Determination of the scaling characteristics of time-dependent optical properties of microalgae using electromagnetic scattering

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Abstract
The time-dependent optical properties of microalgae are crucial for light transfer in photobioreactor (PBR) designs. In this study, the time-dependent optical properties were derived using electromagnetic scattering theory informed by the experimentally measured optical properties of Chlorella protothecoides. The temporal scaling functions (TSFs) of nearly wavelength-independent absorption and scattering cross-sections were demonstrated using electromagnetic scattering theory, leading to the first concrete expression of the TSF. The TSF establishes the relationship between the time-dependent absorption/scattering cross-sections in the stationary and growth phases of microalgal development. The concrete expression of the TSF provides a new means of calculating the time-dependent optical properties of microalgae using electromagnetic scattering theory. The TSF of microalgae has great potential in remote sensing and PBR applications.

1. Introduction
Microalga cultivation can be used to produce medicines, health drinks, animal feed, and other value-added products [1, 2]. Moreover, microalgae represent a sustainable energy source with the potential to help alleviate climate change by providing an alternative to energy sources that generate high CO₂ emissions [3, 4]. The optical properties of microalgae are important parameters that are crucial to their light transfer abilities, photosynthetic efficiency, and electromagnetic scattering behavior.

The light utilization efficiency of microalgae in photobioreactors (PBRs) affects the biofuel production process significantly [5–7]. An optimum irradiance that achieves maximum photosynthesis efficiency in microalgla cultivation has been reported [7]. However, the light irradiance in PBRs depends on the time-dependent optical properties of microalgae, implying that the optimum irradiance varies during the growth process of microalgae. Early research was focused on measuring the optical properties of microalgae in the stationary stage [8–10]. Recently, the time-dependent optical properties of microalgae have been measured in batch cultures [11–13]. A theory for the temporal scaling of growth-dependent optical properties in microalgae was proposed by Zhao et al., which provides a mathematical proof of temporal scaling characteristics but does not give the specific form or determination method of the temporal scaling functions (TSFs) [13]. Nevertheless, time-dependent light transfer analysis is required in order to design and optimize the light distribution in PBRs and thus improve the photosynthetic efficiency.

The objective of this study was to demonstrate a TSF of time-dependent absorption and scattering cross-sections that is largely independent of wavelength using electromagnetic theory. The derived mathematical expression provides deep insight into the temporal scaling characteristics of the time-dependent optical properties and origin of electromagnetic scattering in microalgae. Furthermore, the complex refractive index was retrieved from the measured time-dependent absorption and scattering cross-sections and cell size distribution data for Chlorella protothecoides. The results can facilitate the calculation of the time-dependent optical or radiative properties of multiple microalgae species.
2. Methods

2.1. Light transfer theory

Light transfer within absorbing, scattering, and non-emitting microalgal suspension in PBRs is governed by the radiative transfer equation [14]

\[
\mathbf{s} \cdot \nabla I(\mathbf{r}, s) + \beta I(\mathbf{r}, s) = \frac{k_s}{4\pi} \int_{4\pi} I(\mathbf{r}, s') \Phi(s' \rightarrow s) d\Omega'
\]

(1)

where \( I \) is the radiative intensity in direction \( s \) at location \( \mathbf{r} \), \( \mathbf{s} \) is the direction vector, \( k_s \) is the scattering coefficient, \( \beta = \kappa_a + \kappa_s \) is the extinction coefficient, \( \kappa_a \) is the absorption coefficient, \( \Phi(s', s) \) is the scattering phase function, and \( \Omega' \) is the solid angle. The scattering phase function \( \Phi(s', s) \) represents the probability that the radiation transfer in solid angle \( d\Omega' \) around direction \( s' \) is scattered into solid angle \( d\Omega \) around direction \( s \).

The average absorption and scattering cross-sections of a polydispersive microalgal suspension, \( \langle C_{abs} \rangle \) and \( \langle C_{scs} \rangle \), are related to the absorption and scattering cross-sections, respectively, by the cell size distribution function and can be expressed as [14]

\[
\langle C_{abs} \rangle = \int C_{abs}(d_i) f(d_i) dd_i \quad (2a)
\]

\[
\langle C_{scs} \rangle = \int C_{scs}(d_i) f(d_i) dd_i \quad (2b)
\]

where \( C_{abs}(d_i) \) and \( C_{scs}(d_i) \) are the absorption and scattering cross-sections of a single spherical scatterer with diameter \( d_i \), respectively, and \( f(d_i) \) is the cell size distribution function.

The microalgae are assumed to have an axisymmetric spheroidal shape with major and minor diameters of \( a \) and \( b \), respectively. They can be approximated as spheres with an equivalent diameter \( d_e \), so that their surface area is identical to that of their actual spheroidal shape. The equivalent diameter of the sphere with the same surface area as the spheroid can be expressed as [15]

\[
d_e = \frac{1}{2} \left( 2a^2 + 2ab \sin^{-1} \varepsilon \right)^{1/2}, \quad \text{where} \quad \varepsilon = \frac{(e^2 - 1)^{1/2}}{e}
\]

(3)

Here, \( \varepsilon \) is the spheroid aspect ratio, defined as \( \varepsilon = a/b \). Bidigare et al and Pottier et al used a predictive method to determine the spectral absorption coefficient \( \kappa_{a, i} \) by [16, 17]

\[
\kappa_{a, i} = \frac{1}{a} \sum_{i=1}^{n} E_{a,i} \varepsilon_i
\]

(4)

where \( E_{a,i} \) is the in vivo pigment-specific spectral absorption cross-section of pigment \( i \) and \( \varepsilon_{i} \) are the mass concentrations (kg m\(^{-3}\)) of the cell pigments. The specific absorption cross-sections of chlorophyll (Chl) \( a, b, c \), and \( \beta \)-carotene were reported by Bidigare et al in the 400–750 nm spectral region [18].

2.2. Time-dependent optical properties

The radiation characteristics of microalgae are related to the cell morphology, size, internal structure, components, and cell size distribution [19]. During microalgal growth, numerous processes occur, including the division of cells, changes in pigment [11], and variations in the levels of carbohydrates, proteins, and lipids [20]. Hence, the optical properties of microalgae vary during the growth period. The complex refractive index \( m = n + ki \) is close to unity in water because the cells in living microalgae are composed mostly of water.

Following the approach of van de Hulst [21], the anomalous diffraction approximation theory can be used to obtain the absorption efficiency factor under this special condition:

\[
Q_{abs}(\rho) = 1 + [\exp(-2\rho \tan \xi)(2\rho \tan \xi + 1) - 1]/2\rho^2 \tan^2 \xi
\]

(5)

where \( Q_{abs}(\rho) \) represents the absorption efficiency factor, \( \rho = 2\alpha(n - 1) \), \( \tan \xi = k/(n - 1) \), and \( \alpha = \pi d n_w/\lambda \), where \( \lambda \) is the wavelength in a vacuum and \( n_w \) is the refractive index of water. By introducing parameter \( \rho' = 4\alpha k = 2\rho \tan \xi \), the absorption efficiency factor can also be rewritten as [21]

\[
Q_{abs}(\rho') = 1 + \frac{2\exp(-\rho')}{\rho'} + 2\exp(-\rho') - 1
\]

(6)

When \( \rho' \) is much smaller than 1, we can use the Taylor series expansion for \( Q_{abs}(\rho') \) to obtain the following result:

\[
Q_{abs}(\rho') = \frac{2}{3} \rho' + o(\rho'^2)
\]

(7)

Considering the temporal evolution of the optical properties of microalgae, \( \rho' = 4\alpha k \) represents the growth time function \( t \); hence, the temporal scaling factor of absorption can be expressed as
Therefore, we used the Lorentz–Mie theory to calculate the radiation characteristics of the microalgae cell, which

\[ R_{\text{abs}}(t_a, t_i) = \frac{k(t_a) \int d^3(t_a)f(d(t_a))dd}{k(t_i) \int d^3(t_i)f(d(t_i))dd} \]

(8)

where the absorption index \( k \) is a function of wavelength \( \lambda \), \( t_a \) and \( t_i \) represent the arbitrary growth time and time to reach the stationary phase, respectively. According to equation (4), the ratio \( k(t_a)/k(t_i) \) can be expressed as

\[ \frac{k(t_a)}{k(t_i)} = \frac{\kappa_{a,\lambda}(t_a)}{\kappa_{a,\lambda}(t_i)} = \frac{\sum_{i=1}^{n} \frac{E_{a,i}c_i(t_a)}{E_{a,i}c_i(t_i)}}{\sum_{i=1}^{n} \frac{E_{a,i}c_i(t_i)}} \]

(9)

using the relation \( \kappa_{a,\lambda}(t) = 4\pi k(t)/\lambda \) [22] because the microalgal cells are highly forward scattering [9]. In the case that the mass concentrations of the cell pigments increase or decrease consistently with time, that is, satisfying the relation \( c_i(t_i) = K(t) c_i(t) \) for \( 1 \leq i \leq n \), the ratio \( k(t_a)/k(t_i) \) is independent of wavelength \( \lambda \). Furthermore, the temporal scaling factor of absorption \( R_{\text{abs}}(t_a, t_i) \) is independent of wavelength \( \lambda \) over the visible–near infrared (Vis-NIR) spectral region.

The analysis of scattering efficiency factor \( Q_{\text{sc}}(\rho) \) utilizes the transport approximation proposed by Dombrovsky et al for large semi-transparent particles, which can be applied to optically soft large cells of microalgae [19]:

\[ Q_{\text{sc}}^\gamma(\rho) = C \left\{ \begin{array}{ll} \rho/5 & (\rho \leq 5) \\ (5/\rho)^\gamma & (\rho > 5) \end{array} \right. \]

(10)

where \( C = 1.5n(n-1)\exp(-15k) \), \( \gamma = 1.4 - \exp(-80k) \), and \( Q_{\text{sc}}^\gamma(\rho) \) represents the transport scattering efficiency factor. For microalgal cells, the value of \( \gamma \) is usually smaller than 5. Therefore, the following equations can be used [23]:

\[ Q_{\text{sc}}^\gamma(\rho) = 1.5n(n-1)\exp(-15k)/5 \]

(11)

\[ Q_{\text{sc}}(\rho) = \frac{Q_{\text{sc}}^\gamma(\rho)}{1 - g} \]

(12)

Considering the temporal evolution of the optical properties of microalgae, \( \rho = 2\alpha(n-1) \) is a function of growth time \( t \); therefore, the temporal scaling factor of scattering can be expressed as

\[ R_{\text{sc}}(t_a, t_i) = \frac{\frac{n(t_a)}{4} \int Q_{\text{sc}}(\rho(t_a))d^2(t_a)f(d(t_a))dd}{\frac{n(t_i)}{4} \int Q_{\text{sc}}(\rho(t_i))d^2(t_i)f(d(t_i))dd} \]

(13)

Inserting the expression for \( Q_{\text{sc}}(\rho) \) leads to \( R_{\text{sc}}(t_a, t_i) \) being rewritten as

\[ R_{\text{sc}}(t_a, t_i) = \frac{n(t_a)(n(t_a) - 1)^2}{n(t_i)(n(t_i) - 1)^2} \int d^3(t_a)f(d(t_a))dd \exp\{15[k(t_i) - k(t_a)]\} \]

(14)

Here, the term \( \exp\{15[k(t_i) - k(t_a)]\} \approx 1 \), as \( 15[k(t_a) - k(t_i)] \ll 1 \) for microalga cultivation. Alternatively, we can choose a time interval infinitely close to 0. In this event, \( R_{\text{sc}}(t_a, t_i) \) can be rewritten as

\[ R_{\text{sc}}(t_a, t_i) = \frac{n(t_a)(n(t_a) - 1)^2}{n(t_i)(n(t_i) - 1)^2} \int d^3(t_a)f(d(t_a))dd \]

(15)

Assuming that the refractive index \( i \) satisfies some proportional relation, such as the absorption index, then the temporal scaling factor of scattering \( R_{\text{sc}}(t_a, t_i) \) will be independent of wavelength \( \lambda \) over the Vis-NIR spectral region. In addition, it is noted that the refractive index remains approximately constant over the Vis-NIR region [24]. Kandilian et al [10] verified that the scattering phase function demonstrated near-independence with respect to wavelength over the photosynthetically active region. Ma et al [25] reported that the scattering phase function is nearly time-independent during culture cultivation. Thus, the scattering phase function can be considered independent of the wavelength and growth time.

3. Results and discussion

3.1. Optical constants of microalgae

In this study, microalgae were considered to be approximately spherical in shape, as shown in figure 1.

Therefore, we used the Lorentz–Mie theory to calculate the radiation characteristics of the microalgae cell, which
can be characterized by the homogeneous sphere approximation [26]. Figure 2 shows a schematic diagram of the inverse procedure of the optical constants. The Lorentz–Mie theory was employed in the forward model to calculate the average extinction and absorption cross-sections. The trust region algorithm was used to minimize the difference between the simulated and measured absorption and extinction cross-sections of the microalgae. For each wavelength, the objective function $\eta_\lambda$ is defined as

$$
\eta_\lambda = \left( \frac{\langle C_{\text{abs},\lambda,\text{prod}} \rangle - \langle C_{\text{abs},\lambda,\text{exp}} \rangle}{\langle C_{\text{abs},\lambda,\text{exp}} \rangle} \right)^2 + \left( \frac{\langle C_{\text{ext},\lambda,\text{prod}} \rangle - \langle C_{\text{ext},\lambda,\text{exp}} \rangle}{\langle C_{\text{ext},\lambda,\text{exp}} \rangle} \right)^2 \quad (16)
$$

The convergence criterion was set as $\eta_\lambda < 10^{-4}$. To verify the theoretical approach, the complex refractive index of *Chlorella sp.* was retrieved using the experimentally measured cell size distribution and the absorption and extinction cross-sections reported in [8]. Comparing (not shown) the inverse identified complex refractive index of *Chlorella sp.* with that reported by Lee et al. [15] indicated that the relative errors associated with the real and imaginary parts were less than 0.43% and 33.14% (absolute errors less than $5 \times 10^{-4}$), respectively, for the...
400–750 nm wavelength range, confirming the validity of the inverse model. This model assumes that the microalga solution has a refractive index equal to that of distilled water in the 380–850 nm spectral region.

Figure 3(a) shows the wavelength dependence of the complex refraction index of microalgae at different growth stages, which was inversely identified by using experimentally measured major and minor diameters, via equation (3), as well as the absorption and scattering cross-sections [13]. The complex refraction index varies significantly with the growth time, reflecting the change in microalga composition at different growth stages. The absorption index, shown in figure 3(b), displays peaks at approximately 435 and 676 nm, corresponding to absorption peaks of Chl a, and at 485 nm, which is indicative of carotenoids [11]. Interestingly, dips in the refractive index can be observed around the wavelengths corresponding to the absorption peaks; these features are attributed to the fact that the real and imaginary parts of the complex refractive index are not independent of each other, but rather are correlated by the Kramers-Kroenig relation [22].

3.2. Temporal scaling characteristic root from electromagnetic scattering
Zhao et al [13] reported that a relation exists between the time-dependent optical properties of microalgae, which is called the TSF. Here, the temporal scaling characteristics of absorption and extinction are largely invariant over the Vis-NIR spectral region, as demonstrated using electromagnetic scattering theory. Figure 4 presents the temporal scaling factors of the absorption (figure 4(a)) and scattering (figure 4(b)) predicted using equations (8) and (15), respectively. As shown, these temporal scaling factors are nearly independent of the wavelength 5 and 14 days into the growth process. Conversely, wavelength-independent behavior is not obviously observed at 4 and 9 days, which is likely due to the variation of the components and pigments present in the microalgae at these growth stages [11]. It can be inferred that the optical properties of microalgae vary faster than the experimentally measured time interval. The growth process of microalgae is extremely complex, and the objective of this study was to develop a method of calculating the time-dependent optical properties.
4. Conclusions

The time-dependent optical properties of C. protothecoides batch cultures were presented in the 380–850 nm spectral range. These time-dependent optical properties were shown to vary significantly with the growth time. The evolution of the time-dependent optical properties is attributed to changes in cell composition. To elucidate the evolution of microagal optical properties, the temporal scaling characteristics between the growth phase and stationary phase were established using electromagnetic scattering theory. The temporal scaling characteristics discussed in this report are likely generally applicable to microalga owing to the fact that most strains possess complex refractive indices that are near unity in water. Moreover, the temporal scaling characteristics of microalgae may have important applications in remote sensing and PBR design. Future investigations should focus on isolating the specific impacts that various microalgae components, e.g., pigments, proteins, lipids, etc, have on the complex refractive index of microalgal colonies.

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Declaration of competing interest

The authors report no declarations of interest.

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