Experimental Determination of Radiation Characteristics of *Nannochloropsis oculata* During Nitrogen Starvation

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Abstract: The marine microalgae *Nannochloropsis oculata* is known to accumulate lipids, mainly in the form of triglycerides making them an attractive species for renewable biodiesel production. Lipid productivity can be increased by nitrogen starvation of cells causing them to progressively decrease their replication rate in favor of accumulating lipids (up to 70% in dry weight). In addition to nitrogen starvation, recent studies have shown light availability in photobioreactors (PBR) and light received per cell to be important factors in the lipid productivity of the microalgae. These depend on the incident photon flux density, microalgae cell concentration, and the radiation characteristics of the microalgae. The latter complicates the optimization of the lipid production protocols as nitrogen deprivation also causes the cells to progressively decrease their pigmentation thus changing their radiation characteristics. These effects were quantified by the progressive changes in the average cell absorption and scattering cross-sections caused by modifications in specific chlorophyll and carotenoid concentrations, and cell size distribution.

Motivations

Nitrogen starvation in *Nannochloropsis oculata* leads to large percentage of lipid accumulation[2,3,4] in form of triglycerides (TAG) ideal for biodiesel production[3]

- Previous studies have shown that lipid accumulation strongly depends on the light availability and fluence rate inside the photobioreactor (PBR)
  - Incident light intensity[2,4]
  - Biomass concentration[4]
  - PBR thickness[4]
- To optimize lipid production and design large scale PBRs we need to perform radiative analysis inside the PBR requiring accurate quantitative measurements of the optical and radiation properties of the microalgae[1].
  - Dramatic variations in radiation properties during nitrogen starvation
    - Specific pigment concentration decreases.
    - Carotenoid to chlorophyll *a* ratio increases.
    - Microalgae size increases with lipid accumulation
  - ➢ Changes in radiation characteristics that need to be quantified

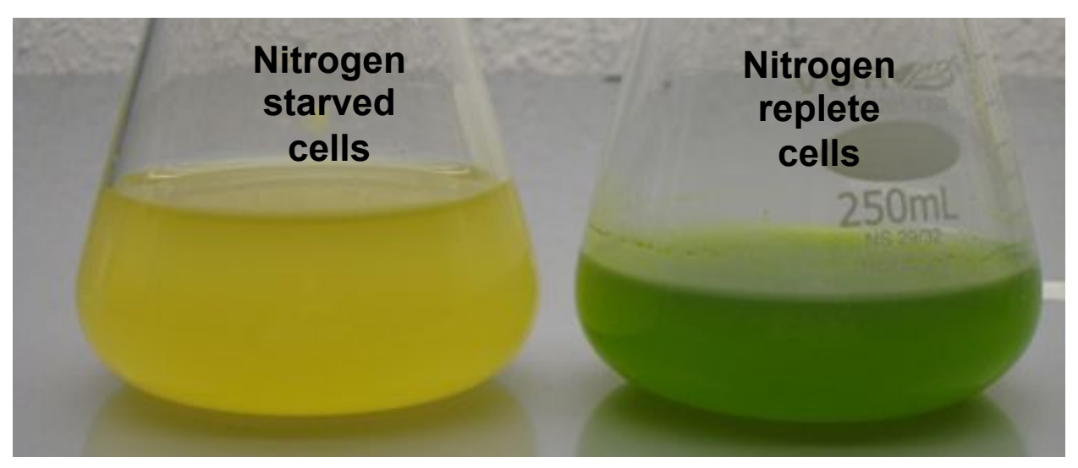


Fig. 1: An illustration of the difference in pigmentation and absorption of nitrogen starved (left) and nitrogen repelete (right) *N.oculata* cells.

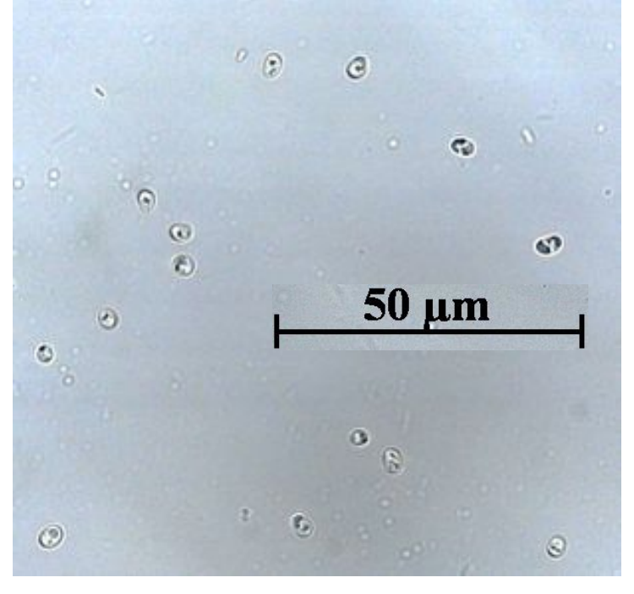
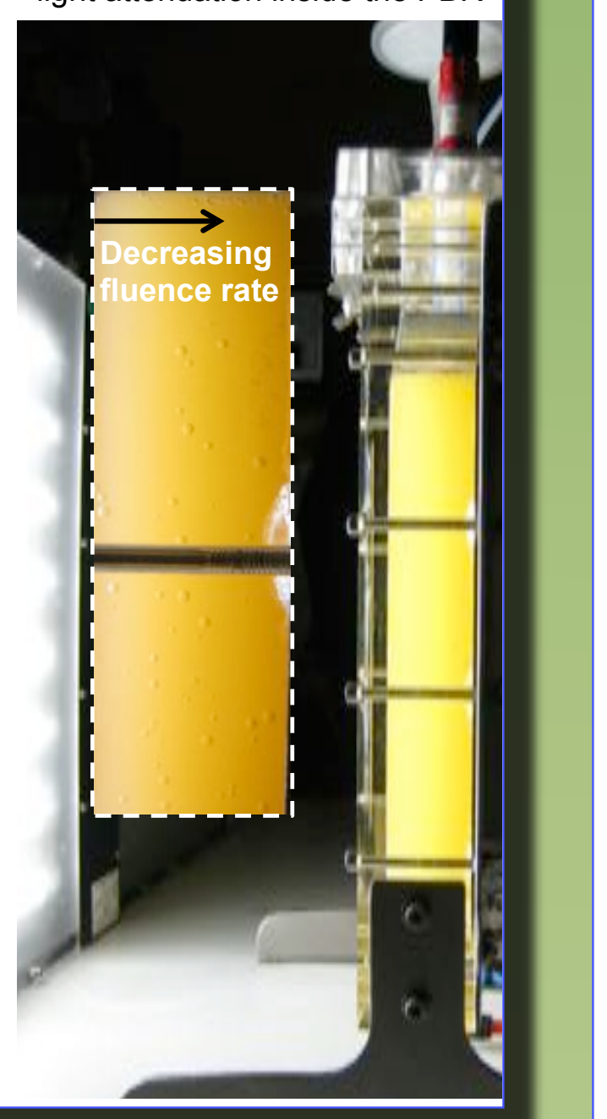


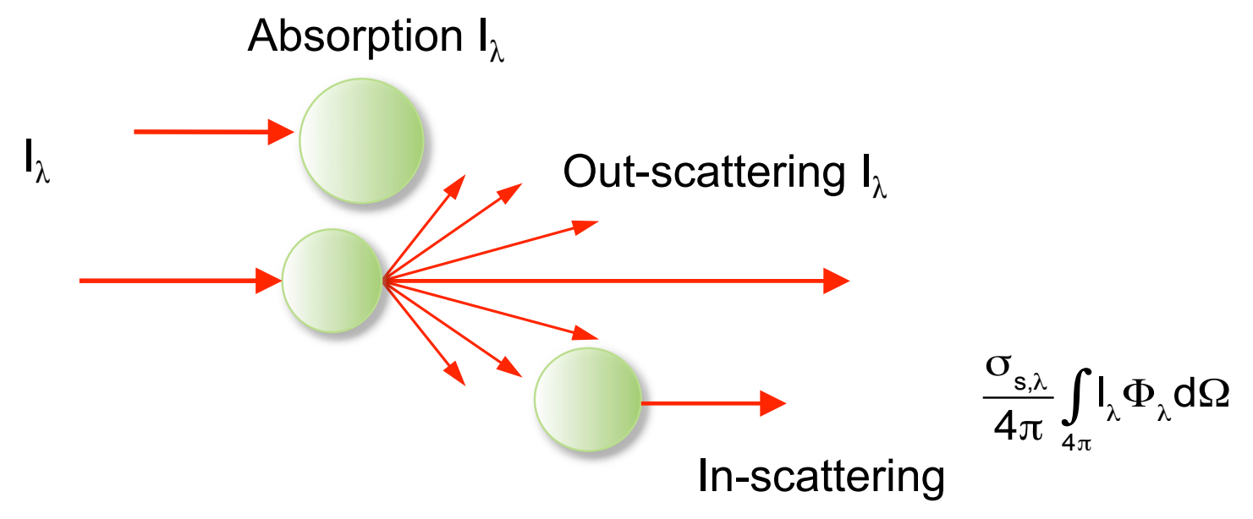
Fig. 2: Microscope image of *N.oculata* during the later stages of progressive nitrogen starvation

Fig. 3: A 1L airlift reactor used to cultivate *N.oculata* showing light attenuation inside the PBR



- The purpose of this study is to experimentally characterize optical and radiative properties of *N.oculata* during nitrogen starvation in order to link lipid productivity to light absorption and light availability in the PBR.

Measuring Radiation Characteristics

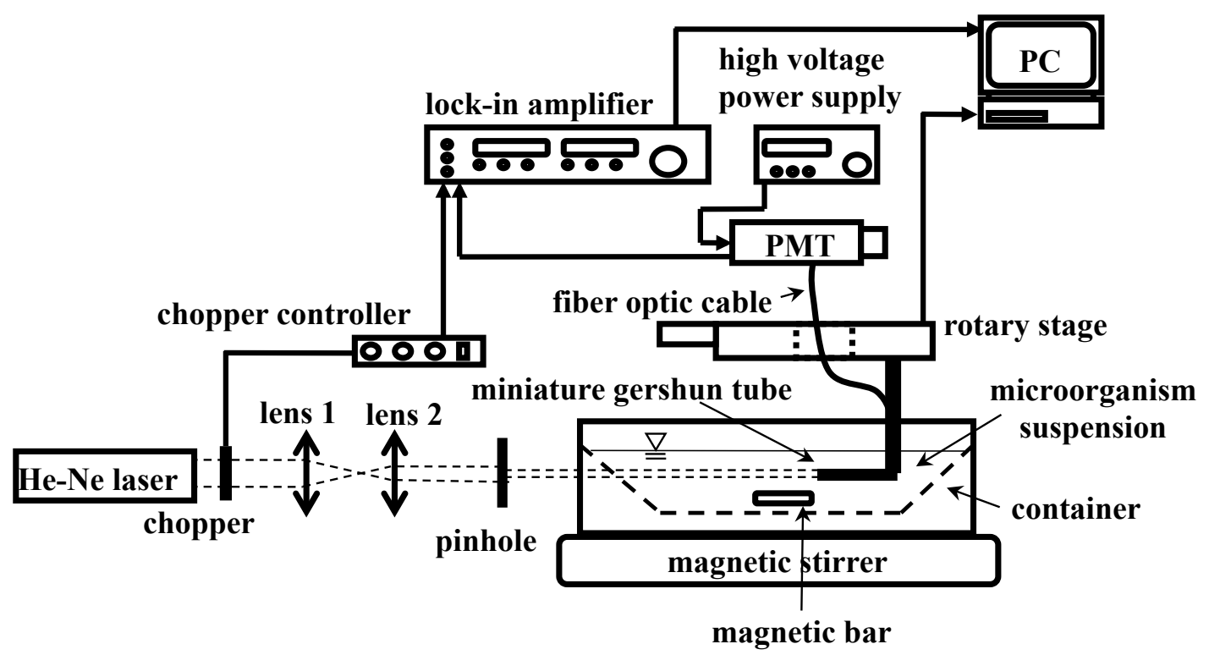


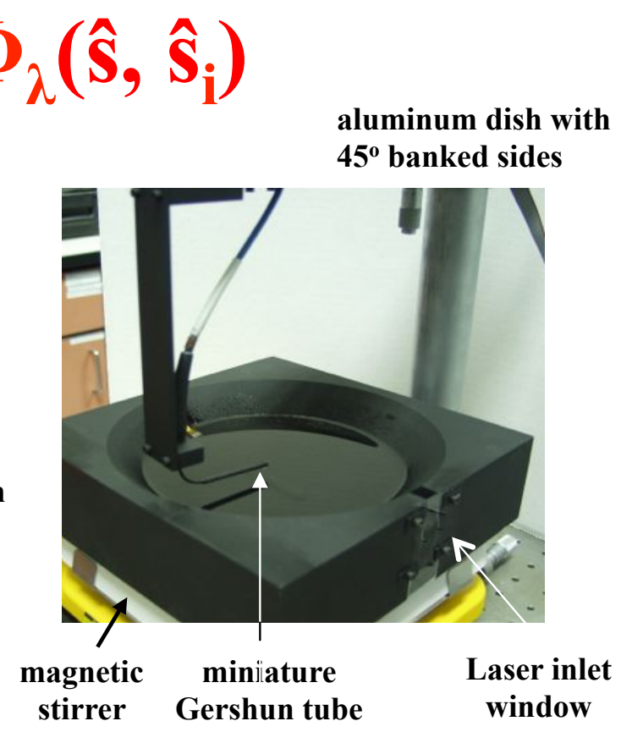
Notations:
β_λ: Extinction coefficient (1/m)
$C_{abs,\lambda}$: Absorption cross-section (m^2/kg)
$C_{ext,\lambda}$: Extinction cross-section (m^2/kg)
$C_{sca,\lambda}$: Scattering cross-section (m^2/kg)
Θ: Scattering angle (rad)
I_λ: Spectral intensity (W/m^2 µm·sr)
κ_λ: Absorption coefficient (1/m)
λ: Wavelength (nm)
N: Number density (#/m^3)
$\sigma_{s,\lambda}$: Scattering coefficient (1/m)
Φ_λ: Scattering phase function
Ω_λ: Solid Angle (sr)

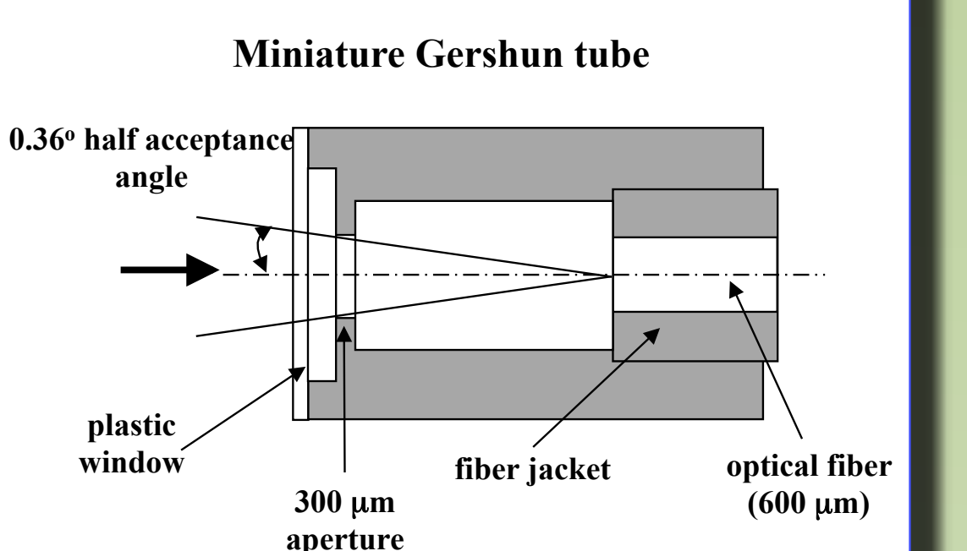
➢Steady State radiative transfer equation

$$\hat{s}\cdot\nabla I_\lambda = -\kappa_\lambda I_\lambda(\hat{r},\hat{s}) - \sigma_{s,\lambda} I_\lambda(\hat{r},\hat{s}) + \frac{\sigma_{s,\lambda}}{4\pi}\int_{4\pi} I_\lambda(\hat{r},s_i)\Phi_\lambda(\hat{s},s_i)\,d\Omega_i$$

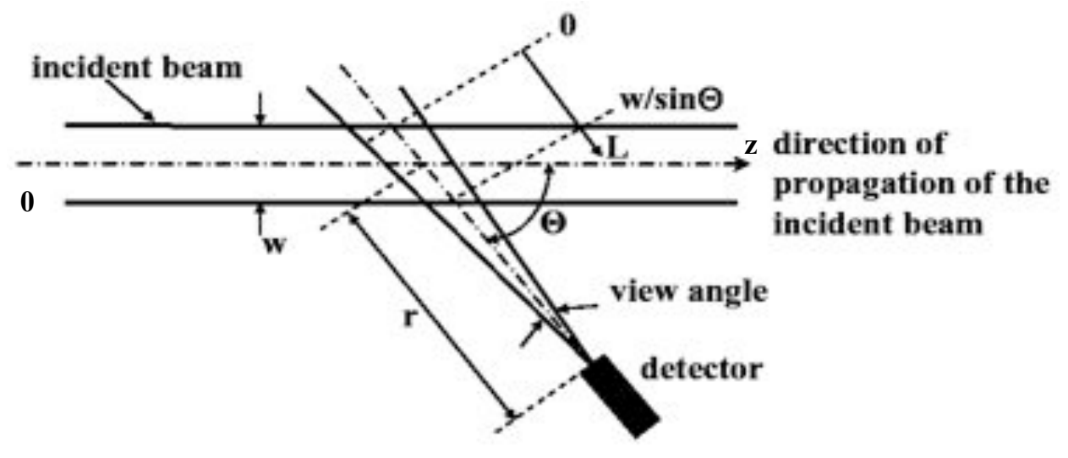
➢Scattering Phase Function $\Phi_\lambda(\hat{s}, \hat{s}_i)$







Capabilities of the setup
(1) Low signal detection using lock-in amplifier.
(2) Small acceptance angle (1.4°) fiber-optic detector.
(3) Sample holder has 45° banked sides to eliminate internal reflection.
(4) Magnetic stirrer ensures homogeneous and random orientation of microorganisms.
(5) High precision motorized rotary stage for accurate angular positioning.
(6) Computer controlled operation.



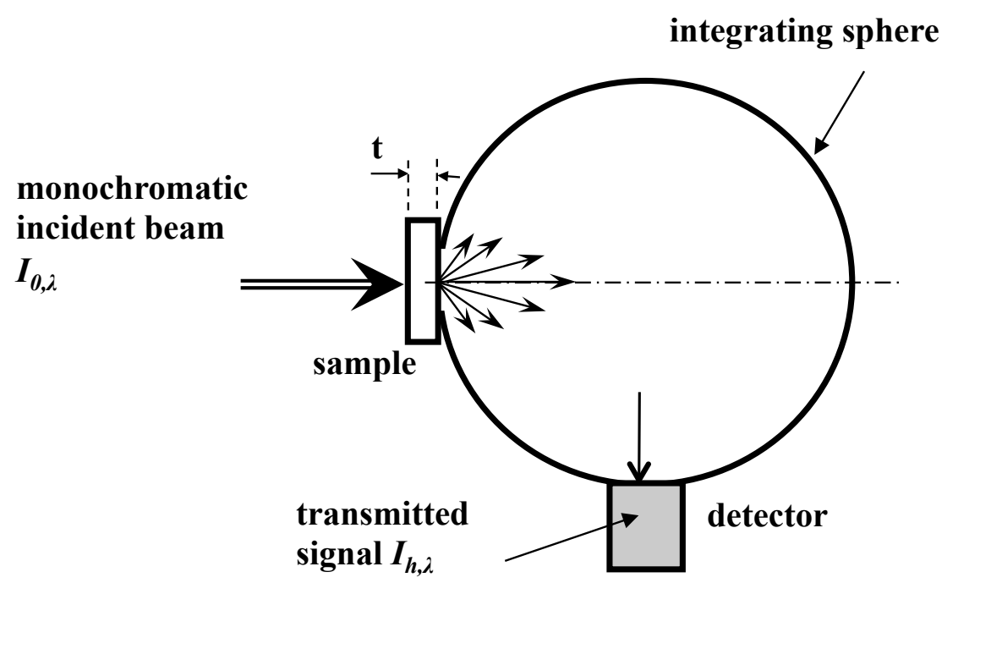
Phase function: $\Phi(\Theta) = \dfrac{2I(\Theta)[U(\Theta)]^{-1}}{\int_0^\pi I(\Theta)[U(\Theta)]^{-1}\sin\Theta d\Theta}$ where

$$U(\Theta) = \int_0^{w/\sin\Theta}\left[1+\beta\frac{w}{2}c\tan\Theta-\beta L\cos\Theta\right]\left[1-\beta\left(r-\frac{w}{2\sin\Theta}\right)\right]\left[1-\beta\left(\frac{w}{\sin\Theta}-L\right)\right]dL$$

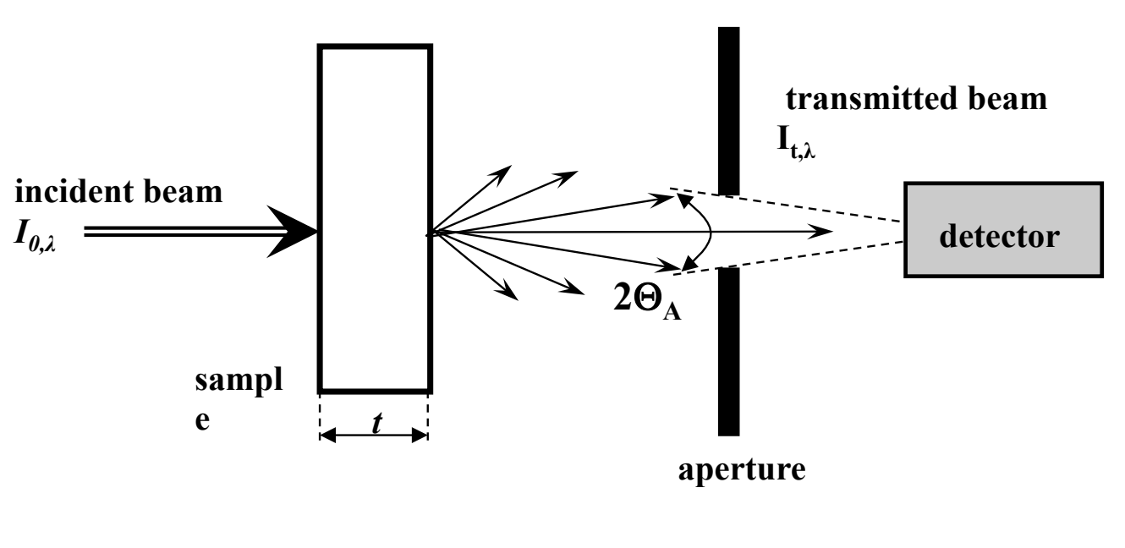
and

$$\beta_{N,\lambda} = \frac{\ln\left|F_\lambda(z_2)/F_\lambda(z_1)\right| + \ln\left|z_1^2/z_2^2\right|}{z_1 - z_2}$$

➢Absorption and Scattering Cross-sections



(a) normal-hemispherical transmittance



(b) normal-normal transmittance

- Apparent absorption coefficient

$$\chi_{h,\lambda} = -\frac{1}{t}\ln\left(\frac{T_{h,\lambda,X}}{T_{h,\lambda,PBS}}\right)$$

- Corrected absorption coefficient

$$\kappa_\lambda = \chi_{h,\lambda} - \chi_{h,\lambda_0}\frac{\chi_\lambda - \chi_{h,\lambda}}{\chi_{\lambda_0} - \chi_{h,\lambda_0}}$$

- Absorption cross-section

$$C_{Abs,\lambda} = \frac{\kappa_\lambda}{N}$$

ε_n represents the portion of the light scattered in the forward direction and detected by the spectrometer in directions other than the normal direction due to the finite size of the acceptance angle:

$$\varepsilon_n = \frac{1}{2}\int_0^{\Theta_a}\Phi_\lambda(\Theta)\sin\Theta d\Theta$$

- Apparent extinction coefficient

$$\chi_\lambda = -\frac{1}{t}\ln\left(\frac{T_{\lambda,X}}{T_{\lambda,PBS}}\right)$$

- Corrected extinction coefficient

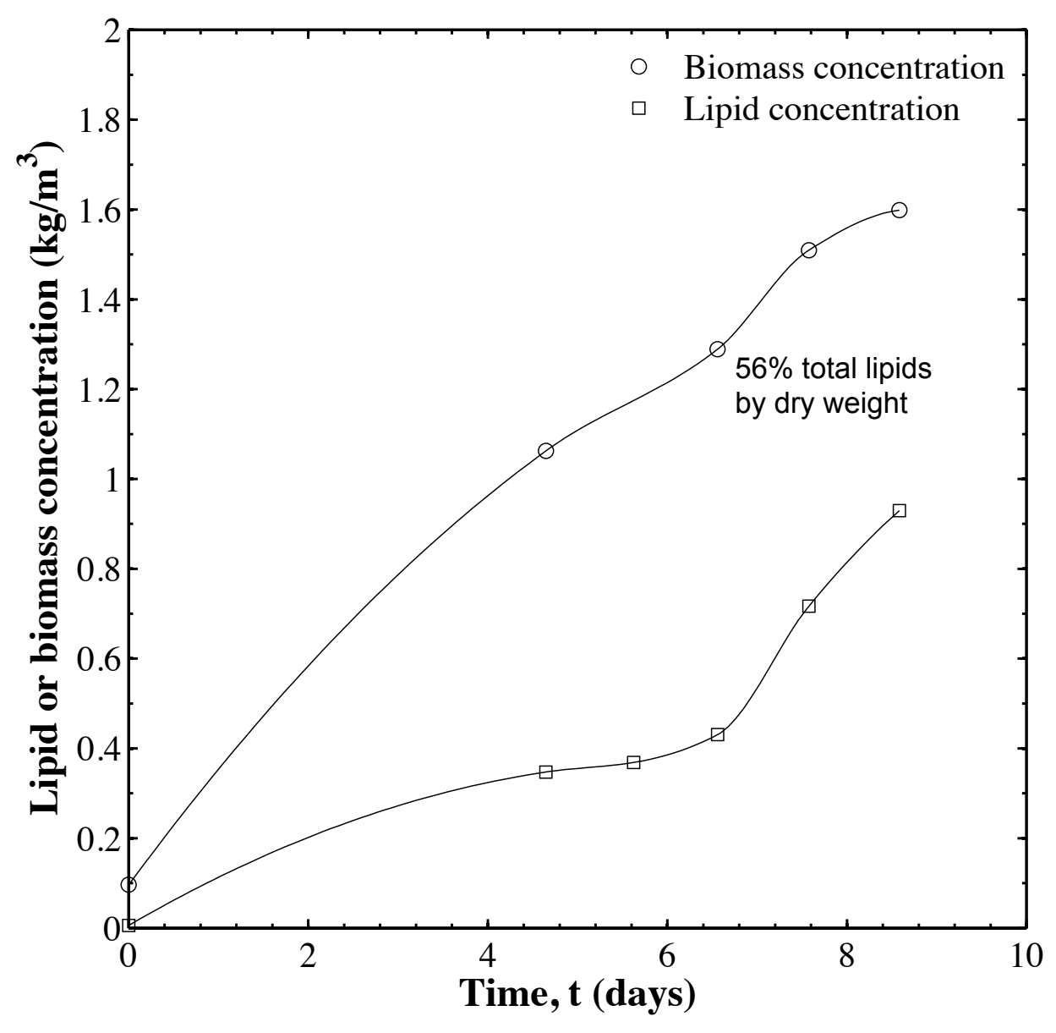
$$\beta_\lambda = \frac{\chi_\lambda - \varepsilon_n\kappa_\lambda}{1-\varepsilon_n} = \kappa_\lambda + \sigma_{s,\lambda}$$

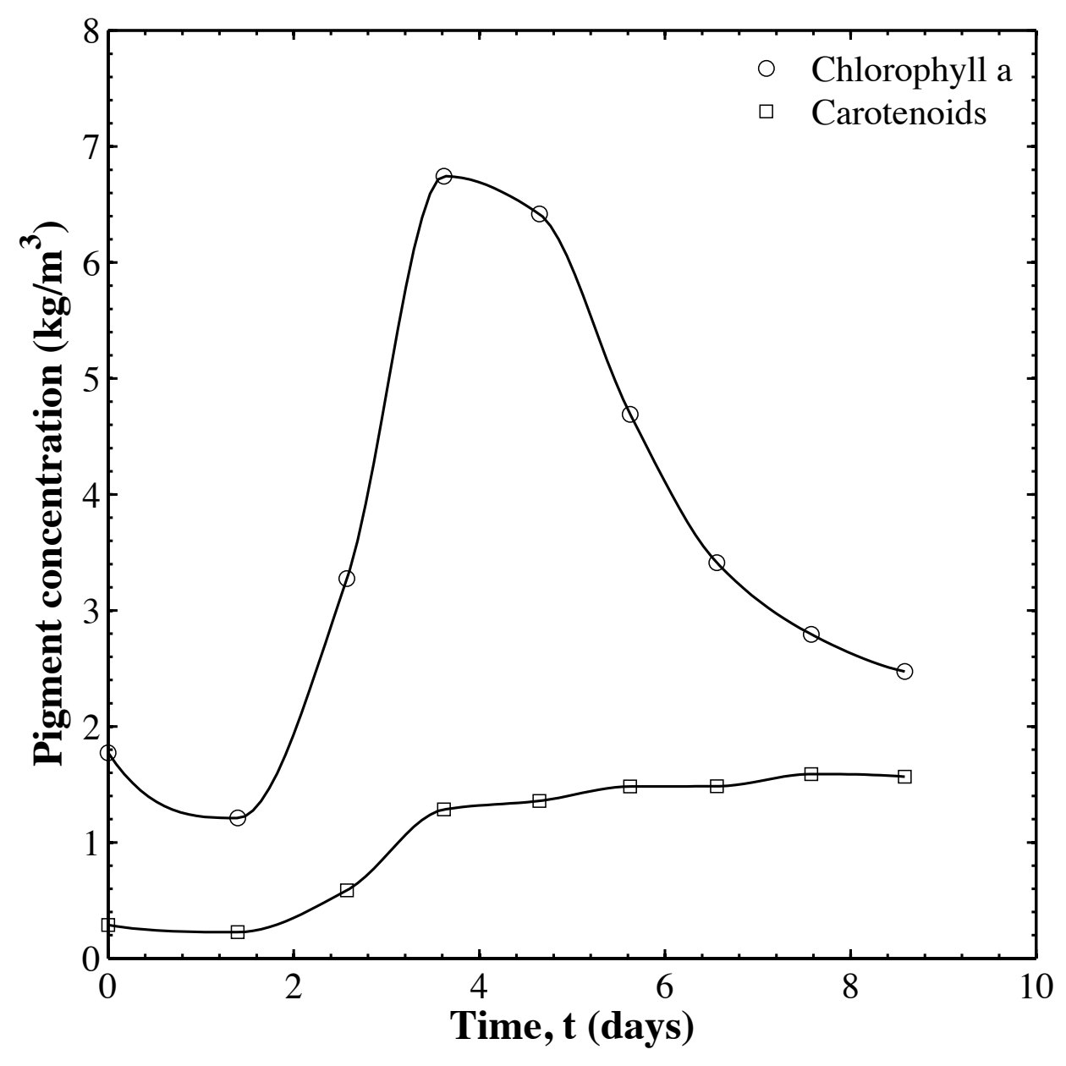
- Extinction and scattering cross-sections

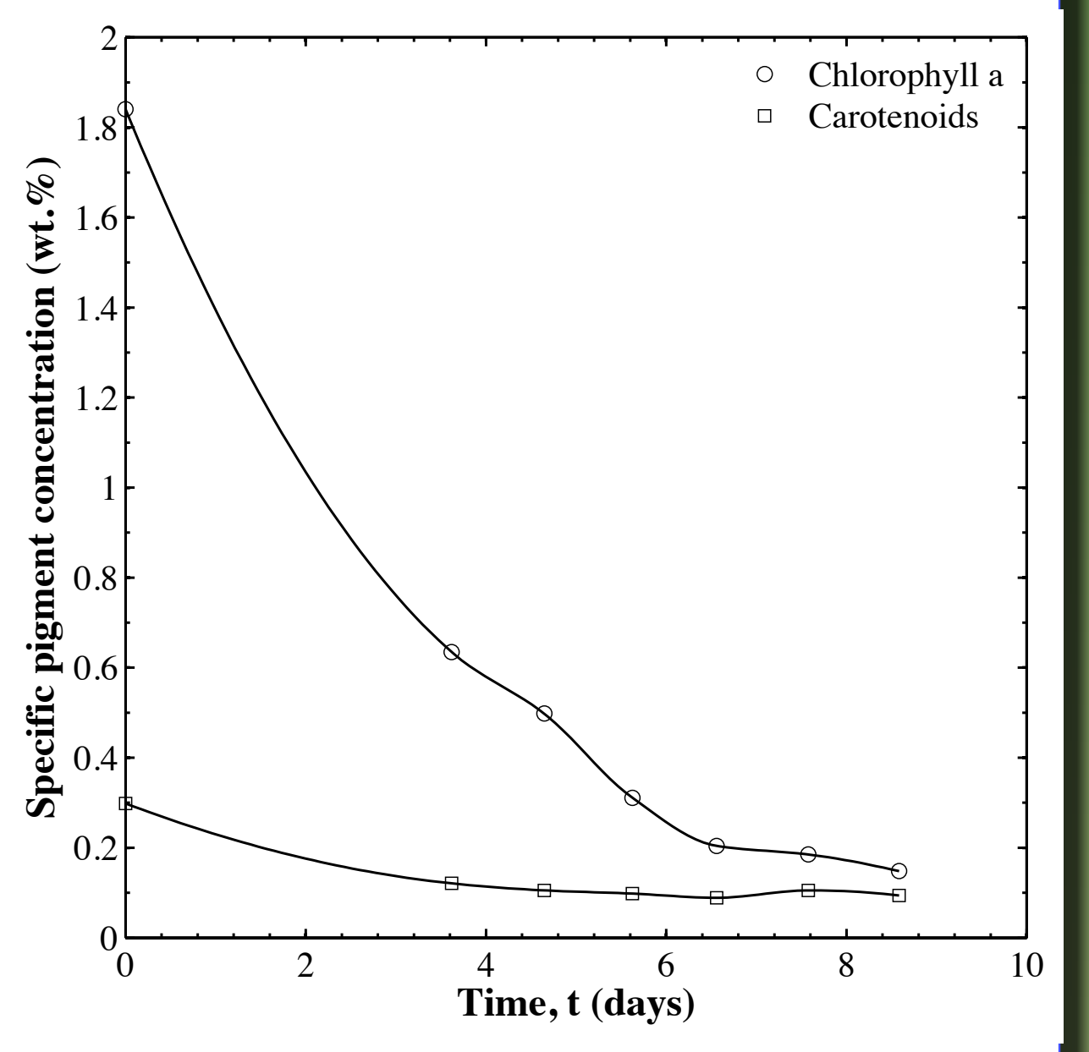
$$C_{Ext,\lambda} = \frac{\beta_\lambda}{N} \quad \text{and} \quad C_{sca,\lambda} = \frac{\sigma_{s,\lambda}}{N}$$

Results

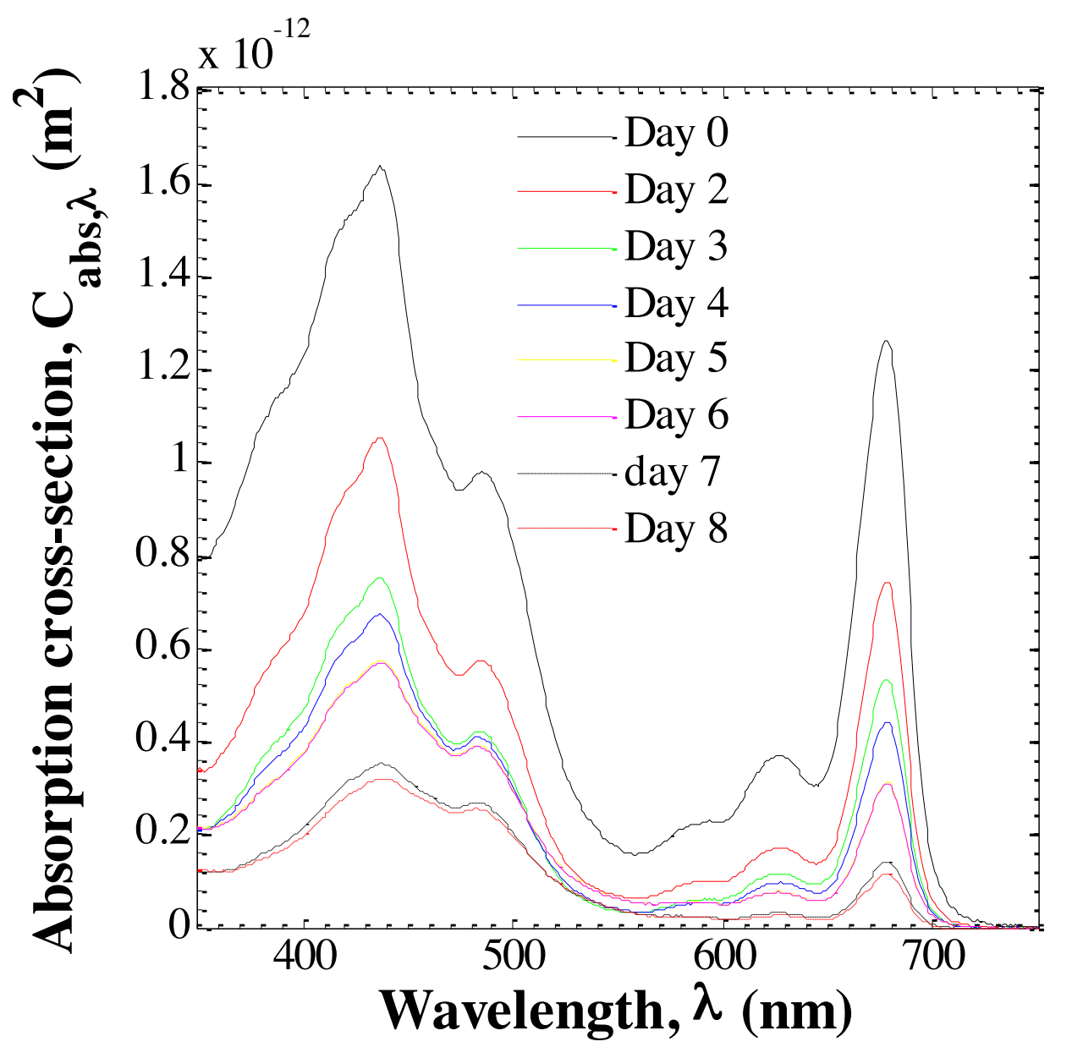
➢ Biomass and pigment concentration

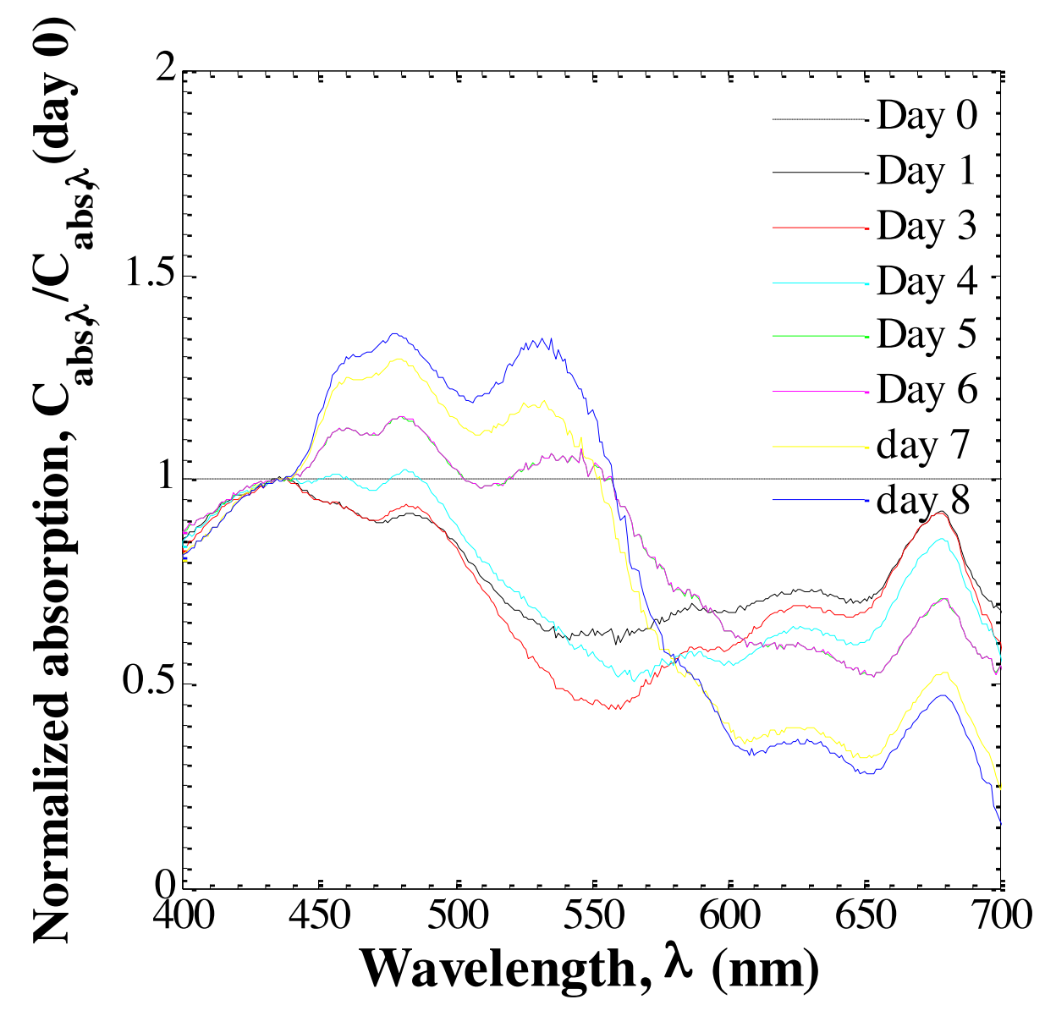


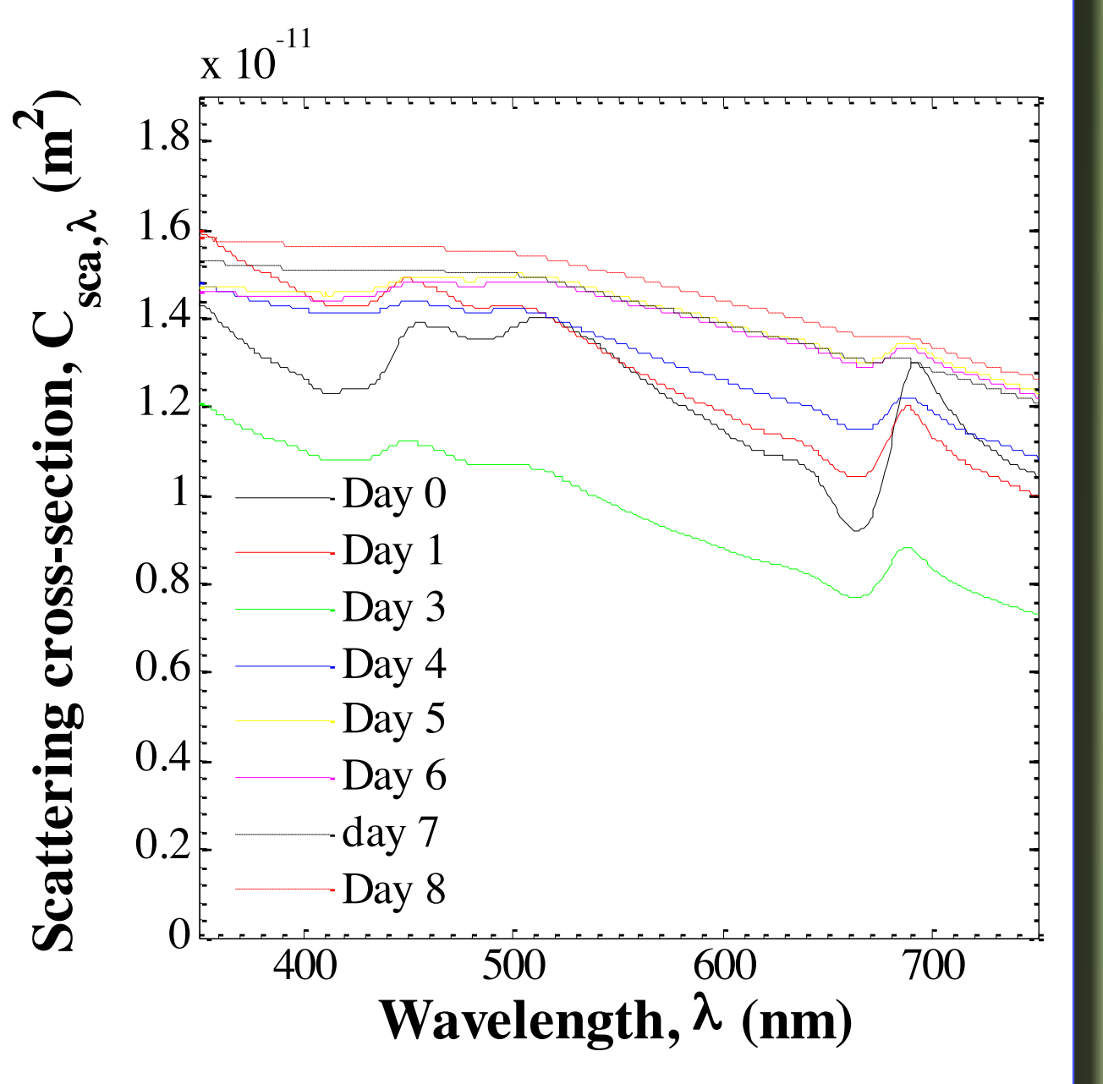




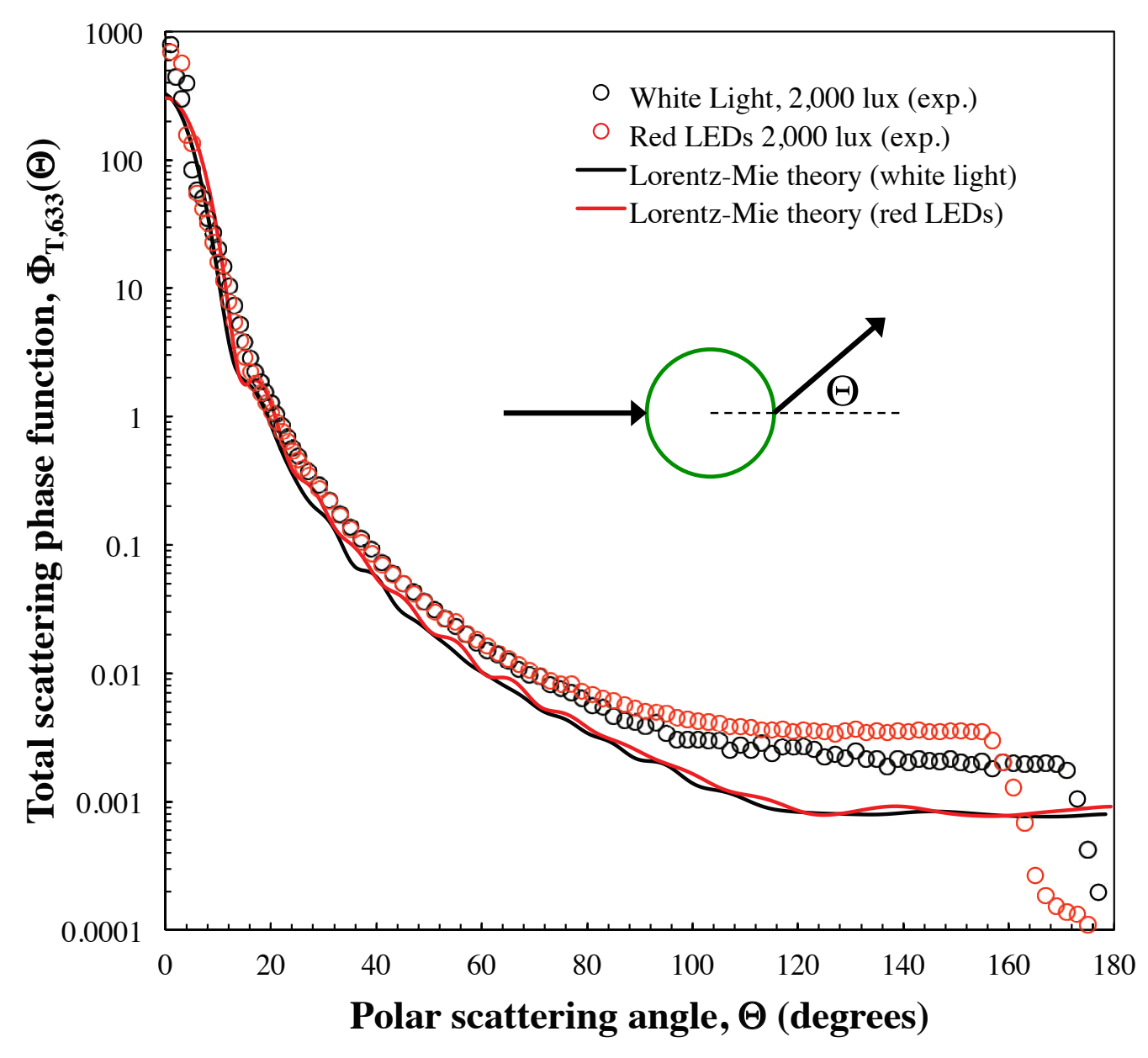
➢ Absorption and scattering cross-sections

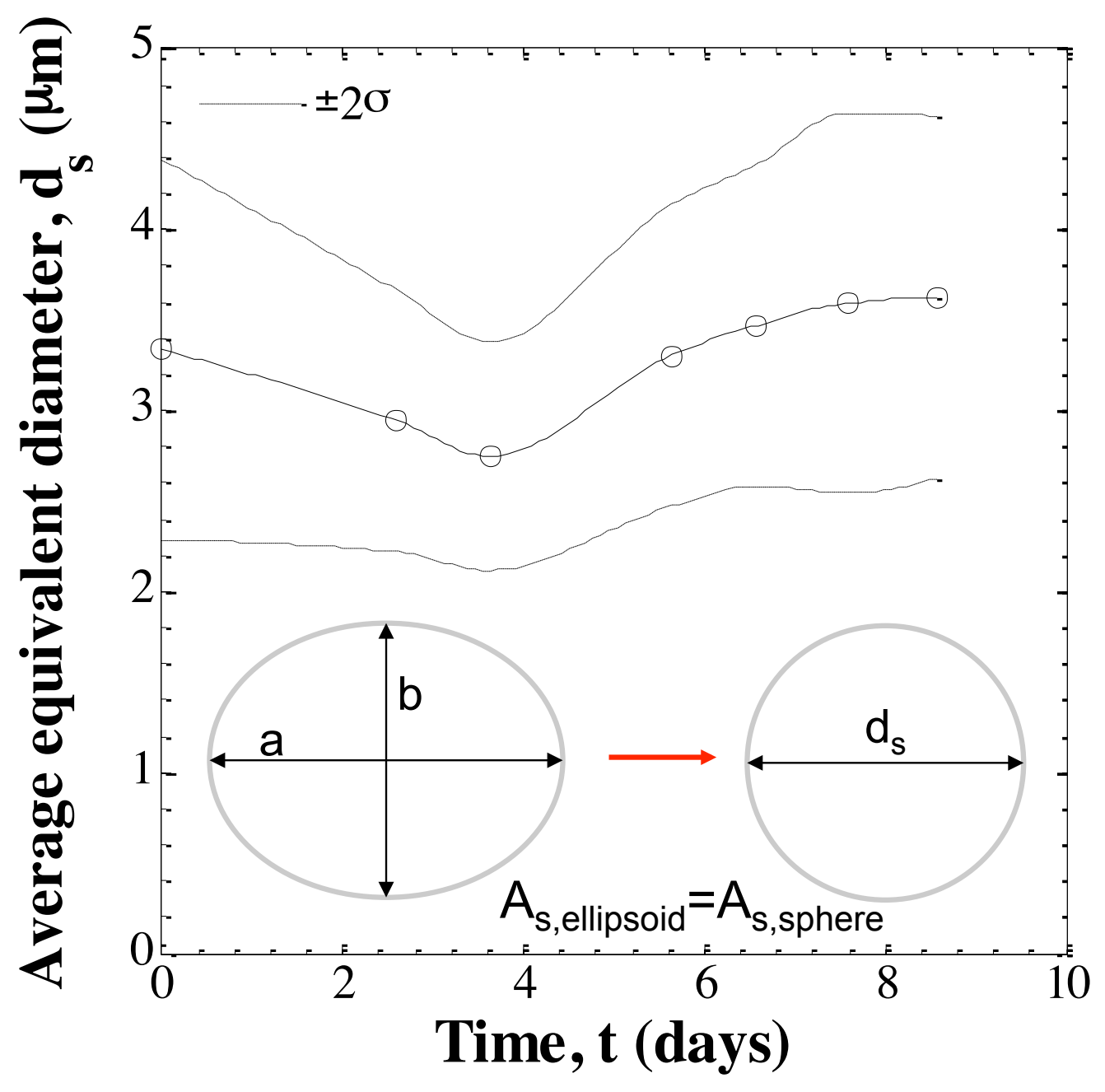






➢Scattering phase function and size distribution





➢ Summary

- This work accurately characterized the evolution of radiation characteristics of *N.oculata* during progressive nitrogen starvation. These included:
  - Biomass, pigments, and lipid concentration, as well as lipid profile
    - Lipid content in cells increased from 8 %dw. to 56 %dw.
    - Specific pigment concentration decreased
  - Absorption and scattering cross-sections
    - Absorption decreased but scattering cross-section increased
      - This correlates with the pigment decrease and the size increase respectively.
  - Size distribution
    - Average equivalent diameter decreased in the cell division phase and increased during lipid accumulation.
- The results obtained will be used to optimize lipid production and design reactors for large scale lipid production.

References

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