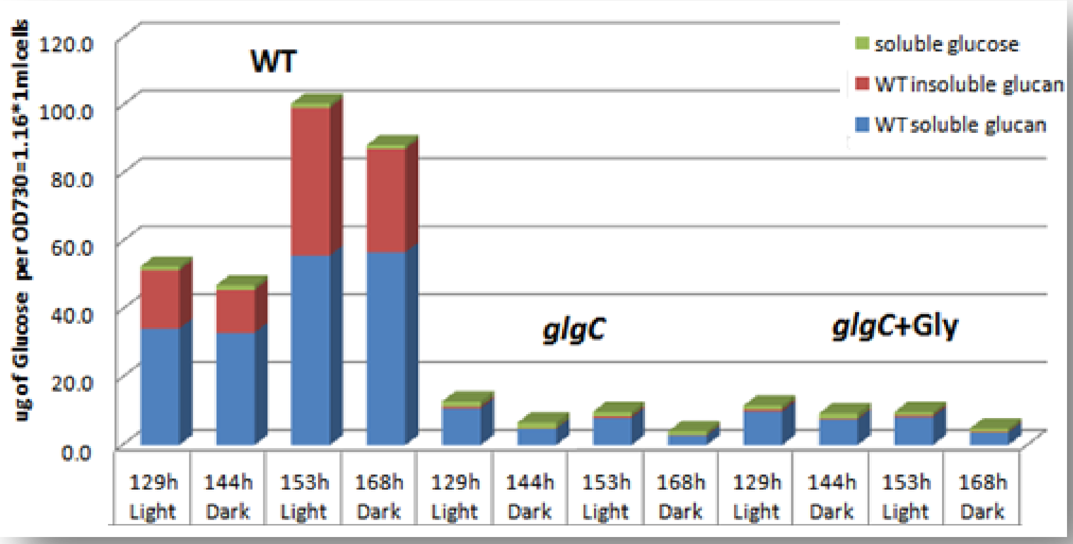

***Rationale:*** Redirect fixed $CO_2$ to mono/ disac-charide derivatives, which can be excreted and used as a carbon and energy source for biofuel synthesis by hetrotrophic organisms.

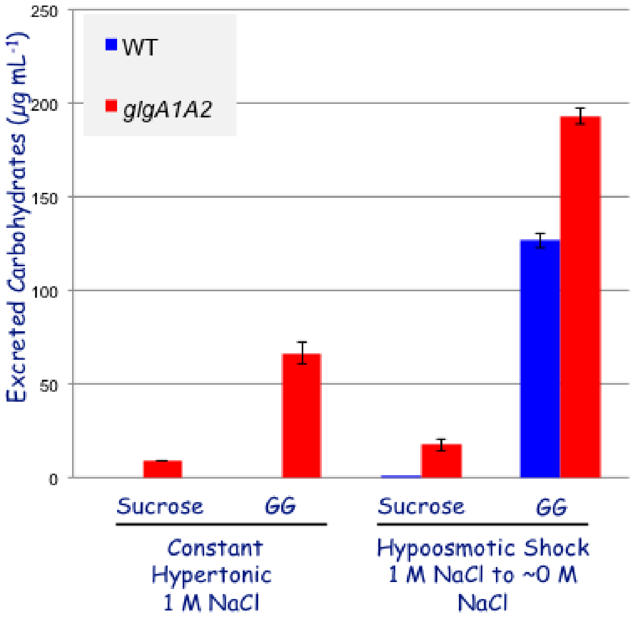
***Approach:*** Eliminate glycogen storage by mutation of *glgA1, glgA2*, and *glgB, and/*or *glgC but* maintain high photosynthetic rate.



*In collaboration with Bryant's Lab (Penn State)*

# Engineering Glycogen Metabolism to Increase Carbohydrate Excretion



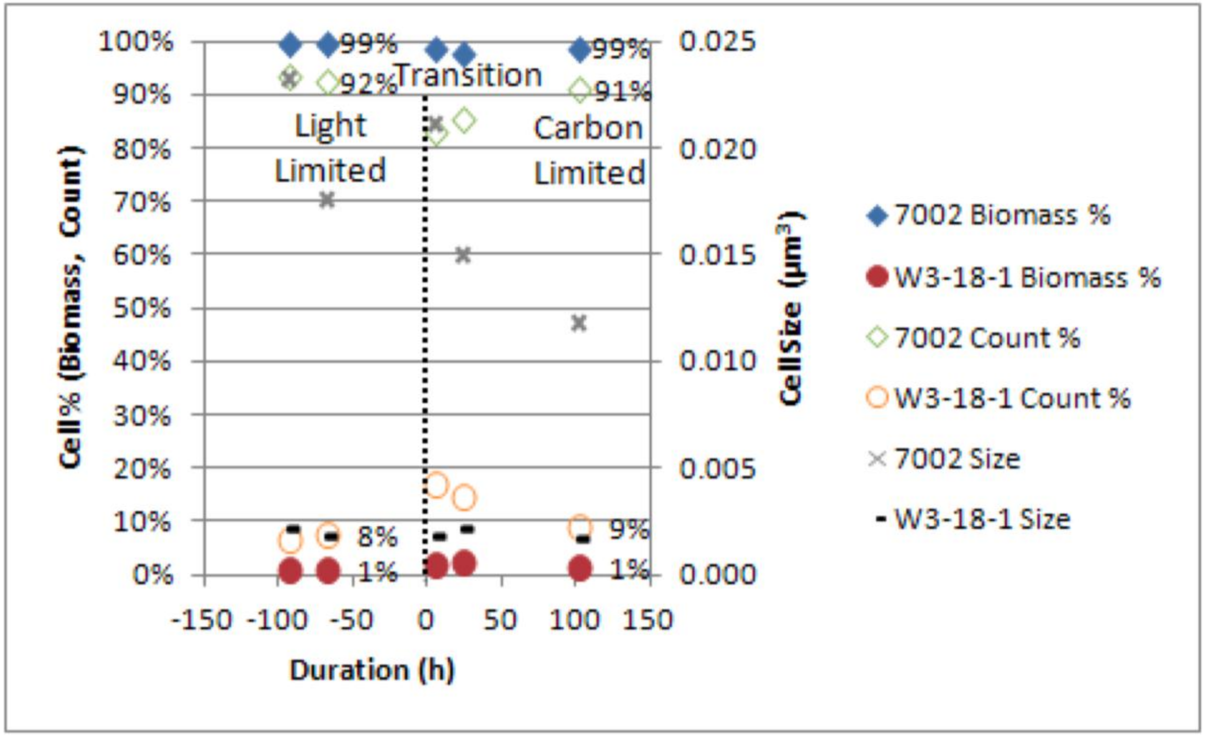




**This strategy works! Glucose, sucrose and glucosylglycerol are excreted in *glg* mutants of *Synechococcus* sp. PCC 7002 that cannot make glycogen.**



*Bryant, Xu et al., 2012 (in prep)*

# Metabolic Coupling through Secreted C



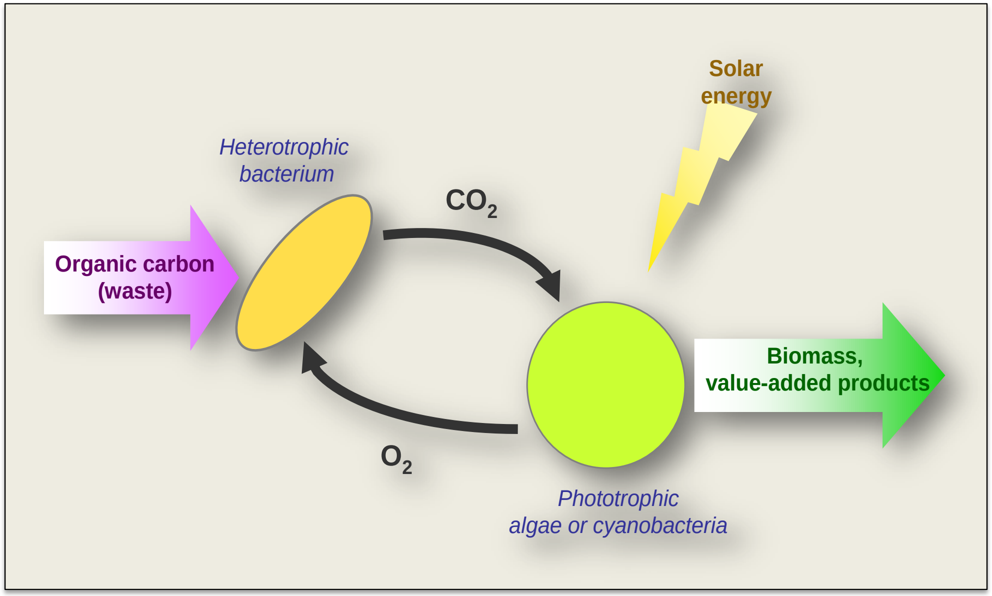

- Heterotrophic growth supported through secretion of sugars and osmolytes (>300hs)
- Biomass concentration can be manipulated by varying growth conditions (light, $CO_2$)

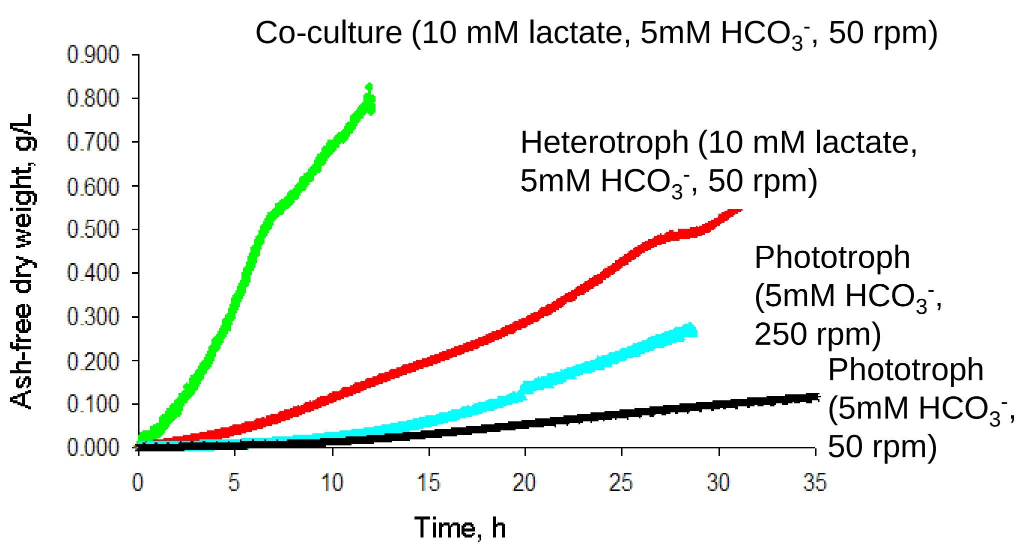
- Plug-and-play approach in which process of photosynthetic carbon fixation and product biosynthesis is spatially separated

    Module A: $CO_2$ -> $C_{org}$ (sugars, organic acids)

    Module B: $C_{org}$ -> target bio-product

# Metabolic Coupling *via* Exogenous C




- Allows utilization of various C sources (including waste streams)

- Limited mass transfer as $O_2$ and $CO_2$ are produced throughout cultivation vessel

- Axenic (pure) cultures display significantly lower biomass productivity and growth rates

- Co-culture displays higher growth & productivity; does not need high mass transfer rates ; utilizes both carbon sources; no $O_2$ accumulation

- Ratio of $C_{org}/CO_2$ affects the proportion heterotroph & phototroph biomass

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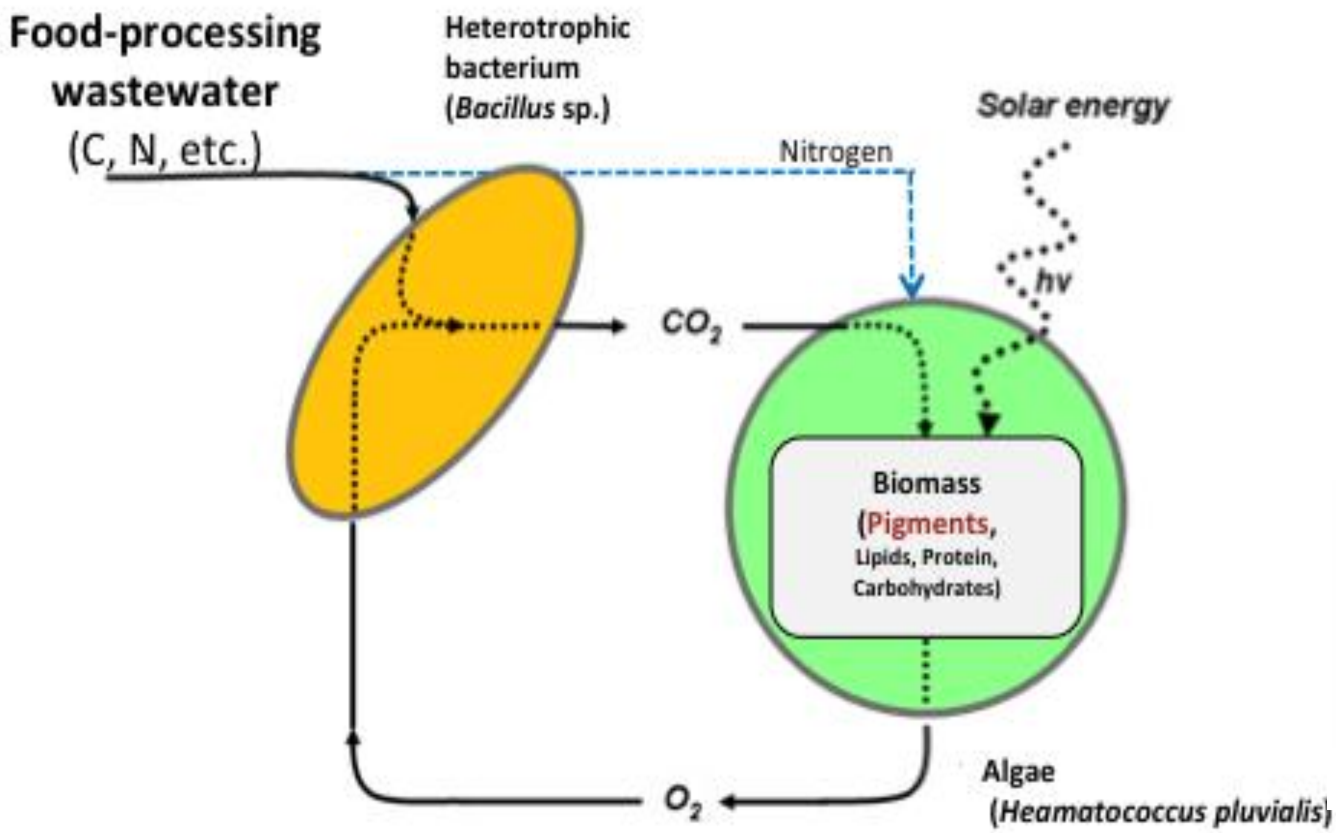
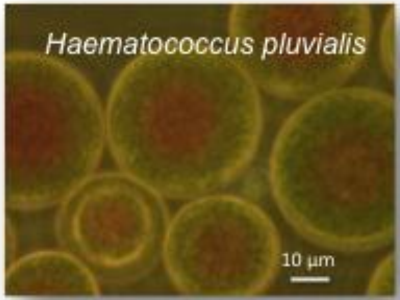
# Waste Treatment using Algal Co-cultures



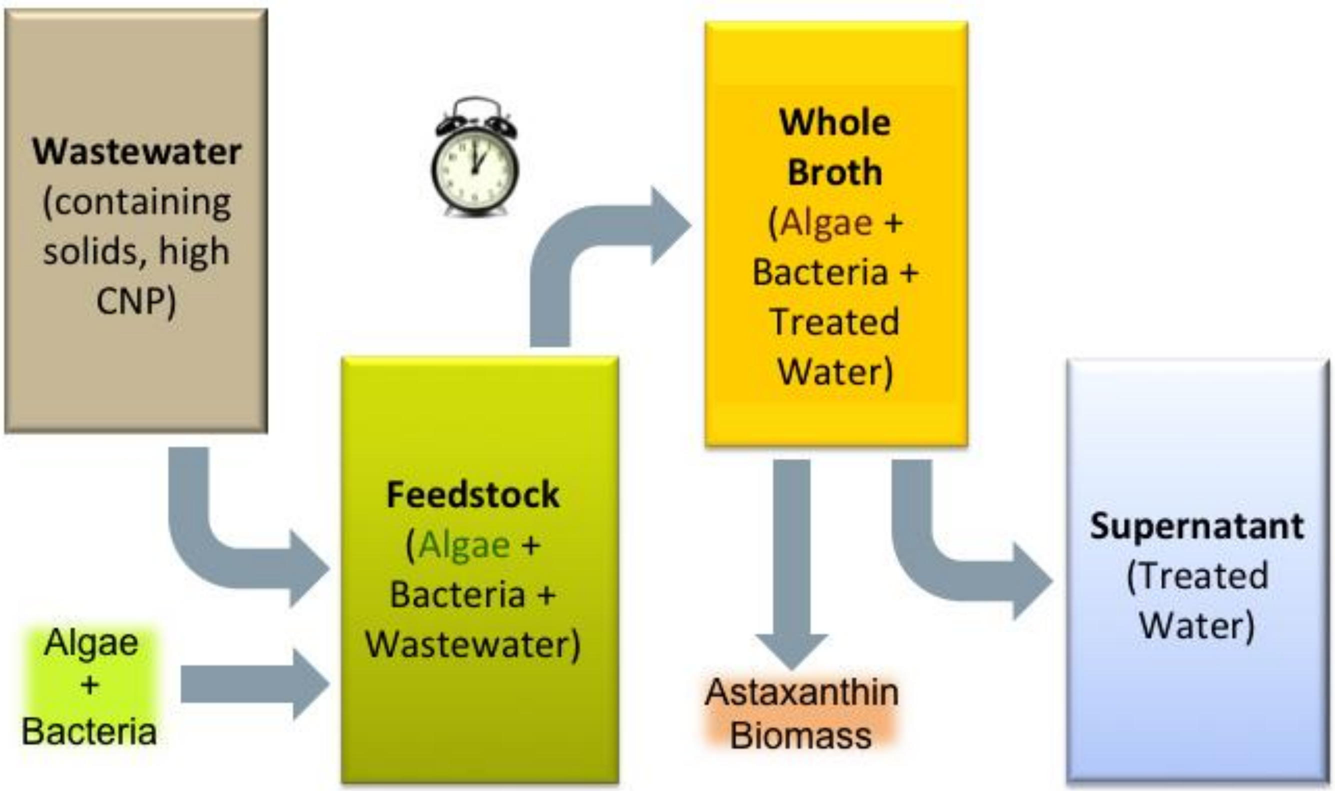

## Astaxanthin

- High-Value Pigment (~$2000/kg)
- Feed: Salmon, Poultry
- Natural vs. Synthetic (95%)



# Wastewater Treatment: Setup

- Wastewater with high concentration of complex carbohydrates, N, and P
- Co-culture *Bacilllus* sp. and *Haematococcus pluvialis*
- Light, no bubbling, low agitation

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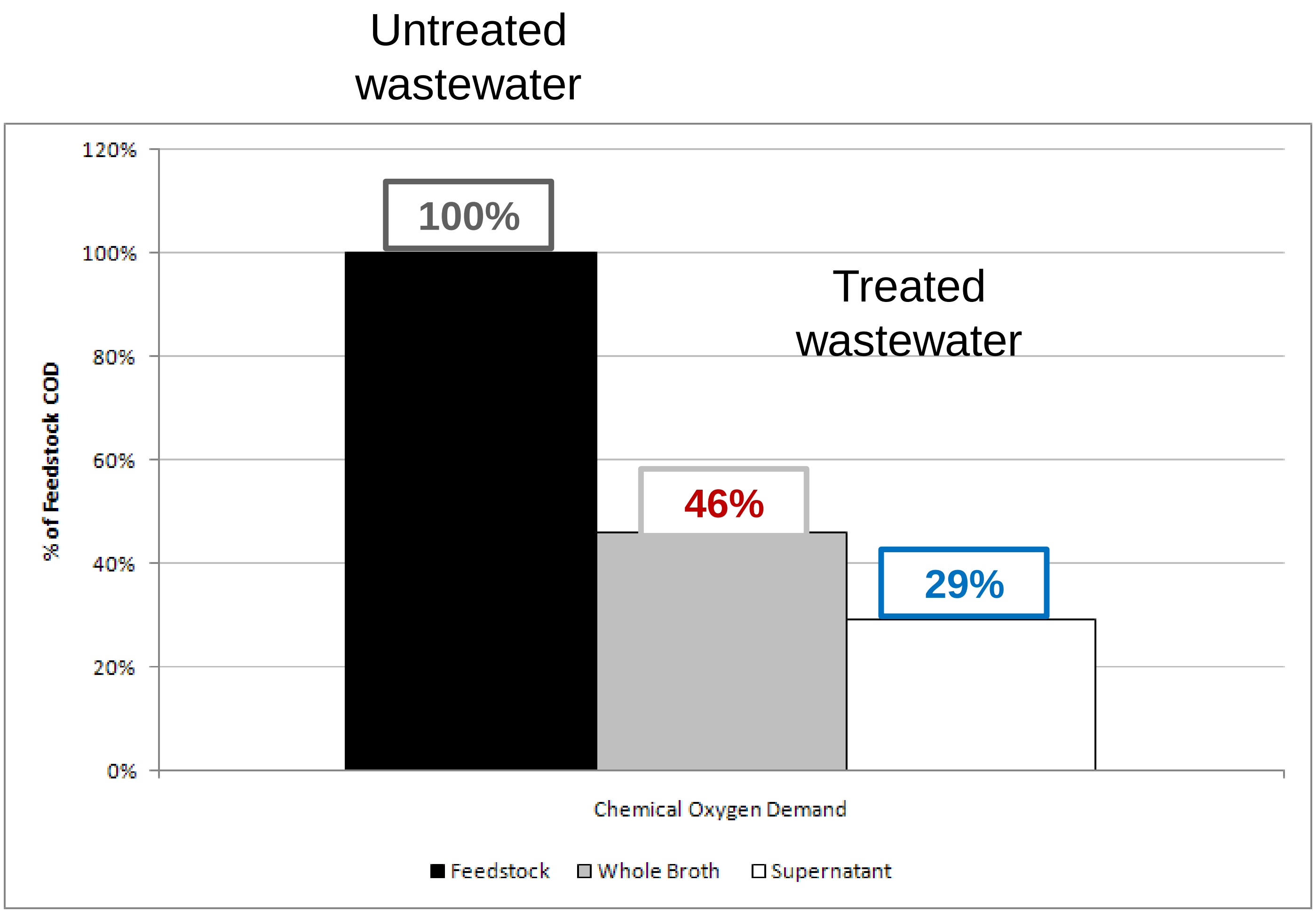
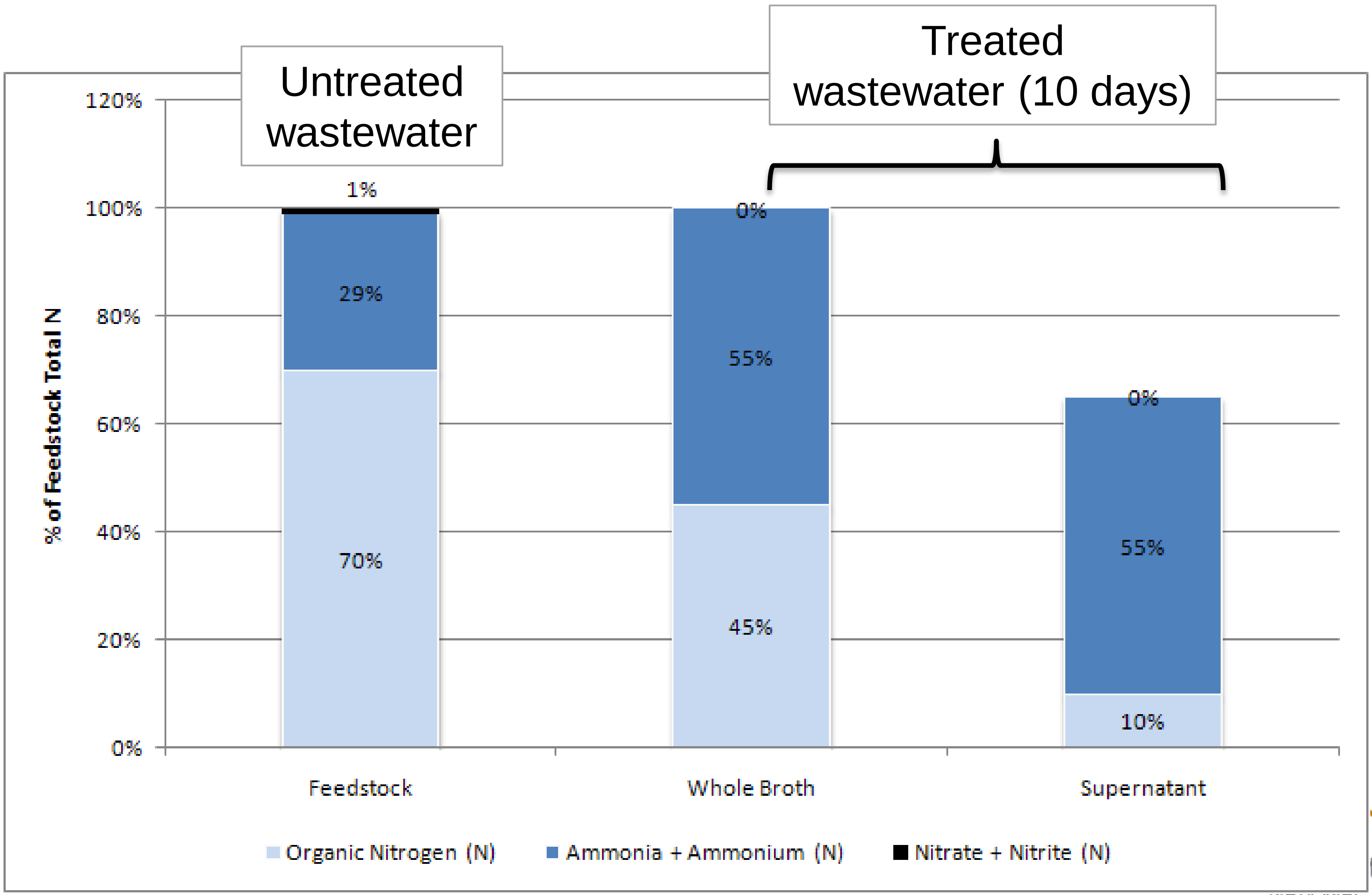
# Wastewater Treatment: COD

## Results after 200 hr incubation:



# Wastewater Treatment: Nitrogen

## Results after 200 hr incubation:

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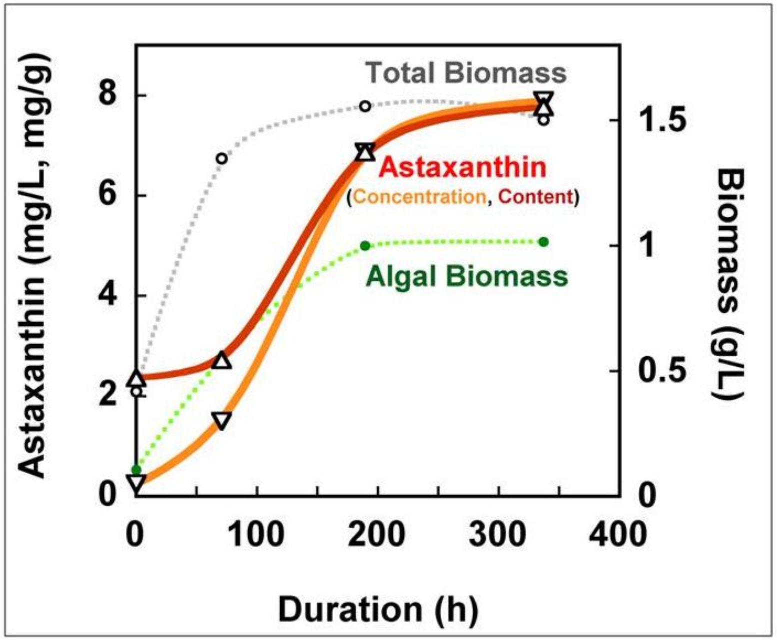
# Production of High-Value Biomass

## Results after 200 hr incubation:

Biomass: 2.2 g/L
Algae: 1.4 g/L
Astaxanthin: ~ 0.8%



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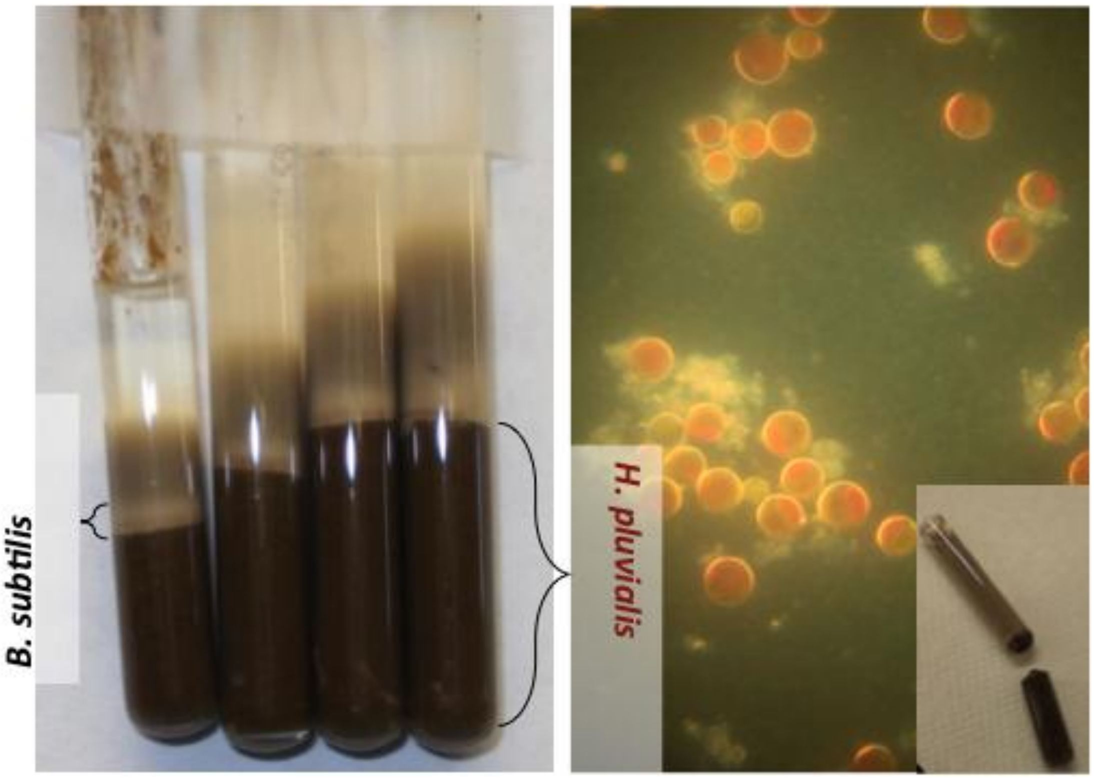
# Astaxanthin Accumulation




**Value proposition:**

- *waste treatment (reduction in COD/BOD, N, P)*
- *high-value biomass production*
- *reduced mass-transfer, energy expenditures, as well as C emissions*

# Summary

❑ Phototroph-heterotroph co-cultures present an alternative option for photosynthetic production of value-added products and commodities such as biofuels.

❑ In comparison to axenic (pure) cultures, co-cultures display broader substrate versatility, higher productivities due to decreased of mass transfer requirements, and provide increased engineering flexibility by spatially and/or temporally separating the processes of photosynthesis and photosynthate conversion

❑ We have successfully applied co-cultivation of heterotrophic bacteria with microalgae for wastewater treatment and production of high-value biomass. The approach opens new ways for designing highly-efficient production processes for feedstock biomass production as well as allows utilization of variety of organic agricultural, chemical, or municipal wastes.

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