Continuous TAG production

by the green microalga *Neochloris oleoabundans* under nitrogen limited conditions

- 9th European Workshop Biotechnology of Microalgae -

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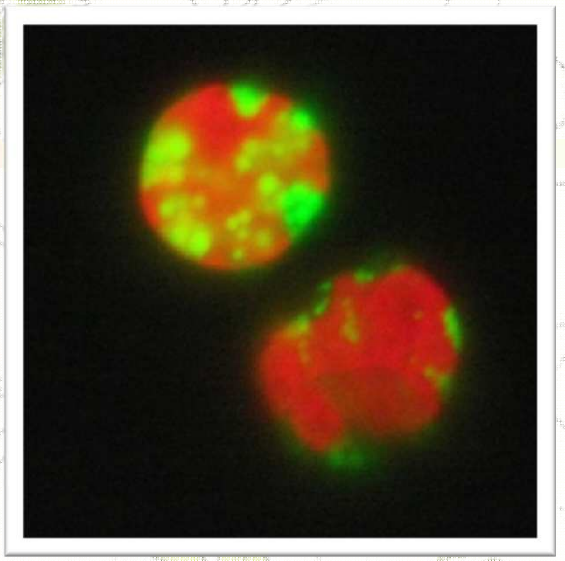
Green microalgae for biofuel



- Microalgae can produce neutral lipids
  - Adverse growth conditions
  - Up to 60% w/w
  - Mainly Triacylglycerols (**TAG**)
  - Stored in ‘lipid globules’
- TAG are **ideal for production of transport fuels**
  - Easily converted using existing technology
- **TAG content** is main target for process optimisation
  - DSP is easiest at highest TAG content
- What is often forgotten:

  TAG Productivity is equally important!

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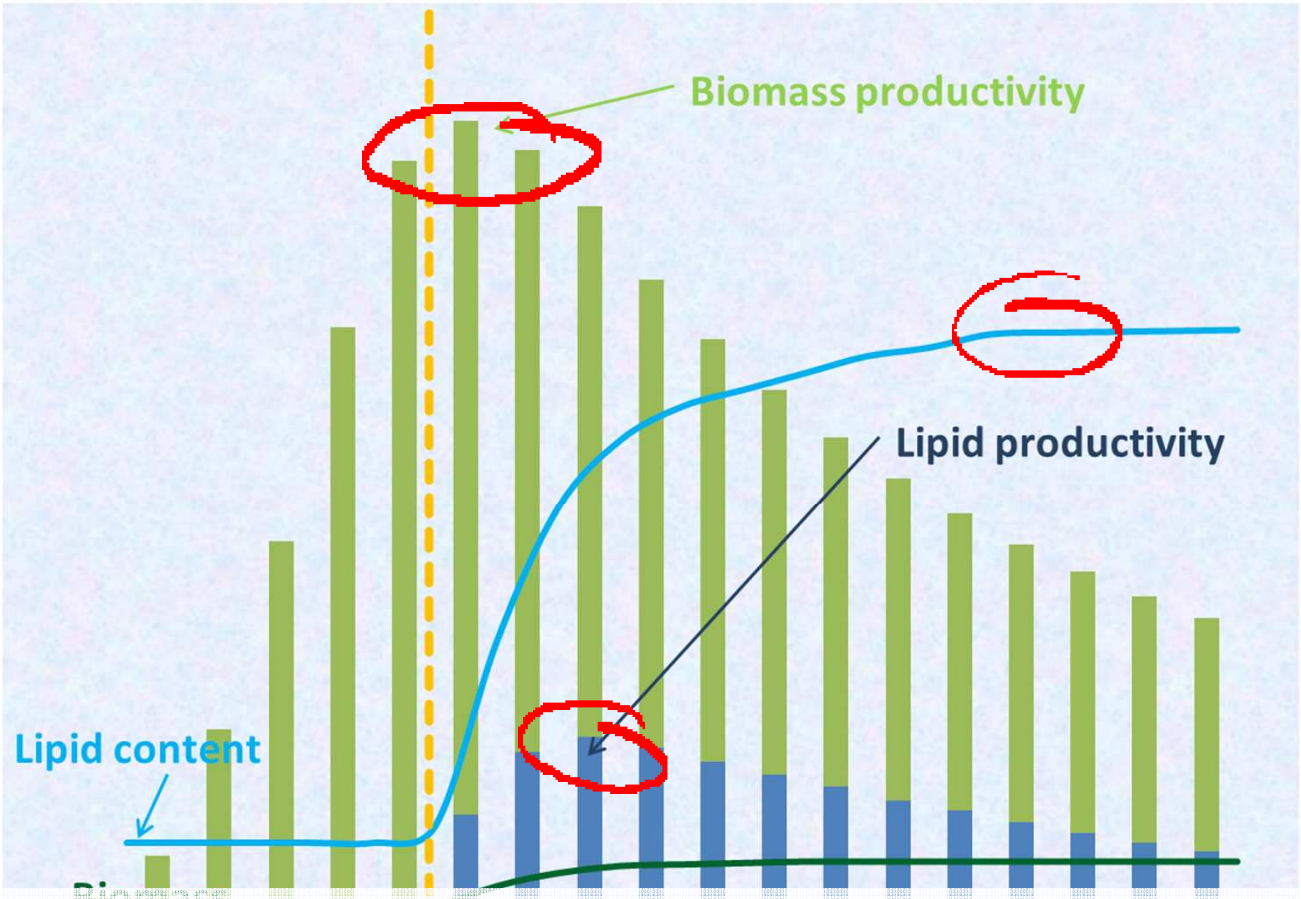
TAG production: the classic approach



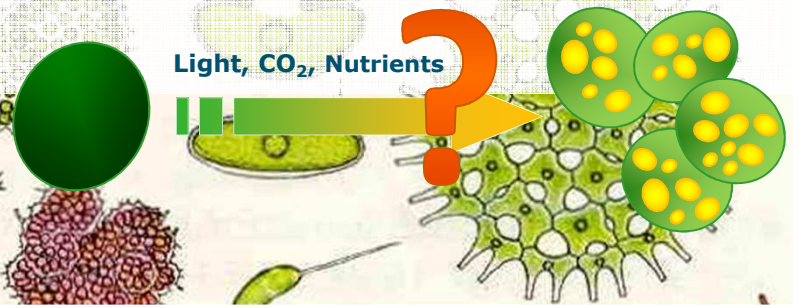
Both TAG productivity and TAG content are important in realizing sustainable production of algal biofuel

TAG accumulation
What is (not) known…

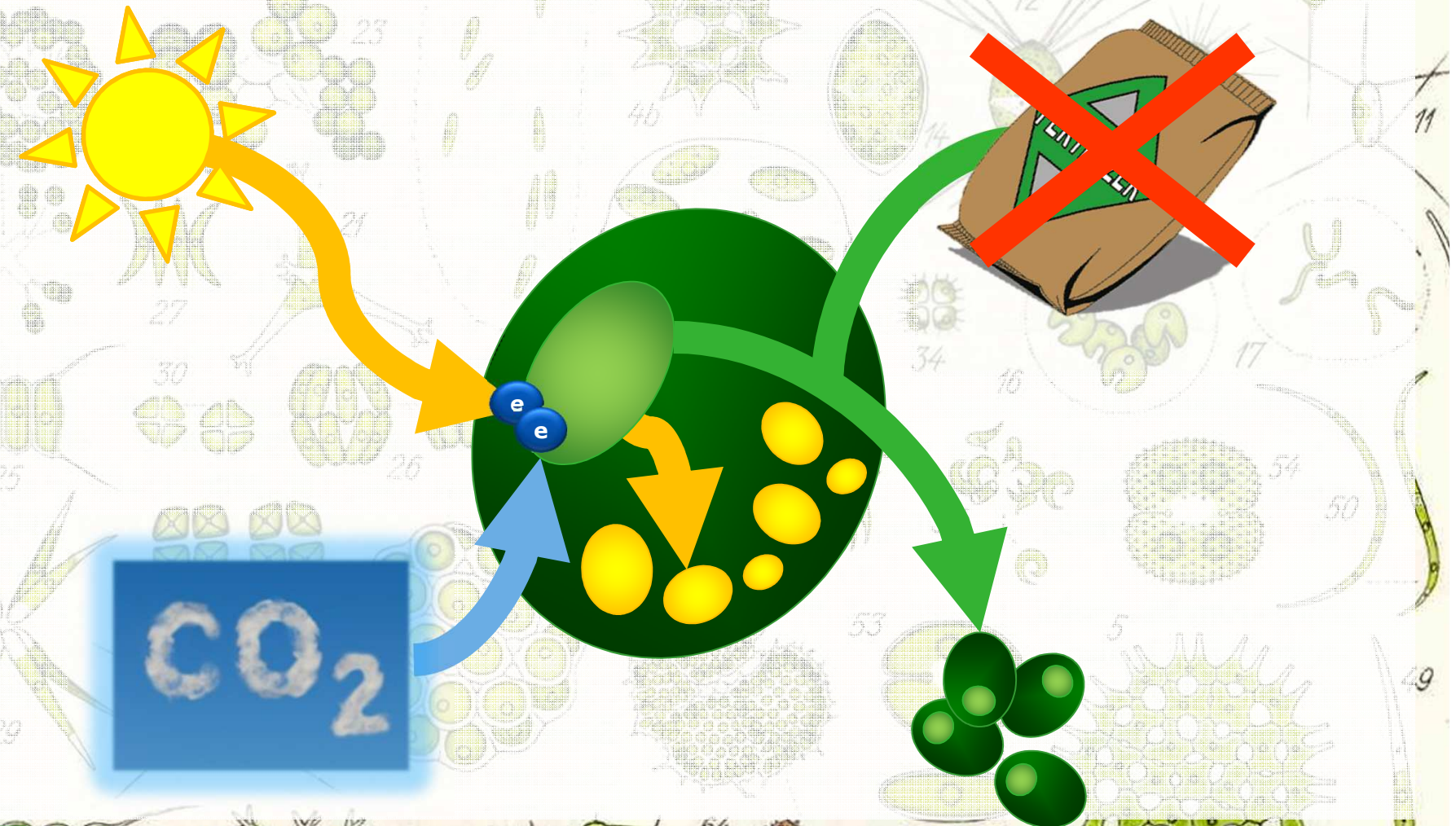
- Exact mechanism unknown
- Literature
  - TAG fraction increases when **nutrients become limiting**
  - Accumulation is more severe at **increased light intensities**

Light and nutrients are very important players in TAG accumulation

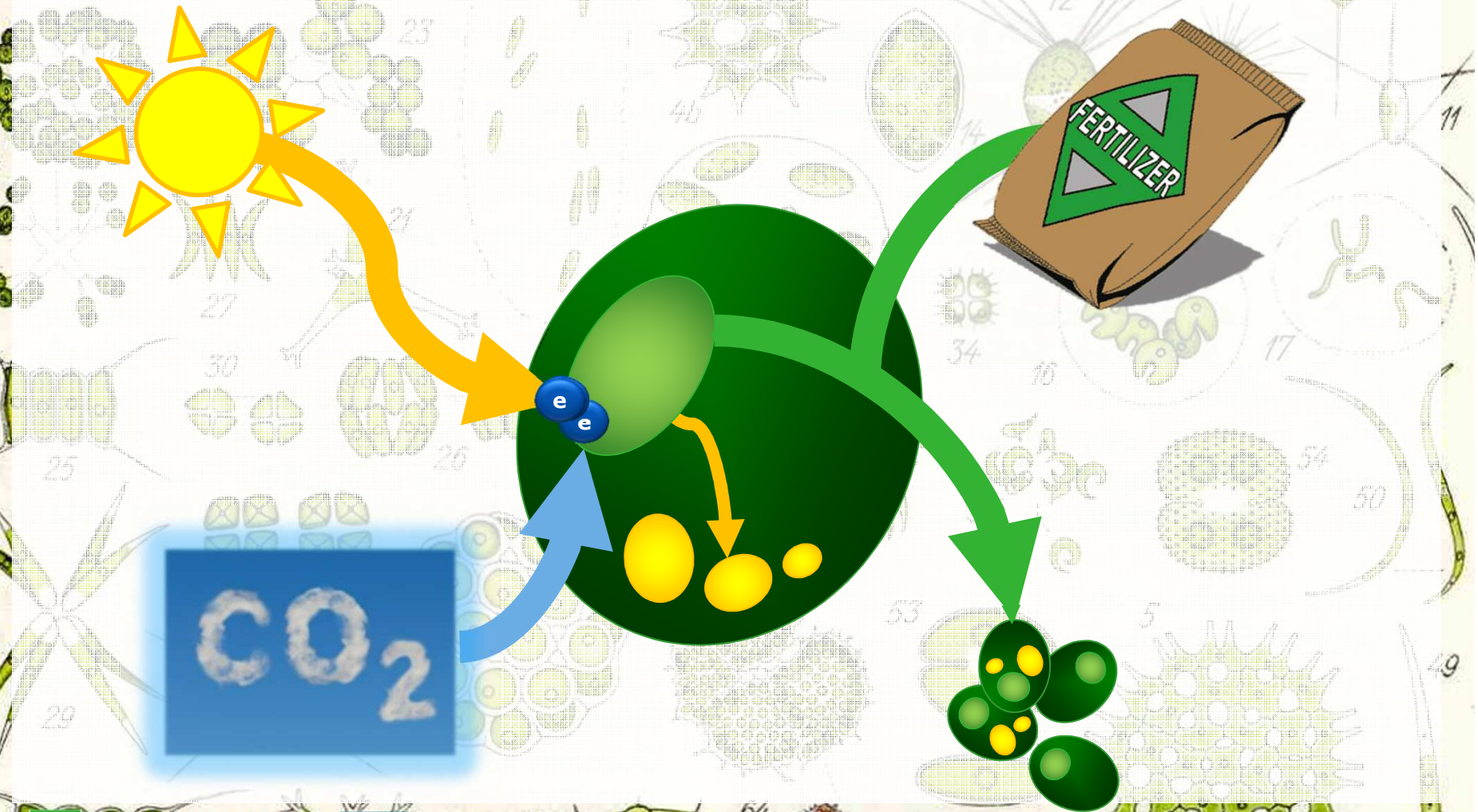
- Current approach: nutrient depletion
  - **Changing conditions** : Light intensity and nutrient concentration
  - **Difficult to study separate effect**



Hypothesis: TAG act as e^- sink



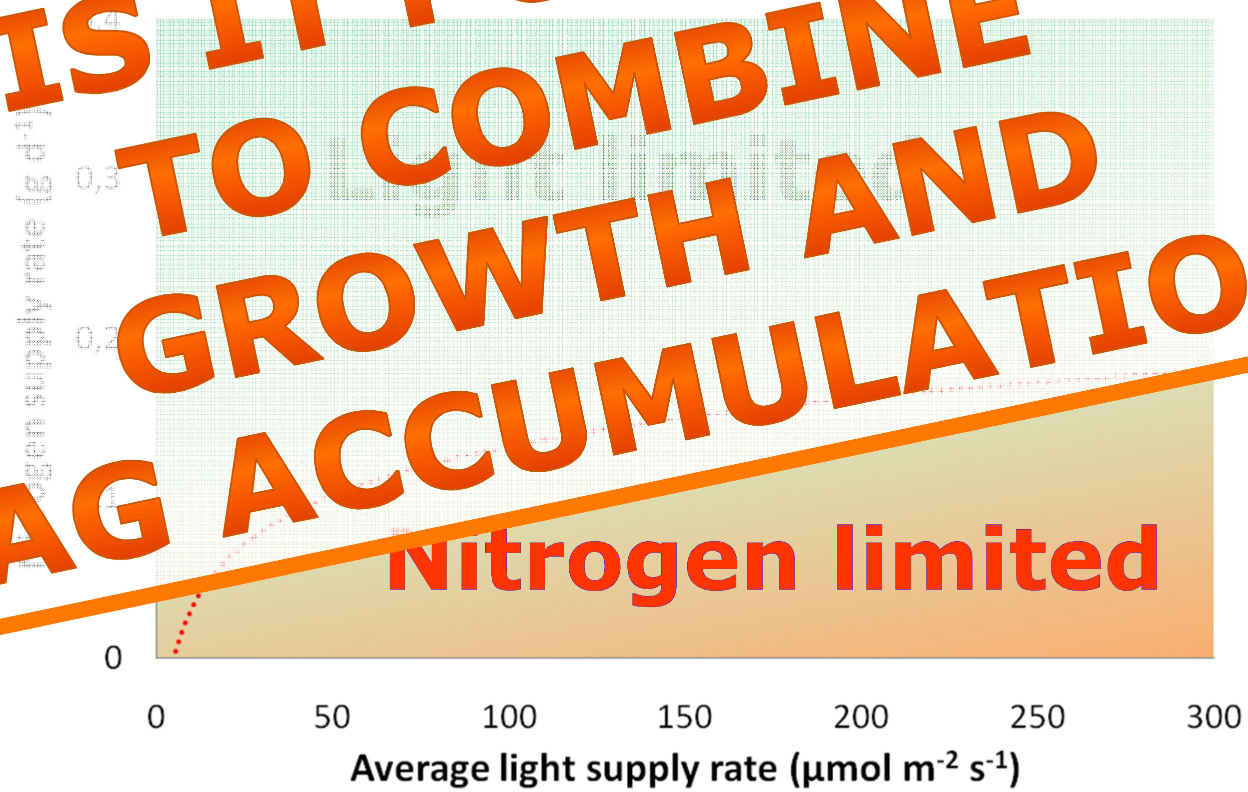
A new way of thinking
Playing around with metabolic fluxes



TAG accumulation
What do we want to know!

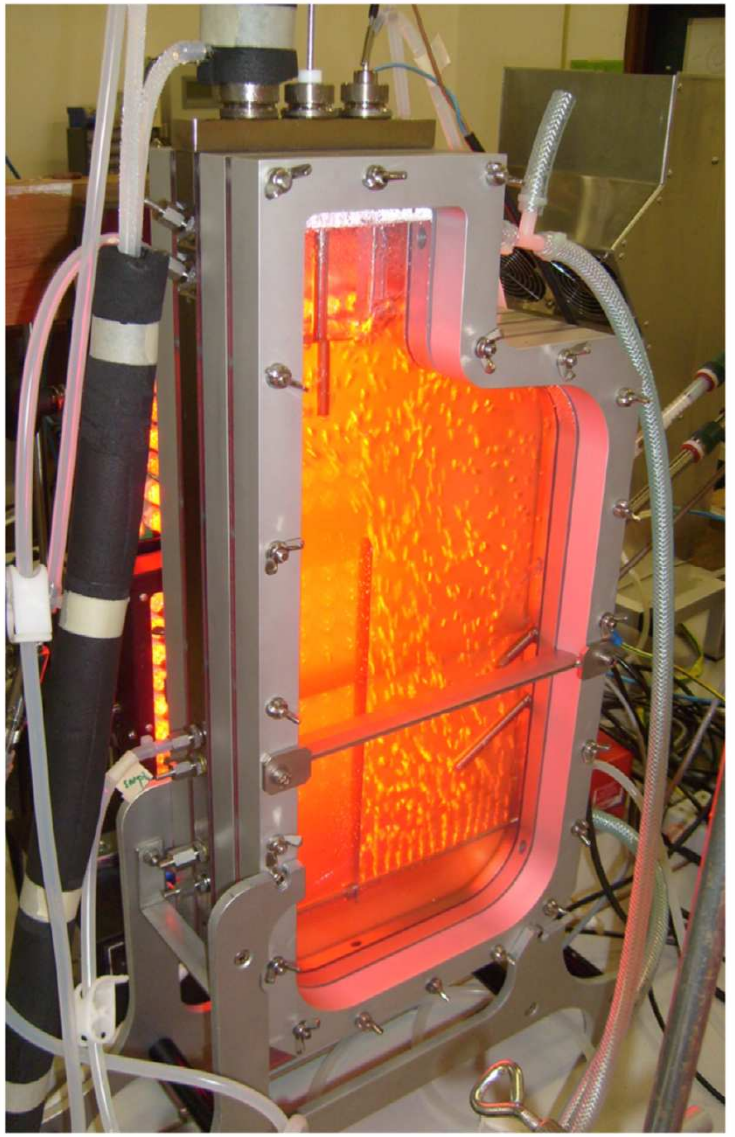
- **Nitrogen and light supply** are tw... major players ... growth and lipid accumulation
- They directly influence the e...

IS IT POSSIBLE TO COMBINE GROWTH AND TAG ACCUMULATION?



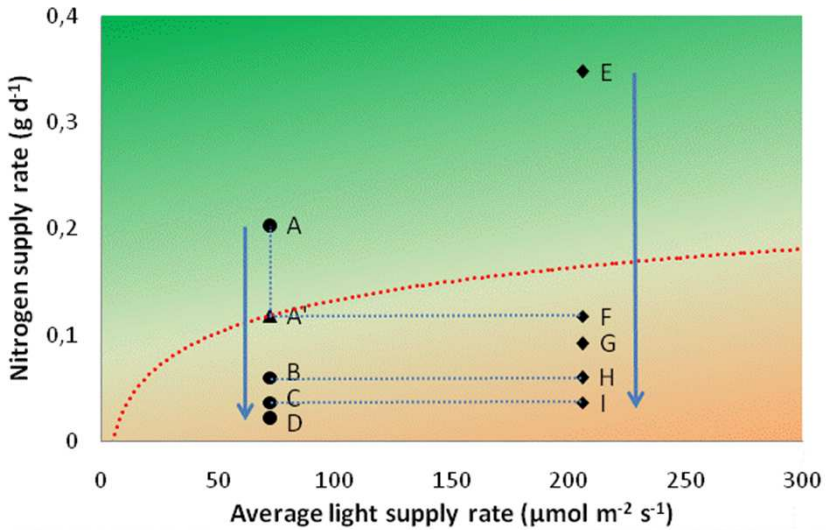
Experimental setup

- *Neochloris oleoabundans*
- **Light** is kept constant by turbidostat control
  - Dilution with N free medium upon increased turbidity
  - $\mu = D$
- **Nitrogen** is supplied separately from diluting medium at a constant rate
  - N stock with same composition as dilution medium
  - N supply is not influenced by small deviations in growth rate



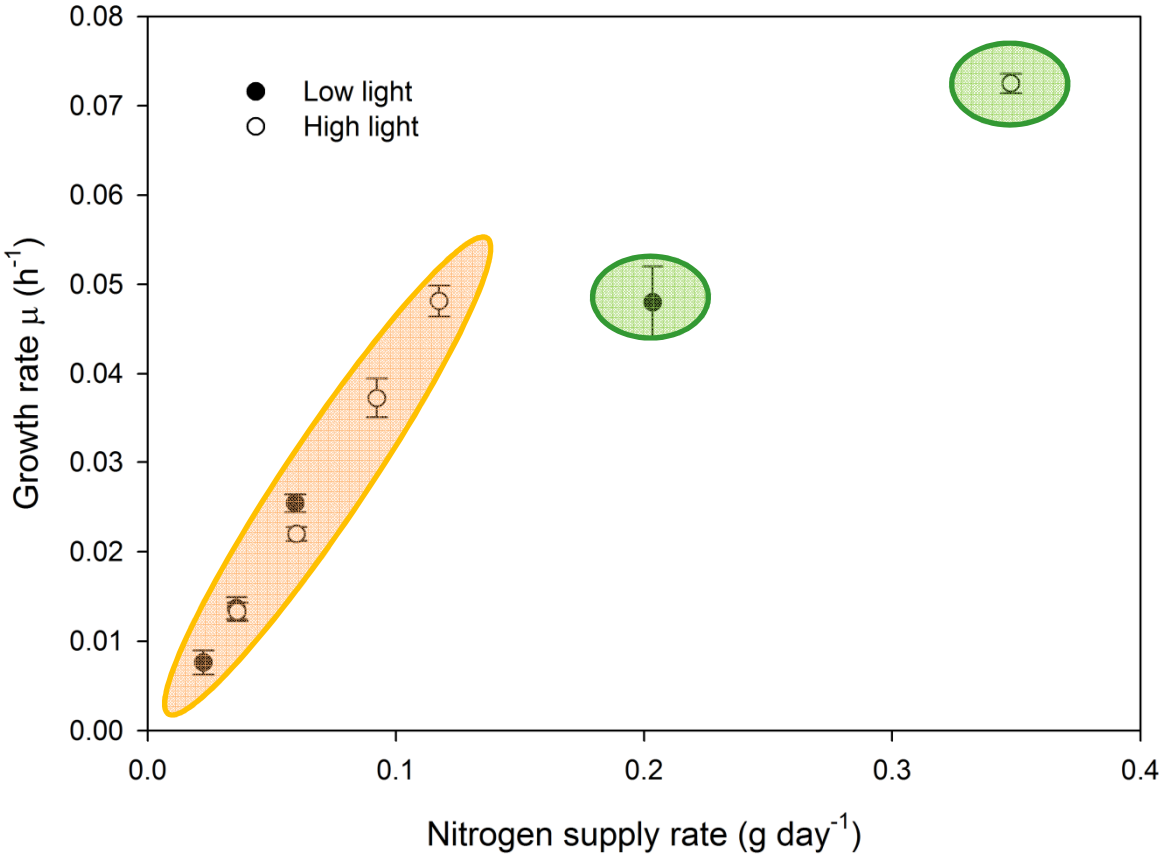
Continuous nitrogen limitation

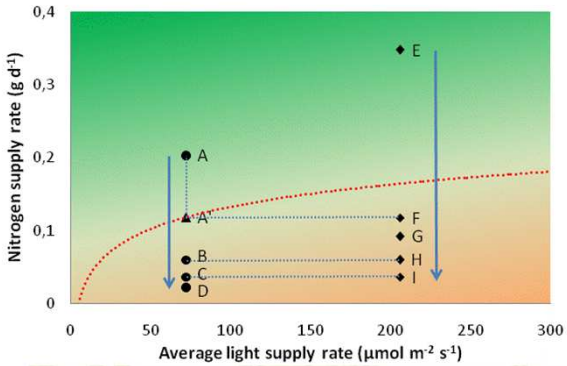
- A constant nitrogen and light supply ensure stable growth conditions
  - 9 Steady states at 2 light supply rates (HL and LL) and several N supply rates
  - 2 Light limited steady states
  - 7 Nitrogen limited steady states with $[NO_3^-] = 0$
  - Continuous experiments
    - **supply rates:** not concentrations!



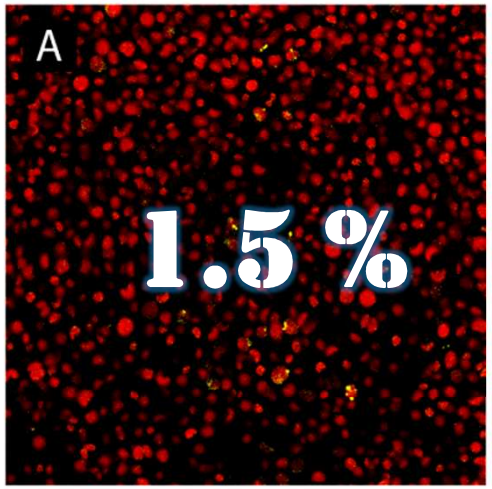
Continuous nitrogen limitation: GROWTH

- Growth is determined by limiting substance



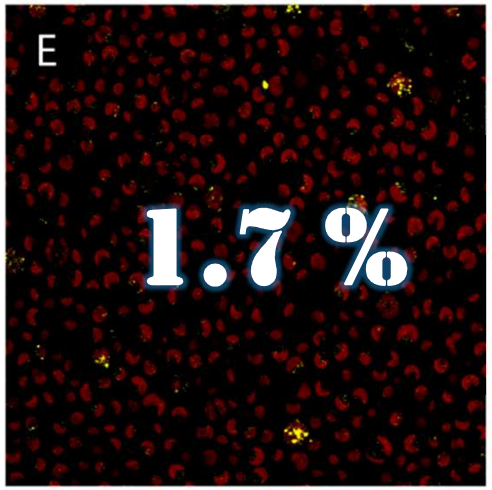


Continuous nitrogen limitation: TAG content



3.7 %

7.5 %

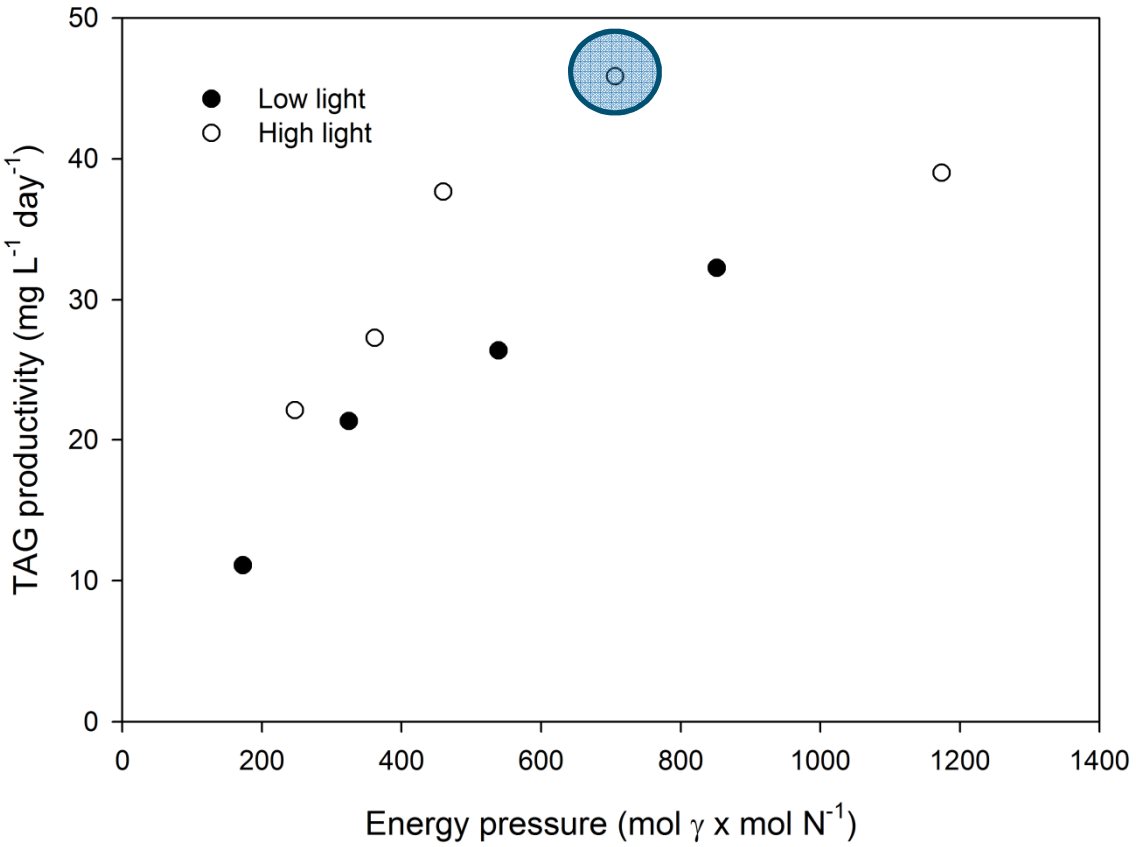


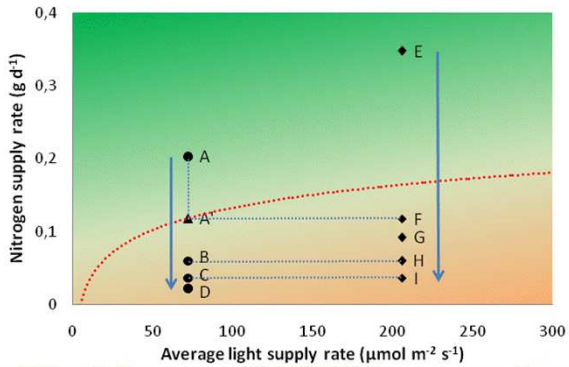
7.0 %

8.7 %

Continuous nitrogen limitation: TAG productivity

- TAG productivity increases with enhanced nutrient stress





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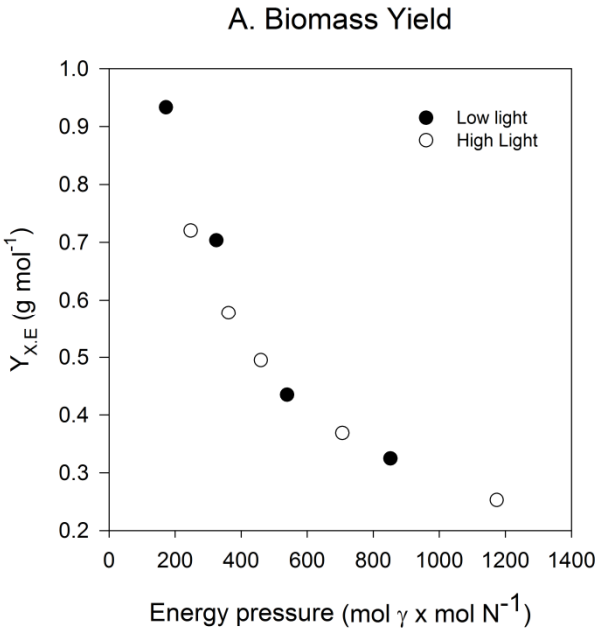
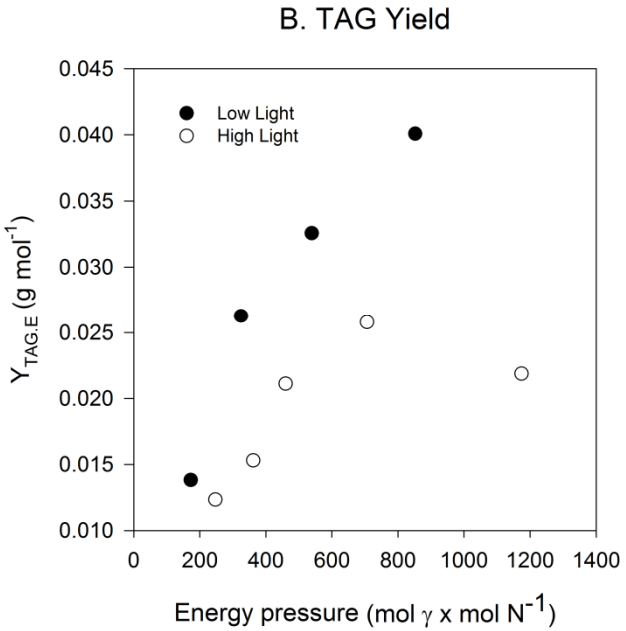
Continuous nitrogen limitation: Yield





- Light is used less efficient because:
  - Biomass is more 'expensive'
  - Maintenance increases
  - Light dissipation increases

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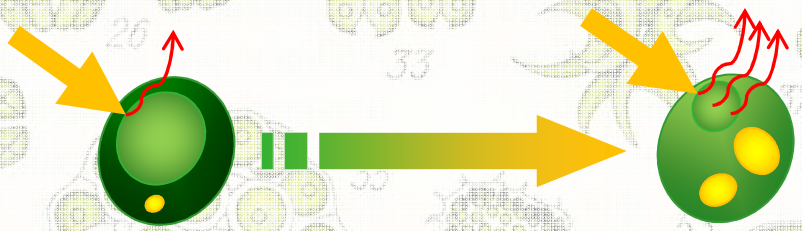
TAG accumulation comes at a price

- Other observations
  - Decrease in **pigmentation**
  - Decrease in **N content** (less protein): 7.3% → 3.0% w/w
  - Decrease in **membrane lipids**: 9.0% → 4.2% w/w
- Overall yield on light decreases because photosystems are less abundant and less efficient at increased stress levels
- Same observations as under classic nitrogen depletion experiments

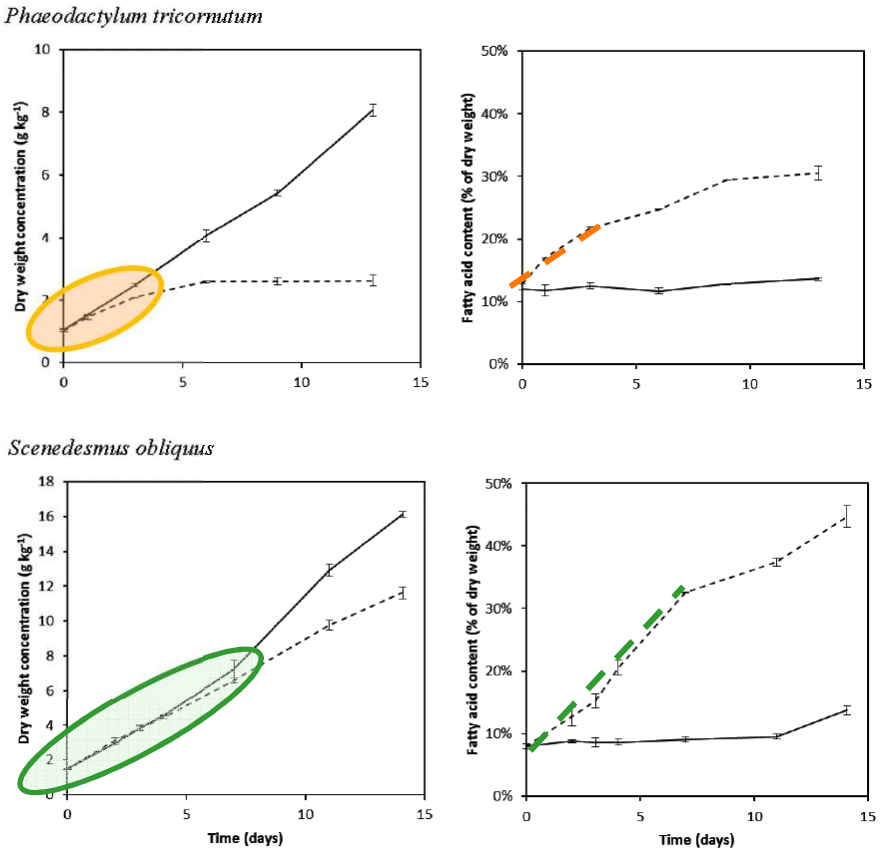


ALGAE RESTRICT THEIR ENERGY INTAKE!

Implications

- High TAG fraction and high productivity is probably found in algae with most efficient photosystem under nutrient stress
  - Energy can be directed towards TAG accumulation

Breuer et al. (2012) submitted



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Summarizing

- TAG accumulation is a result of **an energy imbalance**
- **It is possible to combine growth and TAG accumulation**
  - by creating an imbalance in nutrient demand and supply
- **Algae counteract the imbalance by energy dissipation**
  - thus restricting their energy intake
  - Indications of decreasing size and efficiency photosystem
- **Efficient light use crucial** when aiming at high TAG productivities

Thanks to

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  Centre of excellence for sustainable water technology

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wetsus

and thank YOU for your attention!

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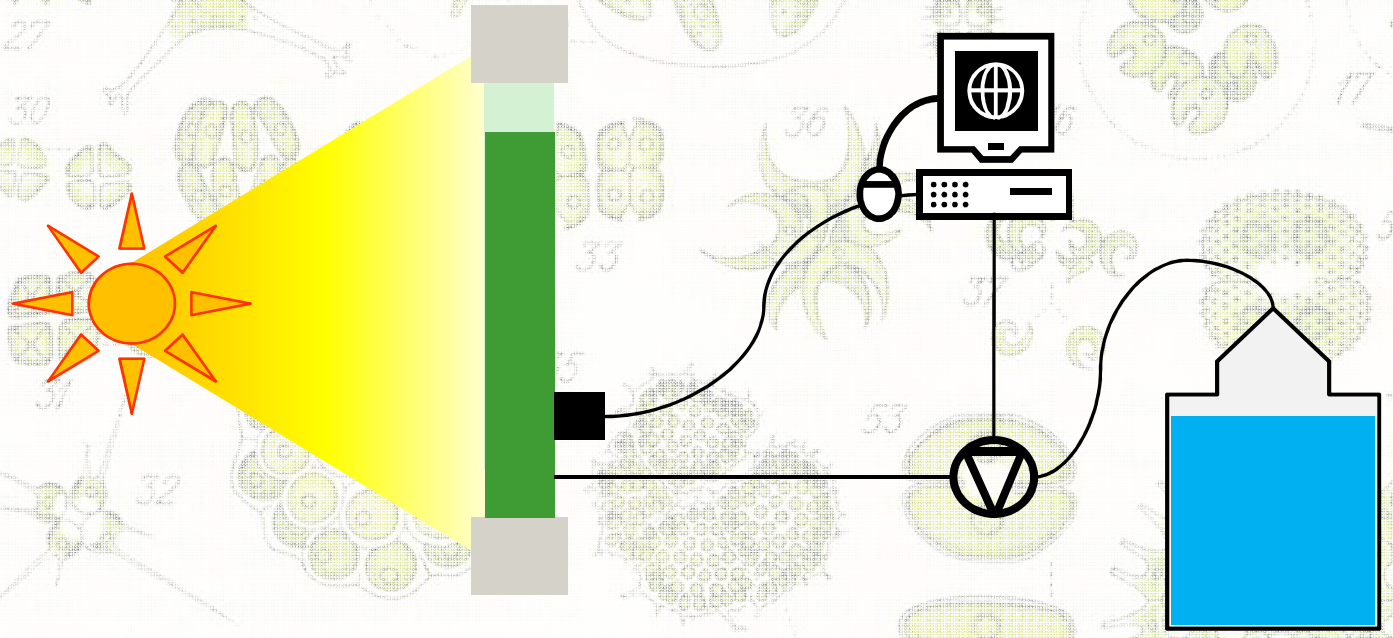
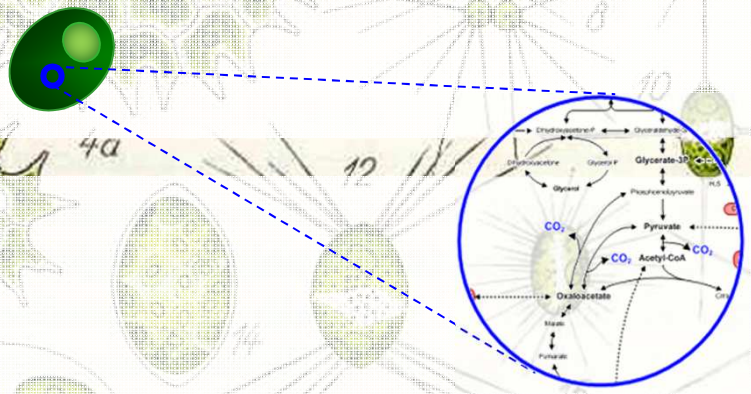
Turbidostat regulation

- Maintaining **constant turbidity**
  - Light per cell is the same throughout experiment
  - Control loop with light sensor



Further research



- RNA sequencing
- Metabolic flux analysis
  - Convert existing model *Chlamydomonas* using DNA sequence
- What is happening on a cellular level
  - Gene regulation
  - Flux regulation

FINDING THE BIOLOGICAL MECHANISM OF LIPID ACCUMULATION