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Contract ESA-ESTEC PRF 141315

MODELING OF THE MELiSSA ARTIFICIAL ECOSYSTEM

Model Parameters for Growth of *Rhodospirillum rubrum* Under Light Limitation in Photobioreactors.

TECHNICAL NOTE 23.4.
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Content

Introduction and Objectives

In Technical notes TN 19.1 and 19.2 (Cornet *et al.*, 1993), a knowledge model for light limited growth of *Spirulina platensis* in cylindrical photobioreactor radially illuminated was established. This model provided basic information in order to perform simulations of the MELiSSA photosynthetic compartment and its model based predictive control. The ability of the model to predict steady states at different incident radiant light fluxes and different dilution rates was validated (Binois, 1994; Cornet *et al.*, 1995). The validation of dynamic predictions under steps in incident light flux is currently under investigation at UAB.

Such a monodimensional model relies mainly on the assumptions of Schuster (1905) for describing radiative light transfer inside the reactor, and on the concept of working illuminated volume for coupling light transfer and biological kinetics (Cornet *et al.*, 1992; Cornet *et al.*, 1995).

The aim of this Technical note is to provide a similar knowledge model for the MELiSSA photoheterotrophic compartment cultivating *Rhodospirillum rubrum* under light limiting conditions. This requires to determine the model parameters for this microorganism, the mathematical formulation of the model being unchanged. As previously decided, kinetic parameters will be obtained from the experimental results already available in the ESA report of Albiol (1994) whereas light transfer parameters determination will need further experimentation.

1- Kinetic Parameters

Two kinetic parameters are used in the model in order to take into account saturation curves of photosynthesis for growth rate (μ) versus radiant light energy locally available inside the reactor ($4\pi J$). Because the model does not consider short time exposure photoinhibition inside the reactor, a simple Monod law is assumed:

$$\mu = \mu_M \frac{4\pi J}{K_J + 4\pi J} \quad (1)$$

The maximum growth rate μ_M and the half saturation constant K_J have been determined according to the experimental results of Albiol (1994). The results of the identification procedure are as follows (Figure 1):

$$\mu_M = 0.17\ h^{-1}$$
$$K_J = 7\ W/m^2$$

It is obvious that it will be necessary to check the consistency of these parameters on further batch and continuous culture experiments.

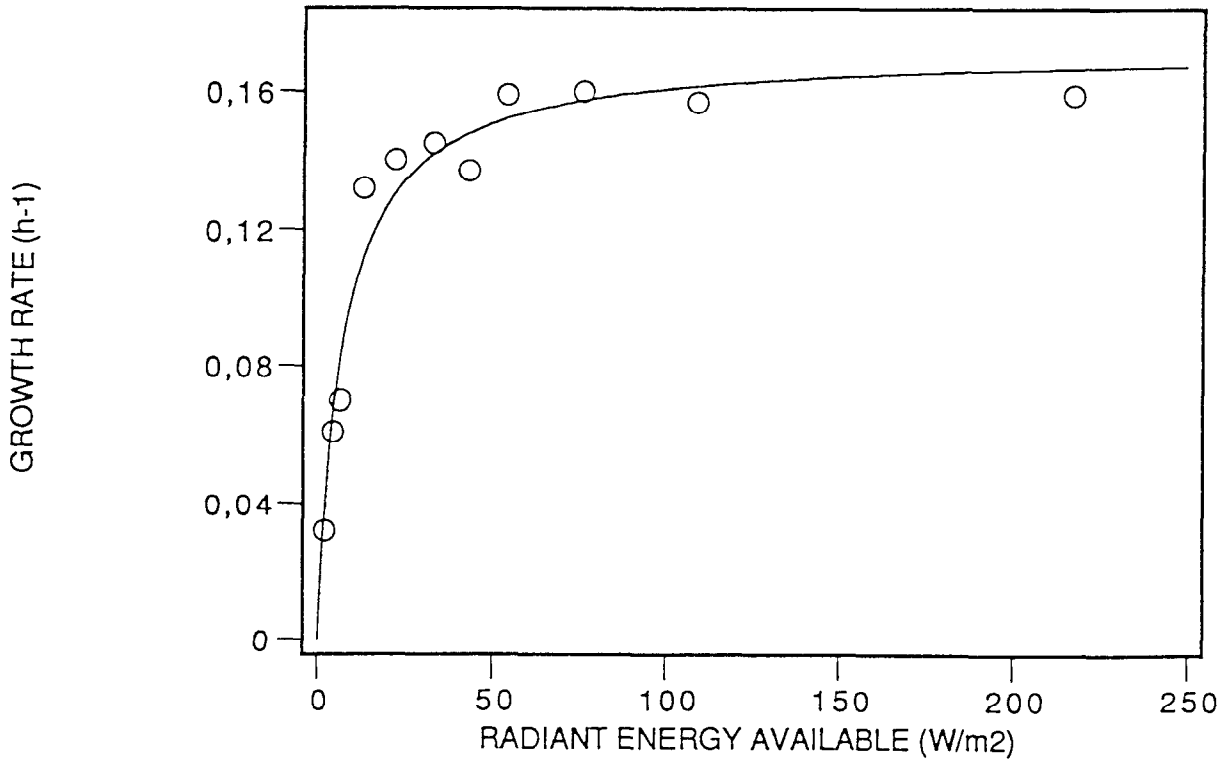

Figure 1: Monod law for radiant light energy available for *Rs. rubrum*. Open circles: experimental results of Albiol (1994).

2- Radiant Light Transfer Parameters

2.1- Introduction

The description of attenuation and availability of radiant light energy inside the medium of the photobioreactor is a complicated problem because of the existence of two dependent phenomena: absorption and scattering of light by microorganisms. This implies to determine from independent experiments the absorption and scattering coefficients for each considered wavelength (respectively $Ea(\lambda)$ and $Es(\lambda)$), then to average them to obtain mean coefficients (respectively Ea and Es) on the total absorption spectrum (350 - 950 nm for *Rs. rubrum*).

The consistency of these parameters is strongly linked to hypotheses made to describe radiant light transfer inside the medium. The proposed model is based on the Schuster hypotheses (1905) which are mainly:

- one considers a main traveling direction for the radiation (monodimensional approximation);
- it exists an homogeneous radiant flux at the bounded surface of the reactor;
- the radiation field inside the reactor presents an isotropic phase function (sometimes called scattering diagram), that means that the scattered light is the same in all directions.

If the absorption and scattering coefficients are determined with the same hypotheses, such a simplified and monodimensional model enables to predict with a sufficient accuracy the profiles in local available radiant light energy inside the reactor.

2.2- Determination of Absorption and Scattering Coefficients from the Method of Aiba - Shibata

This method was described by Aiba (1982) and relies on an hypothesis of Shibata (1958); thus it is called in this study "Aiba - Shibata" method. It was used by Cornet (1992) to determine the Ea and Es coefficients for *S. platensis* provided in TN 19.1 and TN 19.2.

The method is based on spectrophotometric measurement of the true transmission of a microorganism suspension. The word "true transmission" implies that it is necessary to know exactly the transmitted radiation that leaves the sample and not the transmitted radiation that reaches the photodetector (the intensity of the diffuse transmitted light decreases until it reaches the detector). This requires special experimental attention as described by Shibata (1958) and Cornet (1992). The two unknows $Ea(\lambda)$ and $Es(\lambda)$ are determined applying the two equations giving the expressions of transmission and reflection on the sample from Schuster hypotheses (Cornet, 1992):

$$T(\lambda) = \frac{4\alpha}{(1+\alpha)^2 e^{\delta} - (1-\alpha)^2 e^{-\delta}} \quad (2)$$

$$R(\lambda) = \frac{(1-\alpha^2)(e^{\delta} - e^{-\delta})}{(1+\alpha)^2 e^{\delta} - (1-\alpha)^2 e^{-\delta}} \quad (3)$$

with $\alpha = \sqrt{\frac{Ea(\lambda)}{Ea(\lambda)+Es(\lambda)}}$ and $\delta = C_X L \sqrt{Ea(\lambda)(Ea(\lambda)+Es(\lambda))}$

C_X being the biomass concentration and L the optical path length of the sample.

As the measurement of the reflection is very laborious, the Shibata hypothesis is assumed (Shibata, 1958): the ratio of transmission on reflection is considered as a constant which is wavelength independent and may be measured at any nonabsorbed wavelength in the considered spectrum (if the wavelength is only scattered it comes $T(\lambda) + R(\lambda) = 1$):

$$\frac{T(\lambda)}{R(\lambda)} = \beta \quad (4)$$

For *Rs. rubrum*, the constant β may be obtained at a wavelength of 950 nm or, if it is practically impossible at 720 nm (nonabsorbed wavelength). As the spectrophotometer used enables measurements only up to 850 nm, the results obtained by this method are given in the range [350-850 nm] in Figure 2.

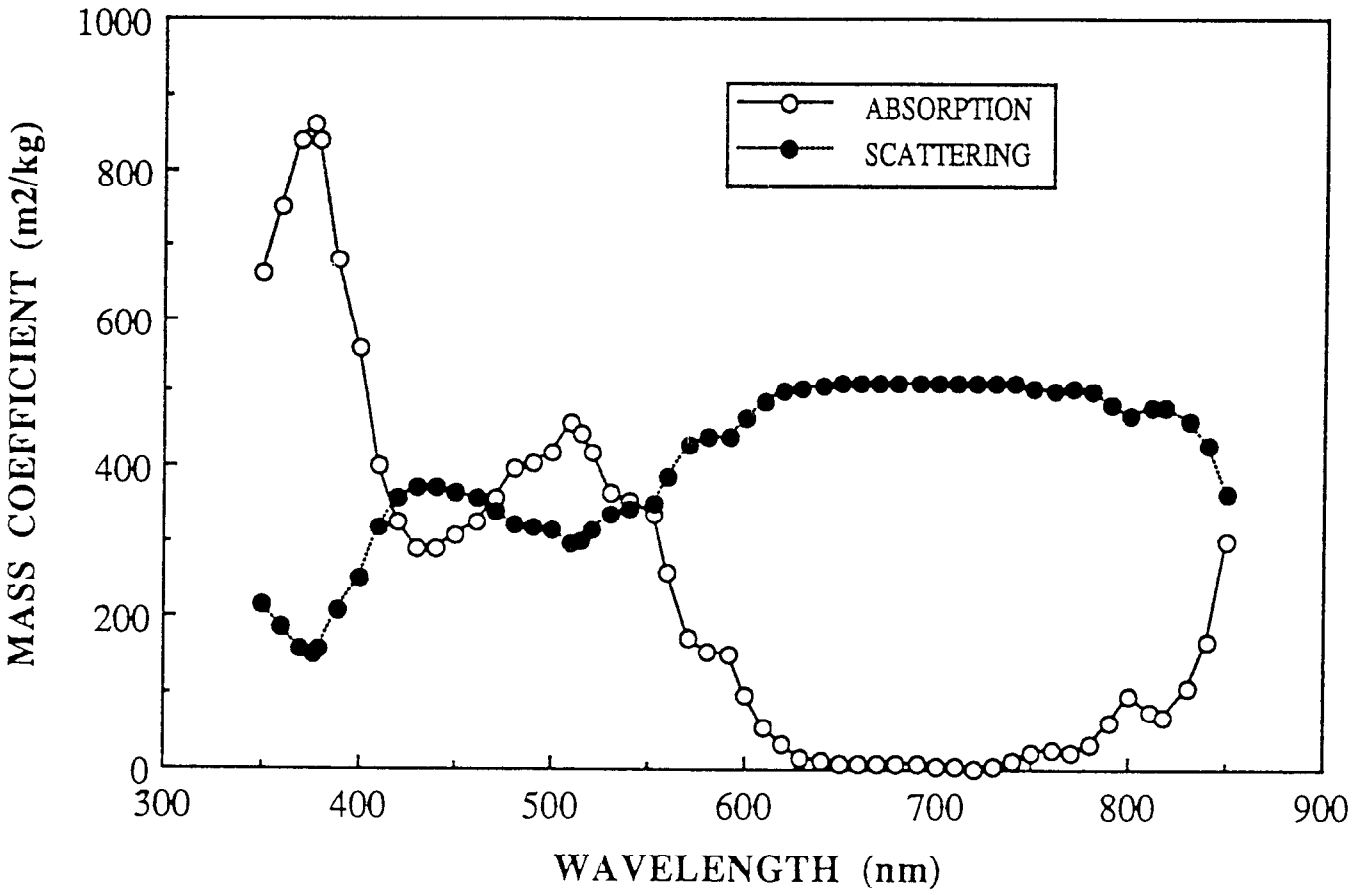

Figure 2: wavelength dependent absorption and scattering coefficients obtained by the method of Aiba - Shibata for *Rs. rubrum*.

2.3- More Realistic Approach for Determination of Absorption and Scattering Coefficients

From a theoretical point of view, the Shibata hypothesis for scattering of light by microorganisms involves two different assumptions:

- the particle (i.e. the microorganism) scatters light as a perfect dielectric (a perfect dielectric is a particle which does not absorb radiation and can then be considered to have a real refractive index) at each considered wavelength of the spectrum, so the phase function appears wavelength independent;

- the scattering coefficient is wavelength independent and its value is fixed at the reference nonabsorbed wavelength choosen for β calculation. This corresponds to the geometrical optics approximation.

Even if it is not the case in reality, the first assumption is compatible with the constant and isotropic phase function proposed by Schuster (1905), so it does not modify the consistency of the model. At the opposite, the second assumption is true only if the characteristic size of the considered microorganism (e.g. the diameter for a spherical particle) is very important compared to the considered wavelength. Theoretical calculations based on the electromagnetic theory of Mie (from Maxwell equations; Van de Hulst, 1957) have shown that this assumption was valid with less than 10% of deviation only if the characteristic size of the microorganism is higher than 100 μm (this value falls to 10 μm if the microorganism absorbs light strongly). As most microorganisms have a lower characteristic size (for example, *Rs. rubrum* has a characteristic size of about 1 μm), the Shibata hypothesis is inacceptable for small microorganisms.

The Mie theory (Van de Hulst, 1957) provides an excellent background for the calculation of $Ea(\lambda)$ and $Es(\lambda)$ but it is quite complex. Additionally, as previously explained, the values of absorption and scattering coefficients are linked to the value of the corresponding phase function which is not isotropic for microorganisms. Consequently, a great difficulty will be to normalize the calculations from Mie theory to an isotropic phase function required for applying Schuster assumptions and keeping the consistency to the model.

Applying Mie theory requires to know two independent informations about particle characteristics:

- the size parameter of the particle $\xi = \frac{\pi D}{\lambda}$ where D is a characteristic size of the particle (e.g. the diameter of a sphere or a cylinder);

- the complex refractive index $m = n - ik$ for the particle (k being proportional to $Ea(\lambda)$ that implies a nonlinear problem) or the real refractive index $m = n$ if the particle does not absorb the radiation at the considered wavelength.

From this point, the Mie theory provides a complex equation which may be used by combination with the transmission equation (equation 2) instead of using the reflection equation both with the Shibata hypothesis (equations 3 and 4). Then, by this way, the prediction of $Ea(\lambda)$ and $Es(\lambda)$ coefficients is possible only from the experimental measurements of true transmission of a sample. Moreover, this approach enables to correctly take into account the wavelength dependence of absorption and scattering phenomena.

2.3.1- Theory Validation on Nonabsorbing Microorganisms

As previously explained, an important parameter of the Mie theory is the real refractive index of the particle n. In this study, the particle (i.e. the microorganism) is considered as homogeneous. Additionaly, the mean real refractive index of the microorganism is supposed to be independent of the considered species. The value of this parameter was determined by identification from experimental measurements of the wavelength dependent scattering coefficient for microorganisms which does not absorb the radiation in the visible spectrum (perfect dielectric). Experimental data for $Es(\lambda)$ have been obtained from true transmission measurements, applying the Schuster model with a Taylor development corresponding to the case where the radiation is only scattered in the medium ($\alpha = 0$, conservative case of radiative transfer).

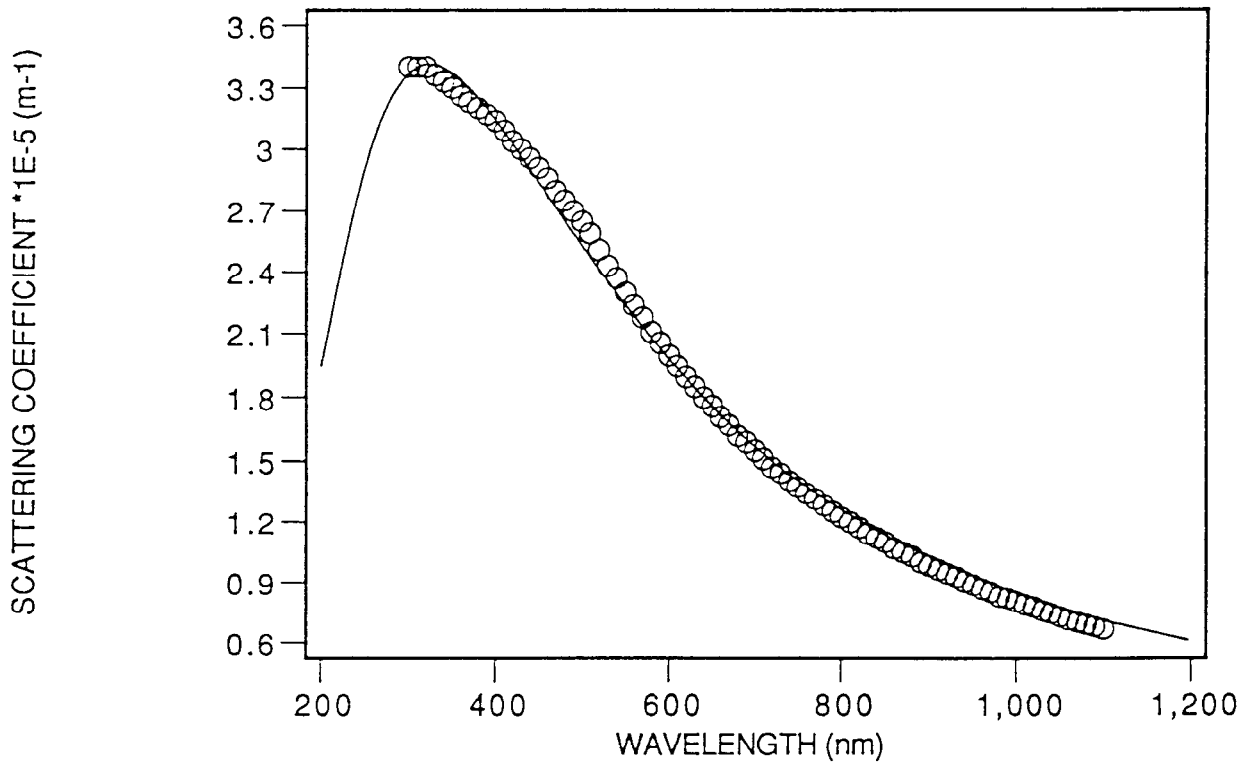

Figure 3: Volumetric scattering coefficient for *S. cerevisiae* in water versus wavelength (n = 1.375). Comparison between Mie theory corrected from an isotropic phase function and experimental data (open circles)

Two microorganisms were used for this study:

- *Saccharomyces cerevisiae* which is a spherical particle of 5 µm diameter. Such microorganism presents a maximum of scattering in the considered wavelength which is of great importance to identify the refractive index with a sufficient accuracy (4 significant numerals).

- *Pseudomonas taetrolens* which is a cylindrical particle of 1.2 µm diameter, then having the same scattering characteristics than *Rs. rubrum*. In this case this microorganism was used to define the wavelength dependent correction for isotropic phase function already discussed.

The result of the identification procedure appears on Figure 3 and gives a value for the mean real refractive index of:

$$n = 1.375$$

This value is compatible with the results for *Ps taetrolens* (Figure 4) and with the previous literature (Wyatt, 1968).

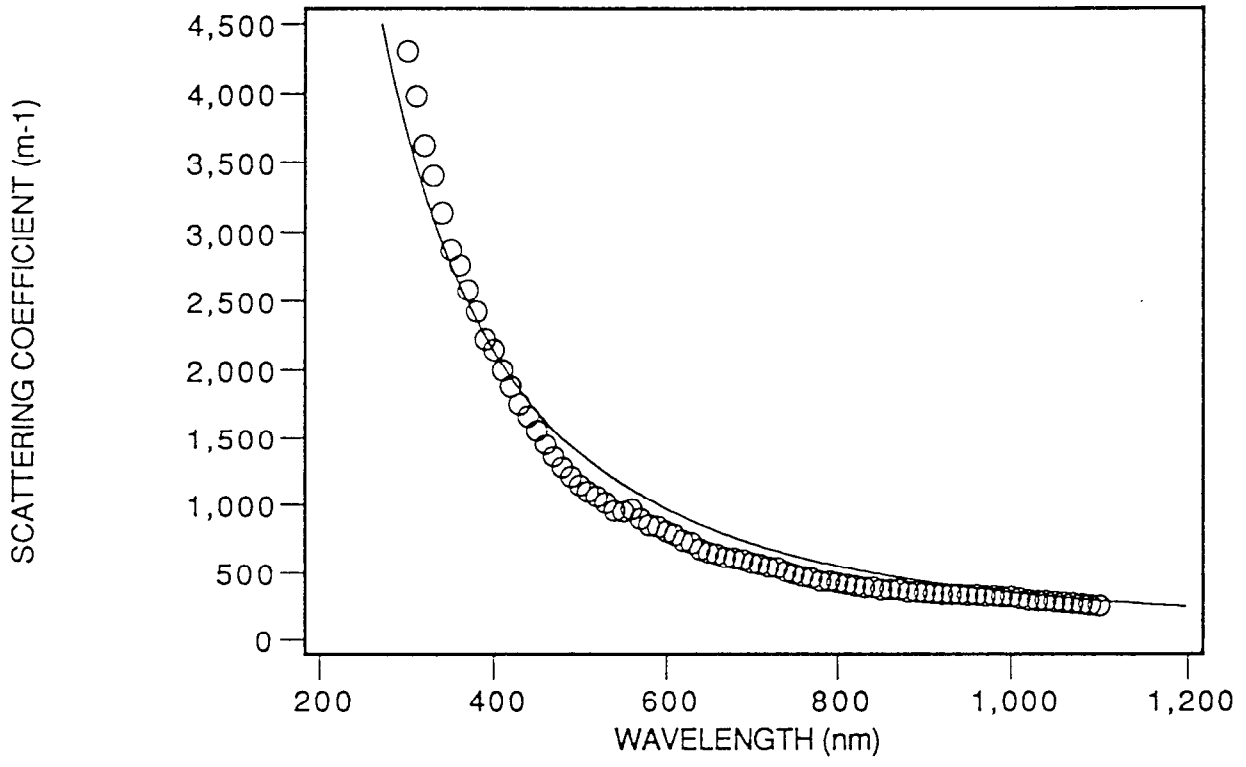

Figure 4: Volumetric scattering coefficient for *Ps. taetrolens* in water versus wavelength (n = 1.375). Comparison between Mie theory corrected from an isotropic phase function and experimental data (open circles)

The sligth difference observed between theory and experimental data for *Ps. taetrolens* comes from the fact that a spherical model of particle has been used instead of a cylindrical one which does not give an analytical solution.

2.3.2- Calculation of the Absoption and Scattering Coefficients

Complex theoretical considerations have shown that it exits a difficult reconciliation between the Schuster - Shibata assumptions and the more rigorous Mie theory to determine absorption and scattering coefficients consistent with the proposed model in TN 19.1 and 19.2. These considerations are out of the scope of this TN and I report here only the main conclusions:

- $Ea(\lambda)$ and $Es(\lambda)$ can be calculated applying Mie theory on a homogeneous microorganism of refractive index equal to 1.375 considering that the particle scatters as a perfect dielectric in order to remain consistent with the assumption of a wavelength independent phase function;
- the results given by Mie theory have to be corrected from the isotropic phase function assumption before combination with Schuster equations to calculate $Ea(\lambda)$ and $Es(\lambda)$.

As already discussed, the wavelength dependent correction for isotropic phase function has been established from the experimental results obtained with *Ps. taetrolens* which presents the same size parameter than *Rs. rubrum*. The characteristic size of *Rs. rubrum* (the diameter of the filament) has been taken equal to **1.17 µm** which is compatible with our own microscopic observations and the literature data (Imhoff and Trüper, 1989). The correction term has then been determined, giving:

$$f(\lambda) = 0.35\left(\frac{\lambda}{8.5\ 10^{-7}}\right)^{0.8} \quad (5)$$

So, $Ea(\lambda)$ and $Es(\lambda)$ may be calculated from the following set of equations (equation 6 is an approximation from Mie theory when the refractive index of the particle is near the refractive index of the surrounding medium, i.e. water for which $n = 4/3$):

$$T(\lambda) = \frac{4\alpha}{(1+\alpha)^2 e^{\delta} - (1-\alpha)^2 e^{-\delta}} \quad (2)$$

$$\frac{\delta}{\alpha} = N_p \pi R^2 L f(\lambda)\left[2 - \frac{4}{\rho}\sin\rho + \frac{4}{\rho^2}(1-\cos\rho)\right] \quad (6)$$

$$\alpha = \sqrt{\frac{Ea(\lambda)}{Ea(\lambda)+Es(\lambda)}} \text{ and } \delta = C_X L\sqrt{Ea(\lambda)(Ea(\lambda)+Es(\lambda))}$$

with N_p the number of particles by unit volume (determined by mean of an hematocymeter i.e. a Malassez cell) and ρ being equal to $\rho = 2\xi\frac{4}{3}(\overline{n}-1) = 2\pi\frac{D}{\lambda}\frac{4}{3}(\frac{1.375\times 3}{4}-1)$.

The results of the nonlinear identification procedure are given on Figure 5 for $Ea(\lambda)$ and $Es(\lambda)$. The comparison of results obtained by the Aiba-Shibata method and by the present method (Mie theory corrected from isotropic phase function) are given respectively on Figure 6 (absorption coefficient) and Figure 7 (scattering coefficient).

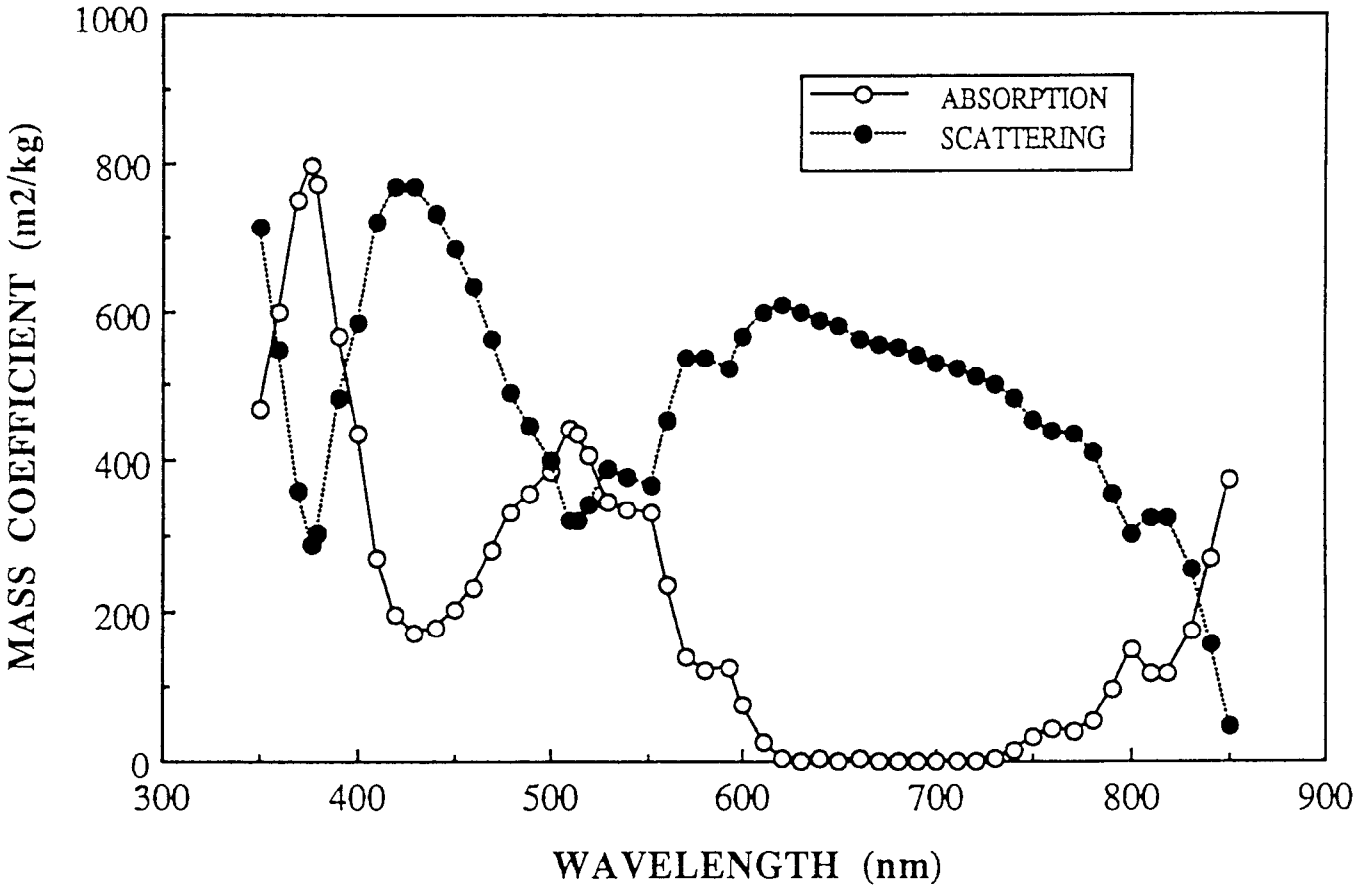

Figure 5: Absorption and scattering mass coefficients obtained by the proposed method (equations 2 and 6) for *Rs. rubrum* in the range 350 - 850 nm.

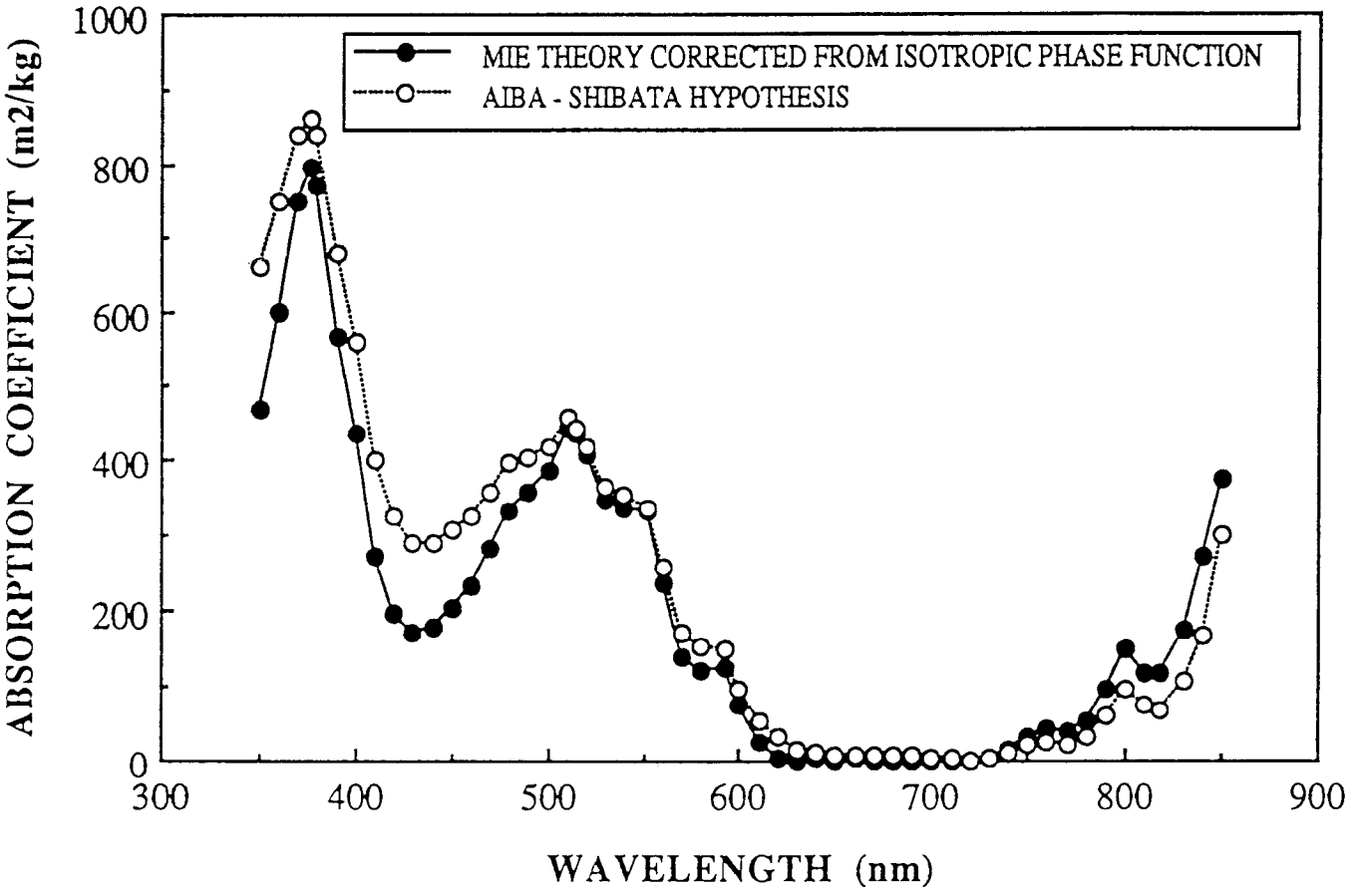

Figure 6: Comparison between the Aiba - Shibata method and the proposed method for the values of the mass absorption coefficient Ea(λ) for *Rs. rubrum* in the range 350 - 850 nm.

From these results, the mean values on the total considered spectrum have been calculated in the range 350 - 750 nm currently used by the UAB, ESTEC and LGCB MELiSSA partners, and in the range 350 - 850 nm which might be used for *Rs. rubrum* (*Rs. rubrum* absorbs radiation up to 950 nm but the spectrophotometer used in this study does not authorize such measurements). The mean coefficients obtained which will be used in the monodimensional model for *Rs. rubrum* are:

In the range 350 - 750 nm:

$$Ea = 220\ m^2/kg$$
$$Es = 480\ m^2/kg$$

In the range 350 - 850 nm:

$$Ea = 200\ m^2/kg$$
$$Es = 440\ m^2/kg$$

These values are likely very close to those required in the range 350 - 950 nm.

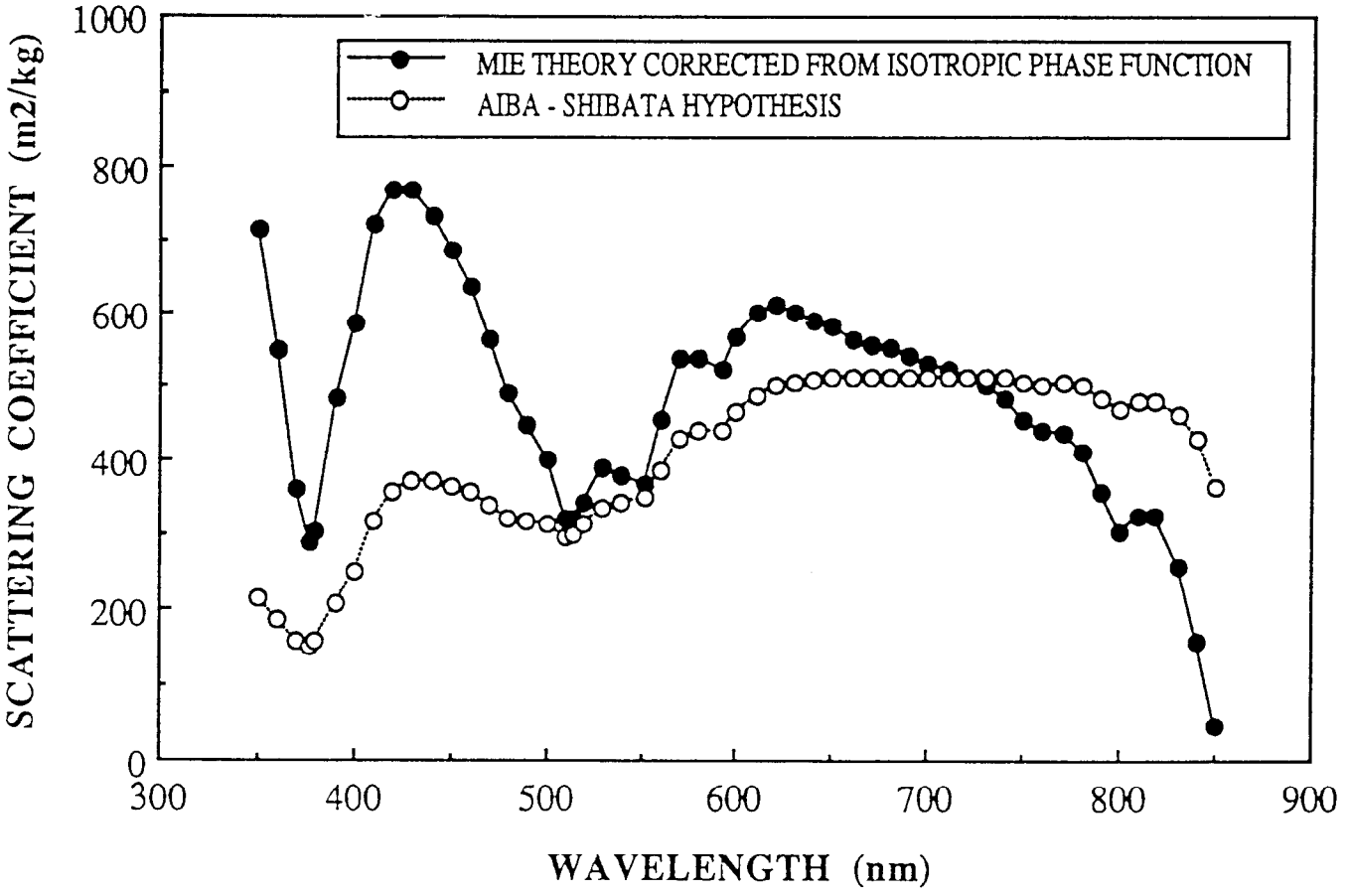

Figure 7: Comparison between the Aiba - Shibata method and the proposed method for the values of the mass scattering coefficient Es(λ) for *Rs. rubrum* in the range 350 - 850 nm.

2.3.3- Remarks and Restrictions

- For some wavelength, the error in values given by the Aiba - Shibata method reaches more than 100 %, specially on the scattering coefficient. It appears that the use of mean coefficients on the entire visible spectrum damps this tendancy. One may consider that the

Shibata hypothesis leads to underestimate *Es* by about 30% and to overestimate *Ea* by about 15% on *Rs. rubrum*.

- An important restriction for the *Ea* and *Es* values given in this study arises from the question of chromatic adaptation. It will be necessary to verify that the pigment content of *Rs. rubrum* is constant under different illuminating conditions in emission quality of the lamps.

- In this study, the *Ea* and *Es* coefficients have been calculated for two considered ranges of wavelength [350 - 750 nm] and [350 - 850 nm] enabling the use of one or other pair of coefficients depending of the emission spectrum of the lamps. Nevertheless, one must keep in mind that the kinetic parameters μ_M and K_J have been obtained with an illumination spectrum in the range [350 - 750 nm].

- The theoretical study led in this TN has shown that the Aiba - Shibata hypothesis $T(\lambda)/R(\lambda) = \beta$ imposes to work with concentrated samples to avoid erroneous values on the scattering coefficient $Es(\lambda)$. It appeared indeed that Taylor developments of equation (4), when biomass concentration was low, gives a wavelength independent scattering coefficient whereas it should be linked to the absorption coefficient.

3- Calculation of Working Illuminated Volume

As previously explained (Cornet *et al.*, 1992, Cornet *et al.*, 1995) the definition of the working illuminated volume is a prerequisite step to ensure robustness of kinetic parameters of the model. The value of the radiant light energy available $4\pi J_{min}$ at which no oxygen consumption or evolution occurs (called compensation point for photosynthesis) and defining the working illuminated volume may be obtained by two ways:

- fundamental oxygen measurements at the cellular scale (Cornet, 1992);
- appearance of the linear phase of growth at the reactor scale; the critical radiant light energy available $4\pi J_{min}$ being calculated applying the radiative transfer equations.

The last solution was considered at UAB and results were obtained on batch cultures in rectangular reactors. Preliminary results (draft of TN 25.7 of UAB) give a critical value of about:

$$4\pi J_{min} = 1\ W/m^2$$

which is the same as for *S. platensis* (Cornet et *al.*, 1992).

4- Model Equations and Parameters

- Model for Radiant Light Energy Available Versus the Radius of the Reactor:

$$\frac{4\pi J}{F_R} = \frac{R}{r}\frac{2\cosh(\delta r/R)}{\cosh(\delta)+\alpha\sinh(\delta)} \quad (7)$$

$\alpha = \sqrt{\frac{Ea}{Ea+Es}}$ and $\delta = (Ea+Es)C_X\alpha R$ with R, radius of the reactor and F_R the radial incident light energy flux.

- Calculation of the Working Illuminated Volume:

$$\text{Solve: } \frac{R_2}{r}\frac{2\cosh(\delta R_2/R)}{\cosh(\delta)+\alpha\sinh(\delta)} - \frac{4\pi J_{min}}{F_R} = 0 \quad (8)$$

The two roots R_2' and R_2 enable to define the working illuminated radius of the reactor from the two parts $(0 - R_2')$ and $(R_2 - R)$.

- Calculation of the mean volumetric growth rate in the reactor:

$$< r_X >=< \mu > \gamma C_X \quad (9)$$

$$\gamma = \frac{R_2'^2}{R^2} + \frac{(R^2 - R_2^2)}{R^2} \quad (10)$$

$$< \mu >= \frac{1}{\pi R_2'^2} \int_0^{R_2'} 2\pi r \mu_M \frac{4\pi J}{K_J + 4\pi J} dr + \frac{1}{\pi(R^2 - R_2^2)} \int_{R_2}^{R} 2\pi r \mu_M \frac{4\pi J}{K_J + 4\pi J} dr \quad (11)$$

-Mass Balance on the Reactor:

$$< r_X > - \frac{C_X}{\tau} = \frac{dC_X}{dt} \quad (12)$$

where τ is the residence time in the reactor.

- Parameters:

$\mu_M = 0.17\ h^{-1}$

$K_J = 7\ W/m^2$

$Ea = 220\ m^2/kg$ *biomass for (350-750 nm)*

$Es = 480\ m^2/kg$ *biomass for (350-750 nm)*

$4\pi J_{min} = 1\ W/m^2$

- Comparison with *Spirulina platensis* parameters:

$\mu_M = 0.073\ h^{-1}$

$K_J = 20\ W/m^2$

$Ea = 150\ m^2/kg$ *biomass for (350-750 nm)*

$Es = 200\ m^2/kg$ *biomass for (350-750 nm)*

$4\pi J_{min} = 1\ W/m^2$

Conclusions

In this Technical Note, the five parameters for the growth model of *Rs. rubrum* under light limitation in photobioreactors have been determined.

The Aiba - Shibata method for determination of mass absorption and scattering coefficients of light has appeared inapropriate for microorganisms of small size. A rigorous treatment of light scattering and absorption by particles has been used (Mie theory) to determine these coefficients from an extensive theoretical and experimental study, whereas the kinetic parameters μ_M, K_J and $4\pi J_{min}$ have to be considered as preliminary results.

These last kinetic parameters will have to be verified from batch culture experiments in rectangular (TN 25.7) and cylindrical photoreactors.

The robustness of these parameters will be checked from further continuous culture experiments and from the dynamic behaviour of the reactor in response to steps in incident radiant energy flux.

The possibility to work with an incident spectrum in the range 350 - 950 nm for *Rs. rubrum* will have also to be investigated and the effects of such a spectrum on the kinetic parameters of the model to be determined.

Notations

C_X	Biomass concentration (kg.m^{-3})
D	Characteristic diameter of a particle (m)
$Ea(\lambda)$	Wavelength dependent mass absorption coefficient (m^2.kg^{-1})
$Es(\lambda)$	Wavelength dependent mass scattering coefficient (m^2.kg^{-1})
Ea	Mean mass absorption coefficient (m^2.kg^{-1})
Es	Mean mass scattering coefficient (m^2.kg^{-1})
F_R	Mean incident radiant light energy flux (W.m^{-2})
J	Mean radiant light intensity (W.m^{-2})
K_J	Half saturation constant for radiant light energy available (W.m^{-2})
k	Imaginary refractive index of a particle (-)
L	Optical path length of a sample (m)
m	Complex refractive index of a particle (-)
N_p	Number of particles by unit volume (m^{-3})
n	Real refractive index of a particle (-)
$\overline{n}$	Particle refractive index on surrounding medium refractive index ratio (-)
R	Particle radius (m)
R	Reactor radius (m)
$R(\lambda)$	Wavelength dependent reflection of a sample (-)
r	Radius (m)
r_X	Volumetric growth rate for biomass (kg.m^{-3}.h^{-1})
$T(\lambda)$	Wavelength dependent transmission of a sample (-)

Greek Letters

λ	Wavelength (m)
μ	Growth rate (h^{-1})
μ_M	Maximum growth rate (h^{-1})
τ	Residence time (h)

Indices

min	Relative to compensation point radiant light intensity
2	Relative to the working illuminated volume

References

AIBA S., 1982. Growth kinetics of photosynthetic microorganisms. *Adv. Biochem. Eng.* **23:** 85-156.

ALBIOL i SALA J., 1994. Study of the MELiSSA photoheterotrophic compartment. Kinetics and effects of C limitation. In *ESA report ESA-EWP-1808. ESA/YCL/2148.JAS.*

BINOIS C., 1994. Automatisation d'un écosystème artificiel utilisé comme système de support vie. Première interaction modèle / système de contrôle. *Conservatoire National des Arts et Métiers, Thesis*, Paris

CORNET J.-F. 1992. Etude cinétique et énergétique d'un photobioréacteur. Etablissement d'un modèle structuré. Applications à un écosystème clos artificiel. *PhD Thesis*, Université Paris IX Orsay, n° 1989.

CORNET J.-F., DUSSAP C.G., DUBERTRET G. 1992. A structured model for simulation of cultures of the cyanobacterium *Spirulina platensis* in photobioreactors: I. Coupling between light transfer and growth kinetics. *Biotech. Bioeng.* **40:** 817-825.

CORNET J.-F., DUSSAP C.G., GROS J.-B. 1993. Modeling of physical limitations in photobioreactors. Adaptation of the light energy transfer model to cylindrical geometries. In *Technical note 19.1. ESA contract PRF 130-820.*

CORNET J.-F., DUSSAP C.G., GROS J.-B. 1993. Modeling of physical limitations in photobioreactors. Modeling of exopolysaccharide synthesis in cultures of *Spirulina platensis.* In *Technical note 19.2. ESA contract PRF 130-820.*

CORNET J.-F., DUSSAP C.G., GROS J.-B., BINOIS C., LASSEUR C. 1995. A simplified monodimensional approach for modeling coupling between radiant light transfer and growth kinetics in photobioreactors. *Chem. Eng. Science.* **50:** 1489-1500.

IMHOFF J.-F., TRÜPER H.G. 1989. Purple non sulfur bacteria. In *Bergey's manual of systematic bacteriology*. 9th edition. **3:** 1658-1673.

SCHUSTER A. 1905. Radiation through a foggy atmosphere. *Astrophys. J.* **21:** 1-22.

SHIBATA K. 1958. Spectrophotometry of intact biological material. *J. Biochem.* **45:** 599-623.

VAN DE HULST H.C. 1957. *Light scattering by small particles.* Wiley. New-York.

WYATT P.J. 1968. Differential light scattering: a physical method for identifying living bacterial cells. *Applied Optics.* **7:** 1879-1896.