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Understanding Precision Nitrogen Stress to Optimize the Growth and Lipid Content Tradeoff in Green Algae

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Understanding precision nitrogen stress to optimize the growth and lipid content tradeoff in green algae

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Is nitrogen deprivation a viable method for production of lipid feedstock algae?

- It’s well-established that stress—especially N deficiency—promotes lipid formation in some green algae
  - But N deficiency limits growth and thus lipid productivity

- Viability depends on proper tradeoff—not extremes
  - High growth increases yield per unit culture volume
  - High lipid content decreases processing costs per unit biomass
  - Precision stress is needed
An example of precision stress in agriculture

• The tradeoff between vegetative and reproductive growth is managed by precision N stress in tomatoes
  - Too much N = big plants, few fruit
  - Too little N = loss of yield

• Tomato production is a big, profitable industry that has faced a similar challenge

A perspective on nitrogen deprivation for algae lipid production

- The Aquatic Species Program suggested mutual exclusivity of nutrition favoring growth and lipids (Sheehan et al., 1998):
  - Increased lipid content in N stress led to cessation of cell division
  - As a result, despite increased oil content, lipid productivity was equal or lower with N deficiency
- This conclusion was generally based on extreme nutritional conditions
- The perspective in the field has changed little since 1998
Where is research needed?

• We have a poor quantitative understanding of the effects of N deprivation on lipid production in algal cultures
  • Growth and lipid content tradeoffs
    • In particular with intermediate levels of stress

• Timing of lipid accumulation

• Magnitude of stress required

• Species differences
  • What characteristics make one species better than another as a lipid feedstock?
Methods

- In 12-day, axenic batch cultures (1% CO₂), we took daily measurements of:
  - Growth – Spectral measurements and filtration
  - Biomass lipid content – Lipids extracted and converted to FAME (biodiesel), quantified by GC
  - Tissue N – Perkin-Elmer CHN Analyzer (Model 2400)
  - Solution N – Lachat QuikChem 8500 Automated Ion Analyzer

- Two N stress treatments:
  - Low N stress (11 mM N) – Not nutrient replete
  - High N stress (4 mM N) – Not severely limiting

- Six species of oleaginous green algae
  - Chlorella sorokiniana, Chlorella vulgaris, Chlorococcum oleofaciens, Neochloris oleoabundans, Scenedesmus dimorphus, Scenedesmus naegelii
Tremendous differences observed in response to N supply

- Three categories of response (with increased stress):
  1. Increase in lipid content exceeded decrease in growth
  2. Decrease in growth exceeded increase in lipid content
  3. 1:1 tradeoff

- The data highlights need for species-specific nutrition
  - Lower level stress for some, higher level for others
The timing of lipid accumulation

- Some algae grow first, then accumulate lipids; others do both at the same time.
- Concurrent growth and lipid accumulation always resulted in higher lipid productivity.
The magnitude of nitrogen stress
The timing of lipid accumulation reflects stress responses

- Wide variation in stress response among species
- A larger range in tissue N indicated better lipid productivity
  - This can be used to identify the best lipid producers
- The most promising species will combine concurrent growth and lipid accumulation with high lipid content
- Cultures can be managed by mass balance
  - Tissue N can be estimated with measurements of solution N and growth
Conclusions

• Precision N stress can be used to optimize growth and lipid content tradeoffs
  • Optimization requires low-level stress for some, high-level for others

• Species selection should be broadened to include:
  • Concurrent growth and lipid accumulation
  • Response to minimal N stress

• N deprivation is a viable method for increasing lipid productivity
  • There is great promise among the natural species