Université Blaise Pascal LGCB
Laboratoire de Génie Chimique et Biochimique

TECHNICAL NOTE 83.3

UPGRADING THE RADIANT LIGHT TRANSFER MODELLING IN RECTANGULAR OR CYLINDRICAL PHOTOBIOREACTORS

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EXPERIMENTAL VALIDATIONS

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1. Introduction and Objectives

As explained in the previous technical note (TN 83.2 – Cornet *et al*., 2006), the models developed in LGCB for the CII kinetic behaviour during the PhD thesis of Lidia Favier (TN 55.1 – Cornet *et al*., 2005) are considered today to be rather qualitative (even if they have a correct structure and an intrinsic coherence) and not sufficiently robust and predictive in regard to the high complexity of the reactor stability when cultivating *Rs. rubrum* in continuous mode (Cornet *et al*., 2003). Consequently, they cannot be used with confidence in the simulation, the design or the predictive control of the CII in the MELiSSA project. These important tasks require indeed to be able to calculate the two critical residence times defining the operating engineering domain for stable cultures with the highest productivity, in relation to the incident hemispherical light flux $q_{\cap}$ and (probably) the hydrodynamics (Cornet *et al*., 2003 and TN 55.1). This will be feasible only if three successive steps may be overcome, that is:

- improving the radiant light transfer description inside rectangular or cylindrical reactor geometries;
- improving the kinetic and stoichiometric coupling with the radiation field, using if possible a predictive method as provided by the linear thermodynamics of irreversible processes;
- improving finally the understanding of the coupling between the hydrodynamics and the metabolic activity of *Rs. rubrum* in short residence time dark zones.

If these two last points represent a considerable amount of work (to plan in the future), the former was performed in LGCB during the last years and was retained as the main objective of the companion technical notes 83.2 and 83.3. In TN 83.2 (Cornet *et al*., 2006), the theoretical analysis of the light transfer problem inside photobioreactors (PBR) of any geometry has been led and up-graded, and important results have been reported as long as the one-dimensional assumption be applicable in solving the radiative transfer equation (RTE). Particularly, a general method enabling to calculate the optical and radiative properties of micro-organisms (absorption and scattering coefficients with the phase function), only from basic information such as their shape, their size distribution and their pigment content has been presented. Different methods have been then envisaged to solve the RTE, examining both analytical approximated generalised two-flux methods and full numerical methods of different orders, the highest leading to a (supposed) exact reference solution. Finally, these results were used to discuss the validity of the assumption using mean coefficients in place of rigorous averaging of each

spectral coefficient in the determination of fields of irradiances or local volumetric rates of radiant energy absorbed (LVREA). These quantities are of prior importance in formulating the kinetic and/or stoichiometric coupling (by a predictive thermodynamics approach) in the framework of the royal route toward PBR modelling proposed in LGCB. As a consequence, the calculation of the radiation field inside a PBR is now became a completely predictive problem, both in rectangular or curvilinear systems of coordinates. This means that, if the basic characteristics of any micro-organism are known, it is possible, from the knowledge of the boundary conditions, to accede to the irradiance or the LVREA fields inside the reactor *a priori*, without any other experimental parameter or determination.
Evidently, all the theoretical developments presented in TN 83.2 needs to be experimentally verified before claiming that we have definitively solved the light transfer problems for the MELiSSA purposes, and it is indeed, the aim of this technical note. More precisely, the initial work package foreseen in this TN 83.3 was focussed on the CII and *Rs. rubrum* experimental validations only. The task involved both an *ex situ* validation of the radiative properties for *Rs. rubrum* using an integrating sphere photometer, and an *in situ* validation of the radiation field inside a cylindrical vessel after developing a new experimental sensor device. Unfortunately, because Jérémy Deremetz left our lab during his PhD thesis, the second part of the work fell down. As a consequence, the new objectives of this TN were limited to *ex situ* experimental validations with the integrating sphere photometer, but including also *Arthrospira platensis* (CIVa) as an important micro-organism for the MELiSSA project. Besides, because the optical bench with the integrating sphere which has been developed in our lab as a prototype still presents today an electronic problem when working near its limit of sensitivity (transmittances close to 1), it revealed impossible to validate the optical properties of each micro-organism using a single scattering measurement as it should be (Tien and Drolen, 1987). Finally, the two main objectives of the present technical note can then be summarised as follows:

i) From measurements of true transmittances in multiple scattering with integrating sphere photometer and using a high accuracy numerical method for solving the RTE, the calculation method proposed (TN 83.2) for optical and radiative properties for the two considered micro-organisms will be experimentally validated for some significant wavelengths in the PAR (photosynthetically active radiation). Clearly, this validation in multiple scattering conditions (which cannot avoid treating the radiative transfer problem in the transmittance prediction) is only pertinent if the method used for solving the RTE can be proved as quasi-exact. Fortunately, even if it is out of the scope of this TN, the proof that the highest order d-S_{96} differential ordinates method provides rigorous solutions for the RTE has been established elsewhere (Cornet, 2007).

ii) In the same manner and from true transmittance measurements for characteristic wavelengths, a comparison of the different assumptions in solving the RTE will be performed, as a validation of the theoretical presentation of this problem (TN 83.2, part 3). First, using rigorous calculations for the radiative properties of the cells (i.e.

using a cylinder as model for the shape), the order of the d-S_N method will be discussed (comparing respectively d-S_{96}, d-S_{32} and d-S_2 or two-flux methods). Second, the same analysis will be conducted but using radiative properties calculated from the volume equivalent sphere for the cells as it is currently done in the literature.

After these experimental validations and a discussion about the choice of the best method suited for a given application (for the CII and the CIVa compartments), a last part will be devoted to a presentation of the important work of computation which is needed if we want to establish a complete MELiSSA bank of radiative properties for *A. platensis* and *Rs. rubrum* in a wide range of experimental conditions (i.e. taking into account the related important composition changes of the biomass as EPS, PHB and pigments contents,…).

2. Materials and Methods

2.1. Strains Cultivation

Arthrospira platensis PCC 8005 and *Rhodospirillum rubrum* ATCC 25903 were subcultured in Erlenmeyer flasks (500 mL) put in an agitated water bath at controlled temperature (30°C). The light was provided by 4 fluorescent lamps giving roughly an incident light flux of 5-7 $W.m^{-2}$ (PAR). This low incident light flux enables to consider a negligible amount of EPS and PHB respectively for *A. platensis* (only active biomass) and *Rs. rubrum* in the biomass composition for the determination of optical characteristics.
For *A. platensis*, the medium used was the classical Zarrouk medium modified by Cogne *et al*. (2003). For *Rs. rubrum*, the medium defined as in the PhD thesis of Lidia Favier-Teodorescu (2004) and TN 49.2 (2003) was used, with MOPS as buffer.

2.2. Strains Characteristics

2.2.1.Image Analysis for Size Distribution

The size distribution of each micro-organism was determined by image analysis, using a microscope (Olympus BX 41) linked to a CCD camera (Kappa PS 30) and the software Image Pro Plus 4.1 (Media Cybernetics). In all cases, the results demonstrated a log-normal law for the polydisperse distribution of micro-organisms in the form:

$$n(r) = \frac{1}{\sqrt{2\pi}\, r \ln(\sigma)} \exp\left[-\frac{(\ln r - \ln \bar{r})^2}{2 \ln^2 \sigma} \right] \quad (1)$$

verifying the density equation (24) in TN 83.2, and then characterised by the mean equivalent radius $\bar{r}$ and the standard deviation σ (see Table 3 in the last part of this document).
The choice of a model shape for each micro-organism was also deduced of such an analysis; clearly, the cylinder was retained for *Rs. rubrum* (a rod) and for *A. platensis* (a spiralled rod with a curvature radius sufficiently high to be considered as a randomly oriented cylinder)

2.2.2. Pigment Content Determination

For the two micro-organisms, the pigment content was determined spectrophotometrically according to the methods described in the PhD thesis of Lidia Favier-Teodorescu (2004) or in TN 49.2 (2003) for *Rs. rubrum*, and in the PhD Thesis of J-F. Cornet (1992) for *A. platensis*.

2.2.3. Real Refractive Index Determination

The real part of the refractive index n_λ of any micro-organism is a fundamental optical data for the calculation of the radiative properties of the particle. As explained in TN 83.2, it can be considered as constant (or more exactly, the relative refractive index $n_r = n_\lambda / n_m$ which account for in the calculations may be considered as constant) over the visible domain of wavelengths. If the volume fractions of the main cellular components together with their respective refractive index are known, it is then possible to calculate the mean refractive index of the cells from composition rules as given by eq. (12) in TN 83.2. This predictive method has been applied for *Rs. rubrum* because the main part of experimental information dealing with optical properties in the literature is available for bacteria (Wyatt, 1968, 1970, 1972). At the opposite, because of the lack of data for the cyanobacteria, the refractive index for *A. platensis* was determined experimentally by inverse method (Cornet, 2007), using a transmittance measurement with the integrating sphere photometer (see hereafter) at a non-absorbed wavelength (e.g. at 820 nm in this case).

2.3. Integrating Sphere Photometer

The optical bench used in this study was mainly composed with a spectrofluorimeter SAFAS Flx, linked to an integrating sphere of 6 inches diameter (the largest commercial size available from Labsphere®) by optical fibres (Figure 1). This combination was possible because of the high energy xenon lamp of the spectrofluorimeter enabling to work with a so high diameter for the integrating sphere (then more accurate), corresponding alone to a light attenuation of five log. This device can measure transmittances, diffuse and specular reflectances of liquid or solid samples.

The liquid suspensions are put in special quartz cells of 15 mL total volume with a light path length of 10 mm and with a large width (40 mm) enabling to catch all the photons scattered at the rear of the sample (transmittance measurement).
This optical bench (a prototype) then authorises to measure very accurately (four significant digits except near unity) true transmittances and reflectances with a collimated incidence in the range 280-850 nm. It has been extensively used in this study to measure only transmittances of suspensions for the two considered micro-organisms.

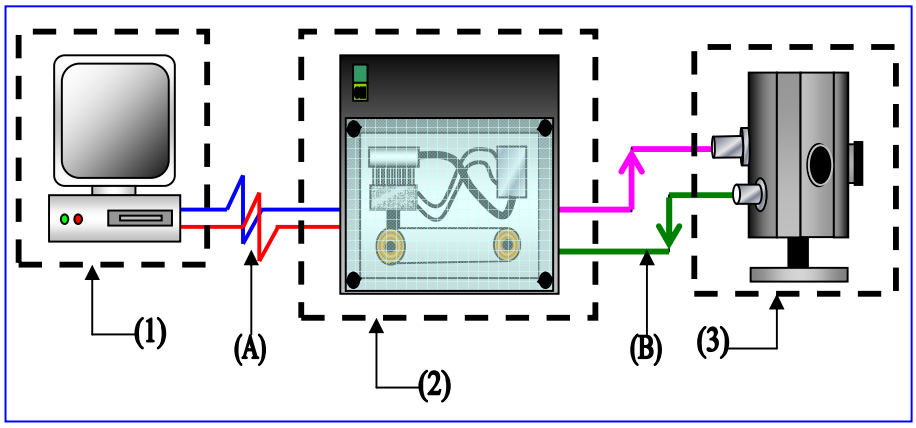
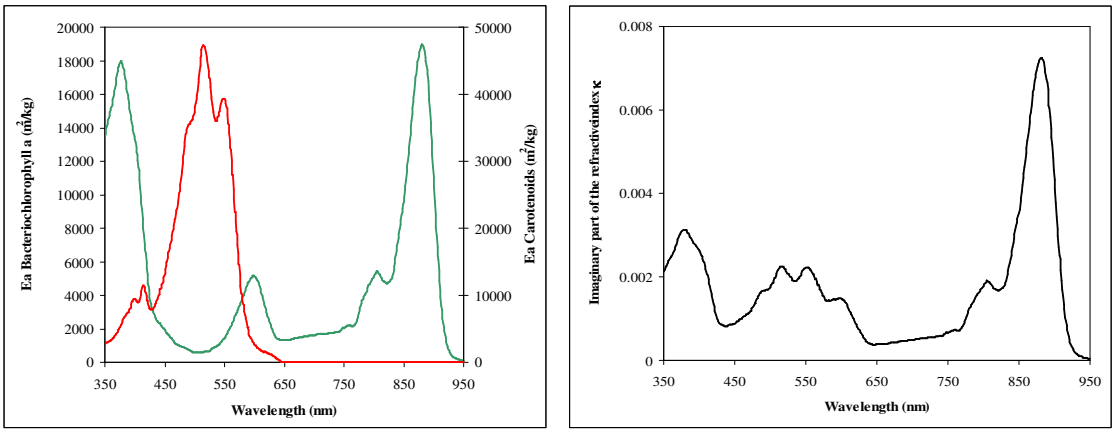


Figure 1: Scheme of the optical bench with a 6 inches integrating sphere of high accuracy: (1) Software interface with computer; (A) RS 232 link; (2) Spectrofluorimeter SAFAS Flx; (B) Optical fibres; (3) Integrating sphere Labsphere® (6 inches).

3. Validation of Optical and Radiative Properties for *Rhodospirillum rubrum* and *Arthrospira platensis*

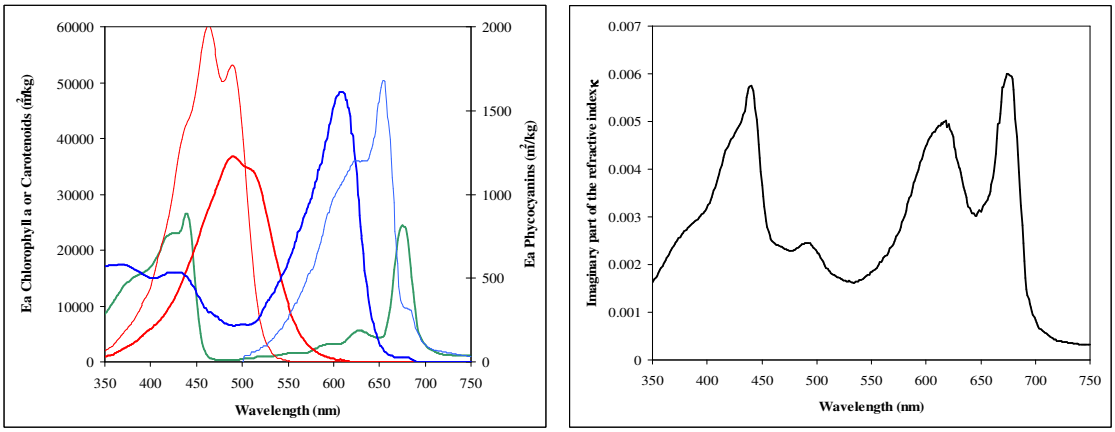
3.1. Calculation of the Imaginary Part of the Refractive Index

The two following Figures (Figure 2 for *Rs. rubrum* and Figure 3 for *A. platensis*) show the calculation of the wavelength dependent imaginary part of the refractive index κ_λ (part *b* of the Figure) obtained from a convolution of the *in vivo* absorption coefficients of each individual pigment (part *a* of the Figure), as given by the equation (8) in TN 83.2.
The data bank of individual *in vivo* spectral absorption coefficients available in LGCB has been built partly from data of the literature (for the main pigments as chlorophylls, phycocyanins and carotenoids used here for *A. platensis* – see Bidigare *et a*l., 1990) and partly from our own data obtained after extraction, separation and *in vivo* corrections (as for the bacteriochlorophyll *a* and special carotenoids of *Rs. rubrum*).



(a) **(b)**

Figure 2: Calculation of the imaginary part of the refractive index by convolution κ_λ **(b)** for *Rhodospirillum rubrum* from the *in vivo* absorption coefficients of pure pigments **(a)**. The mass contents used are respectively 1.75% for Bacteriochlorophyll *a* (green line), and 0.35% for carotenoids (spirilloxanthin and rhodovibrin, red line).



(a) **(b)**

Figure 3: Calculation of the imaginary part of the refractive index by convolution κ_λ **(b)** for *Arthrospira platensis* from the *in vivo* absorption coefficients of pure pigments **(a)**. The mass contents used are respectively 1.2% for Chlorophyll *a* (green line), 10.5% for phycocyanin (thick blue line), 7% for allophycocyanin (thin blue line) and 0.35% for carotenoids with respectively 0.20% for photosynthetic carotenoids (thick red line) and 0.15% for protecting carotenoids (thin red line).

As it can be seen, if a data bank of a wide variety of pure pigments properties is available, it becomes very easy to calculate this important optical intrinsic property of any micro-organism, and for any given pigment content in the cells (in this work, the respective percentages indicated in Figures 2 and 3 correspond, as indicated, to the subculture cultivation conditions, i.e. neglecting the EPS and PHB contents, but could be modified in case of different conditions in PBR).

3.2. Experimental Validation of Radiative Properties from True Transmittance Measurements

The following part of this technical note aims to demonstrate that the theoretical approaches proposed and discussed in the previous TN 83.2 are well suited in calculating the radiative properties of micro-organisms considered as weakly absorbing particles of non-spherical shape. This involves being able to know the size distribution of any micro-organism in given culture conditions, to calculate the spectral refractive indexes (optical properties, see above), and to compute for any size parameter the Lorenz-Mie problem (TN 83.2, Cornet *et al*., 2006) leading to the predictive values of the spectral absorption and scattering coefficients and of the phase function. As explained in introduction, because the integrating sphere photometer of the lab does not permit to operate in single scattering condition in measuring true transmittances (lack of accuracy for transmittances near unity) and then to have a direct experimental validation, we decided to work on concentrated suspensions of micro-organisms, in multiple scattering conditions. In this case, the true experimental transmittance measurement results in a coupling between the intrinsic radiative properties of the particles and the radiative transfer problem in the suspension. This approach, which requires calculating the radiation field in one-dimensional conditions with a collimated incidence, may be considered as a rigorous validation of the radiative properties themselves, only if a quasi-exact method was used to solve the RTE. For this reason, we used only the highest order d-S_{96} differential ordinates method with an exact phase function matrix as developed in TN 83.2 for this validation. The proof that this method provides rigorous solutions for the RTE in all cases has been indeed established elsewhere (Cornet, 2007), for different cases of particles having known radiative properties. As a consequence, obtaining a good agreement between experimental transmittances (with an integrating sphere) and the theoretical predictive calculated values is indeed a validation of the method in calculating the radiative properties.

3.2.1. *Rhodospirillum rubrum*

The experimental true transmittances were measured for *Rs. rubrum* first, using the integrating sphere photometer as described in the materials and methods part (2.3), for two different samples (respective concentrations of 0.41 and 1.23 g/L), and in the largest authorized domain of wavelengths (350 – 850 nm) characterising the PAR for this micro-

organism (the lack of energy of the xenon lamp and the high absorption of *Rs. rubrum* in the domain 850-950 nm limited the domain of validation). The results are depicted on Figure 4 in which we have added the theoretical calculations for the main characteristic wavelengths. On a transmission spectrum indeed, the peaks correspond to mainly scattered wavelengths and the valleys to mainly absorbed one. The limited number of wavelength used for this validation results in the very high computation time needed in obtaining the angular properties (phase function) of the particles for a method of high order (this limiting problem in relation with the computation power of the lab will be again pointed out in the following of the discussion). Nevertheless, as it can be seen on Figure 4, these wavelengths have been chosen to be representative of the two main phenomena (absorption dominant or scattering dominant).

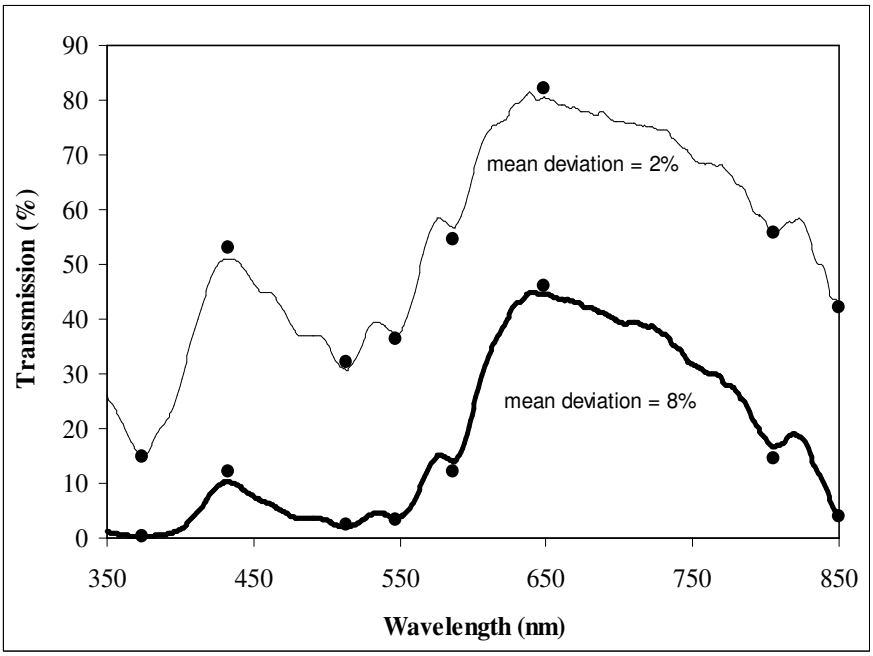


Figure 4: Comparison between experimental transmissions obtained with the integrating sphere for *Rhodospirillum rubrum* ATCC 25903 suspensions (continuous line) and theoretical transmissions calculated from the predictive optical and radiative properties (dots) obtained as explained in TN 83.2 (eqs. 1, 8, 45-50) and using the d-S_{96} differential ordinates method. Two samples are represented: a dilute sample (thin line) with a biomass concentration of 0.41 g/L (optical thickness τ_L roughly 8), and a more concentrated sample (thick line) with a biomass concentration of 1.23 g/L (optical thickness τ_L roughly 25).

One can observe that in all cases (even near the limit of accuracy of the optical bench, with a high optical thickness of 25) the results are in very good agreement taking into account the high sensitivity of the radiative properties in the photon transport phenomenon, validating as well these properties for the particular values and likely over the entire spectrum.

3.2.2. *Arthrospira platensis*

In the same manner as previously depicted, the true transmittances were experimentally measured for two different suspensions of *A. platensis* in the domain 350-750 nm. The maximum concentration used in this case was lower than that of *Rs. rubrum* just because of the availability of the subcultures at a given time (respectively 0.2 and 0.6 g/L). Nevertheless, it should be mentioned that a comparison between different micro-organisms only on their biomass concentration wouldn't have any physical basis and should rather involve mainly the radiative properties.

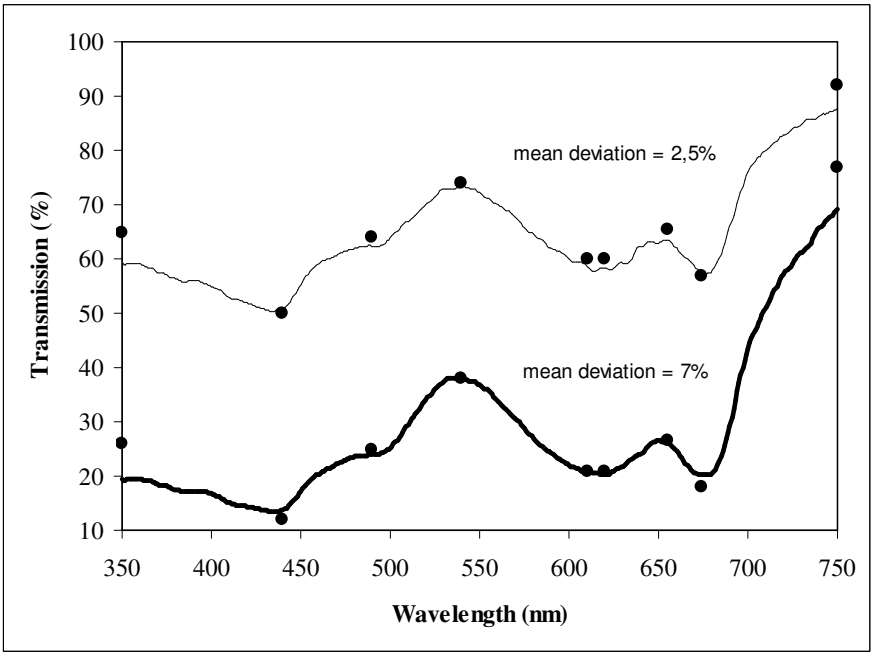


Figure 5: Comparison between experimental transmissions obtained with the integrating sphere for *Arthrospira platensis* PCC 8005 suspensions (continuous line) and theoretical transmissions calculated from the predictive optical and radiative properties (dots) obtained as explained in TN 83.2 (eqs. 1, 8, 45-50) and using the d-S_{96} differential ordinates method. Two samples are represented: a dilute sample (thin line) with a biomass concentration of 0.20 g/L (optical thickness τ_L roughly 1.5), and a more concentrated sample (thick line) with a biomass concentration of 0.60 g/L (optical thickness τ_L roughly 5).

As it can be seen on Figure 5, the results comparing the experimental transmittances and the theoretical calculations for characteristic wavelengths are also in very good agreement, except for the extreme values of the spectrum at 350 and 750 nm, validating again the theoretical approach of the previous TN 83.2. These small differences come probably from the values of the pure *in vivo* absorption coefficients for chlorophyll *a* at these wavelengths which have been established from eukaryotic microalgae data (Bidigare *et al*., 1990) and which could then present some minor differences with those of cyanobacteria at the boundaries of the absorption domain. This is not really a problem first because if necessary, the considered data bank can be modified accordingly and second because we decided to work for the future in the PAR limited to 400-700 nm (see TN 83.2, Cornet *et al*., 2006).

4. Comparison between Different Assumptions Using Radiative Properties and Solving the Radiative Transfer Equation

After the validation of the theoretical approach described in the companion TN 83.2 (2006) for the calculation of the optical and radiative properties of photosynthetic micro-organisms, it was then interesting to compare the respective accuracy of the different assumptions and simplifications which can be made in the description of the radiative transfer in PBR. For sake of convenience, this comparison will be limited to the very practical case of one-dimensional geometry, specially the rectangular geometry, in order to compare the calculations to experimental measurements using the integrating sphere photometer. As explained in TN 83.2 (Cornet *et al*., 2006), it is possible to formulate simplifying assumptions at two levels. Considering first the radiative properties as in the previous part of this TN, it is easy to verify in the literature that most authors being not able to calculate these properties for exact shapes of particles used the (very) simplifying assumption of the equivalent sphere (in surface or volume) for their assessment. Second, for given radiative properties, it is possible to use different assumptions in solving the RTE itself, for example decreasing the order of the quadrature to have shorter calculation times and finally, to use only order two (two-flux method) leading to practical analytical solutions. It must be noticed here that this comparison has already been partly done from the theoretical point of view in TN 83.2 (part 3).

In the following, for six characteristic wavelengths of each considered micro-organism, we compared in Tables 1 and 2 the results of different assumptions with experimental transmittances measured with the integrating sphere photometer of the lab. In the first part of the Table, we used exact radiative properties (the cylinder was used as model of shape) as validated in part 3 of this TN and we compared the results with the quasi-exact solution given by the highest order d-S_{96} method with the lower order (considered as a limit in the specialised literature and used in TN 83.2) d-S_{32} method, and then finally with

the simple two-flux method. In the second part of the Table, we used radiative properties approached by the equivalent sphere (in volume) method as in the literature. Because in this case, the order of the quadrature as been proved without any effect on the result from order higher 32 (Cornet, 2007), we just compared the experimental results with the d-S_{32} method and the analytical two-flux method.

Wavelength (nm)	374	433	513	649	805	850
Experimental Transmission	0.003	0.102	0.021	0.447	0.166	0.040
Exact Radiative Properties						
Albedo ϖ_λ	0.8566	0.9413	0.9021	0.9687	0.8941	0.8102
Optical thickness $\tau_{L,\lambda}$	34.47	25.78	27.76	17.68	12.90	12.65
Backscattered fraction $b_{2,\lambda}$	0.0064	0.0070	0.0080	0.0098	0.0116	0.0123
d-S_{96}	0.003 (-4%)	0.115 (+12%)	0.022 (+5%)	0.458 (+2%)	0.161 (-3%)	0.040 (0%)
d-S_{32}	0.003 (+4%)	0.130 (+27%)	0.024 (+14%)	0.487 (+9%)	0.146 (-12%)	0.032 (-20%)
Two-Flux (S_2)	0.006 (×2,2)	0.184 (+80%)	0.051 (×2,4)	0.603 (+35%)	0.220 (+32%)	0.080 (×2)
Radiative Properties from Equivalent Spheres						
Albedo ϖ_λ	0.9021	0.9631	0.9118	0.9717	0.9003	0.8218
Optical thickness $\tau_{L,\lambda}$	53.37	42.05	32.37	20.62	14.48	14.02
Backscattered fraction $b_{2,\lambda}$	0.0004	0.00055	0.0007	0.0011	0.0017	0.0018
d-$S_{N>32}$	0.003 (+4%)	0.153 (+50%)	0.035 (+67%)	0.467 (+5%)	0.174 (+5%)	0.053 (+33%)
Two-Flux (S_2)	0.005 (×2)	0.207 (×2)	0.056 (×2,7)	0.546 (+22%)	0.231 (+39%)	0.080 (×2)

Table 1: Comparison (for some characteristic wavelengths) between different assumptions to solve theoretically the one-dimensional RTE and the experimental transmission values obtained by mean of an integrating sphere for a suspension of *Rhodospirillum rubrum* ATCC 25903 at 1.24 g/L (i.e. N_p = 1.1 10^{15} m^{-3}). All the calculations are based on a log-normal (σ = 1.14) size distribution law and the results are given using the exact radiative properties for micro-organism [cylinder with asphericity ratio A_R = 3.802], or using a volume equivalent sphere approximation.

Considering first the method using the quasi-exact values for the radiative properties, as it can be seen on Table 1 for *Rs. rubrum*, and on Table 2 for *A. platensis*, and as foreseen in regard of our previous analysis, the high order d-S_{96} numerical method gives quasi-exact results (a mean deviation of 3% for *A. platensis* and 5% for *Rs. rubrum*, but with higher optical thickness). In comparison, the lower order d-S_{32} numerical method gives also (less) accurate results with a mean deviation respectively of 12 and 15% and could be

used in most of the practical cases in which saving computation time is important. At the opposite, the very simple analytical two-flux approximations appears as a correct assumption for low optical thicknesses (around 5) with *A. platensis* (Table 2, best than 20%), but becomes of poor quality at higher optical thicknesses (i.e. between 10 and 30 for *Rs. rubrum*, Table 1) with deviations reaching between 30 to 150%. It is then clear that the use of this method should be restricted to practical cases with low optical thicknesses, or to situations in which the accuracy of the light transfer description was not too critical, as for model based predictive control. Conversely, it would not correct to use this method to predict the appearance of bifurcations and the operating engineering domain in case of the CII continuous functioning, as it has been done in curvilinear coordinates in the pioneer work of L. Favier-Teodorescu (TN 55.1, 2005).

Wavelength (nm)	**440**	**490**	**540**	**620**	**655**	**674**
Experimental Transmission	**0.137**	**0.240**	**0.380**	**0.202**	**0.261**	**0.203**
Exact Radiative Properties						
Albedo ϖ_λ	0.6630	0.7780	0.8296	0.7301	0.8110	0.7385
Optical thickness $\tau_{L,\lambda}$	5.235	4.820	4.320	5.002	5.386	5.445
Backscattered fraction $b_{2,\lambda}$	0.0491	0.0341	0.0286	0.0331	0.0288	0.0327
d-S_{96}	**0.140** (+2%)	**0.251** (+5%)	**0.384** (+1%)	**0.201** (-1%)	**0.269** (+3%)	**0.193** (-5%)
d-S_{32}	**0.117** (-14%)	**0.288** (+20%)	**0.412** (+8%)	**0.210** (+3%)	**0.296** (+13%)	**0.175** (-14%)
Two-Flux (S_2)	**0.145** (+6%)	**0.304** (+27%)	**0.433** (+14%)	**0.230** (+13%)	**0.320** (+23%)	**0.213** (+5%)
Radiative Properties from Equivalent Spheres						
Albedo ϖ_λ	0.5225	0.6036	0.6826	0.5552	0.6119	0.5476
Optical thickness $\tau_{L,\lambda}$	1.998	1.998	2.004	2.010	2.010	2.016
Backscattered fraction $b_{2,\lambda}$	0.0012	0.0019	0.0031	0.0016	0.0024	0.0015
d-$S_{N>32}$	**0.385** (×2,8)	**0.452** (+88%)	**0.528** (+39%)	**0.408** (×2)	**0.458** (+75%)	**0.401** (+97%)
Two-Flux (S_2)	**0.385** (×2,8)	**0.452** (+88%)	**0.528** (+39%)	**0.408** (×2)	**0.458** (+75%)	**0.401** (+97%)

Table 2: Comparison (for some characteristic wavelengths) between different assumptions to solve theoretically the one-dimensional RTE and the experimental transmission values obtained by mean of an integrating sphere for a suspension of *Arthrospira platensis* PCC 8005 at 0.60 g/L (i.e. N_p = 1.3 10^{11} m^{-3}). All the calculations are based on a log-normal (σ = 1.11) size distribution law and the results are given using the exact radiative properties for micro-organism [cylinder with asphericity ratio A_R = 35.71], or using a volume equivalent sphere approximation.

Comparing now briefly the experimental results with the approximation of radiative properties by equivalent spheres shows surprising results comparing *Rs rubrum* at high optical thickness (Table 1) and *A. platensis* at lower optical thickness (Table 2). These results can be explained easily examining other shapes of micro-organisms than rods or cylinders as for example Tchebytchev particles (Cornet, 2007) very close to spheres. This more general discussion can be found in the work of Cornet (2007), but the main conclusions can be summarized here. In fact, the approximation in calculating the radiative properties with equivalent spheres modifies three main and critical quantities for the light transfer problem which are (as indicated in Tables 1 and 2) the optical thickness, the albedo of single scattering and the phase function (or the resulting integral via the back-scattered fraction b_2). We have then demonstrated (Cornet, 2007) that this approximation gives always bad results in comparison to actual values, but the deviation depends if the effects on the three preceding variables are conjugated or opposite. From different shapes of photosynthetic micro-organisms, we observed that these effects were absolutely not predicable, even from a rational analysis. For example, *Chlamydomonas reinhardtii* which is a quasi spherical particle (rigorously a Tchebytchev particle with only 10% deviation from a sphere) gives the worst results using equivalent sphere approximations (deviations up to a factor 10 for transmittances at optical thickness of 25 – see Cornet, 2007). Surprisingly, but in a random way, the results are better with *Rs. rubrum* which is a rod of small size parameter and elongation (around 50% of mean deviation for the d-S_{32} method, Table 1) than for *A. platensis* with a higher size and elongation (roughly a factor 2 for the same method, Table 2). This is because in the case of *Rs. rubrum*, the increase in optical thickness is annihilated by the decrease in the back-scattered fraction, whereas for *A. platensis*, the decrease in optical thickness was not compensated for by the increase of the backscattered fraction. In all cases, the two-flux method linked to equivalent sphere approximation gives very bad results (a factor 2 in Table 1 and 2) but revealed as the same quality than the d-S_{32} method in case of a big size parameter for cylinders (*A. platensis*, Table 2) leading to a phase function strongly peaked in the forward direction.

As a conclusion of this work and of the more general work of Cornet (2007), one can conclude that it is really necessary to link a predictive method using quasi-exact shape of micro-organisms together with a high order numerical method if it is desired to have a good description of the light transfer problem in PBR (except for very low optical thicknesses). Moreover, it appeared from these comparisons, that the use of exact radiative properties is a more accurate choice than the method in solving the RTE itself. In the same idea, it appeared that, using rigorous radiative properties, the simple analytical two-flux method can give correct results with optical thicknesses of maximum 5 (in the case of quasi-collimated incidence particularly convenient with the phase function of micro-organisms). These conclusions on particular transmittance measurements confirmed the theoretical plots already discussed in the companion TN 83.2 (Figures 4 and 5) for complete light profiles in a PBR.

5. Radiative Properties for *Rhodospirillum rubrum* and *Arthrospira platensis* in General Culture Conditions

As explained in part 4 of the companion TN 83.2 (Cornet *et al*., 2006) and as verified in the experimental results above, the optical and radiative properties of any photosynthetic micro-organism are spectral properties, requiring rigorously to solve the RTE for each considered wavelength in the PAR to obtain, after averaging, the LVREA with a high accuracy as the key quantity to formulate the coupling with kinetic rates and stoichiometry (Cornet *et al*., 2003). Nevertheless, this rigorous treatment strongly impairs the calculation time and in some situations, as for example in case of model based predictive control, it can be necessary to have a very fast estimate of the output of the process, leading to work with first averaged radiative properties by different methods which are also explained in TN 83.2 (a rigorous spectral method requiring to know all the radiative properties for any wavelength – eq. 75 -, and a simplified method using mean optical properties – eq. 77 -).
Additionally, we have already mentioned in the first part of this TN that the radiative properties of any micro-organism depend mainly of the size distribution and of the intracellular composition as the pigment content and storage compound content (EPS, PHB,…). Clearly, if these parameters can be influenced by the culture conditions, and especially by the radiation field itself, requires formulating in the model the non-linear coupling between light transfer, kinetic rates and intracellular contents (stoichiometry). Basically speaking, at the first stage of calculating radiative properties of cells for different biomass compositions requires to develop an extensive bank of data for these properties in a wide domain of operating conditions (incident light flux, stirring, lightening fraction…), enabling then to obtain faster estimate of the considered value by interpolation. The constitution of this data bank (which could be spectral considering the previous remark of this paragraph) is indeed absolutely necessary in the future for *Rs. rubrum* and *A. platensis* regarding the critical calculation times necessary if one works with the exact shape of the micro-organisms (see Table 4 for examples) as demonstrated.

5.1. Results and Limits for the Conditions Involved in this Study

Clearly, the aim of this study was to validate the theoretical tools developed in TN 83.2 (Cornet *et al*., 2006) in calculating rigorously optical and radiative properties and then their coupling in the RTE which can be solved in different systems of coordinates and with different simplifying assumptions. For this, as previously discussed, it was just necessary to compare experimental true transmittances for **only some characteristic wavelengths** with theoretical calculation based on cell characteristics (size, composition, and pigment content) obtained **in particular culture conditions**, i.e. those of **subcultures** in Erlenmeyer flasks for this work (see materials and methods part for details). As a consequence and because we know that the biomass composition can be

strongly influenced by the culture conditions and mainly the radiation field, it is only possible to use the radiative properties obtained in this work for a limited range of operating conditions, very close to those of subcultures, i.e. gentle mixing and low incident light flux inputs (this last case corresponding to the active biomass without EPS for *Arthrospira* and without PHB for *Rhodospirillum*). Moreover, in our previous results, the calculations were done only for a maximum of ten chosen wavelengths, which is not sufficient to average them in the aim to obtain mean spectral radiative properties.

	Rhodospirillum rubrum **ATCC 25903** **(without Polyhydroxybutyrate)**	***Arthrospira platensis*** **PCC 8005** **(without Exopolysaccharide)**
Kind of micro-organism	Non-sulphur purple photosynthetic bacteria	Cyanobacteria
Pigments content	Bacteriochlorophyll : 1.75% Carotenoids : 0.35%	Chlorophyll a : 1.2% Phycocyanin : 10.5% Allophycocyanin : 7% Carotenoids : 0.35%
Model of shape	Cylinder Asphericity ratio $A_R = 3.802$	Cylinder Asphericity ratio $A_R = 35.71$
Characteristic size *a* (µm)	0.55	4.19
Mean equivalent radius $\bar{r}$ (µm)	0.983	15.52
Log-normal size distribution σ	1.14	1.11
PAR and λ_{mean} (nm)	[400-900] (650)	[400-700] (550)
Correction factor Q^* (dimensionless) (eq. 10 in TN 83.2)	0.99	0.93
Imaginary part of the refractive index κ (dimensionless) (eqs. 8, 9 and 11 in TN 83.2)	0.001738	0.002643
Real part of the refractive index *n* (dimensionless)	1.386	1.460
Mass absorption coefficient *Ea* ($m^2.kg^{-1}$)	122	162
Mass scattering coefficient *Es* ($m^2.kg^{-1}$)	1345	636
Albedo ϖ (dimensionless)	0.9166	0.7973
Linear scattering modulus α (dimensionless) (eq. 63 in TN 83.2)	0.900	0.900
Backscattered fraction b_2 (dimensionless) (eqs. 54-55 in TN 83.2)	0.0107	0.030
Asymmetry factor *g* (dimensionless)	0.9298	0.8282

Table 3: Summary of the main size characteristics for the two considered micro-organisms with the mean resulting optical and radiative properties. These results correspond to a given pigment content obtained with the subculture conditions (low incident light fluxes) for the two micro-organisms.

5.1.1.Mean Radiative Properties

As explained in TN 83.2 (Cornet *et al*., 2006), the use of mean radiative properties in solving the RTE can save an important computation time with a reasonable loss in accuracy. Rigorously, they should be obtained by averaging 300 (or 500 for *Rs. rubrum*) spectral properties in the PAR (for each nanometre), leading to a tedious computational work (which cannot be done with the current computing equipment of our lab). Nevertheless, it was also explained in part 1.1.3 of this TN that a quite good assumption is possible by working directly with the mean optical properties (i.e. mainly with a mean imaginary part of the refractive index κ) and the method of calculation was explained (eqs. 9-11 in TN 83.2). As an example, this method was applied here for the two considered micro-organisms, in case of the limited domain of application as already explained, and all the radiative properties necessary to solve the RTE for any given assumption were then calculated. These results, together with the basic input data as size distribution and pigment contents, are summarized on Table 3 showing what it is necessary to compute (except the matrices **P** which cannot be presented here) if it is desired to work directly with mean optical and radiative properties for any given culture condition.

5.1.2.Spectral Radiative Properties

If the more rigorous spectral approach is preferred in solving the RTE, it is necessary to compute the same Table (with the phase function and the matrices **P**) for as much as values required, depending of the chosen spectral accuracy (1, 2, 5, or 10 nanometres) in the PAR and for our restricted culture conditions (with a constant pigment content). This would require clearly an important computing power regarding the calculation times for non-spherical particles (see Table 4).

5.2. More Generally Encountered Conditions

As briefly explained above, it will be necessary to take into account the effects of the culture conditions (with first the physical limitation by light) on the biomass composition changes and then on optical and radiative properties changes. The Table 4 gives an example of the required computation times (with the faster computer of the lab) giving the main radiative properties for one wavelength, in comparison with the calculation time for spherical particles. The results show that this time is very important, increases strongly with increasing the size parameter of the micro-organism, and mainly taking into account the size distribution on angular properties (the phase function and derived quantities). Clearly, it is not possible (even with a higher computing power) to envisage a computation of these properties directly in the numerical sequence of the kinetic model. For this reason, we have proposed above to build a data bank of radiative properties,

taking as variables the main process parameters, in which any property (mean or spectral) could be obtained by interpolation with a sufficient accuracy.

	Calculation of the phase function and of scattering matrix P (96×96)		**Calculation of absorption and scattering coefficients with size distribution**
	With size distribution	**Without size distribution**	
***Saccharomyces* ($x \cong 40$) (sphere)**	30 seconds	500 milliseconds	5 milliseconds
***Rhodospirillum* ($x \cong 7$) (cylinder with asphericity ratio $A_r \cong 4$)**	6 days	1 hour	7 minutes
***Arthrospira* ($x \cong 60$) (cylinder with asphericity ratio $A_r \cong 36$)**	60 days	6 hours	3 hours

Table 4: Comparison of the computation times in the calculations of radiative properties of spherical micro-organisms (like *Saccharomyces*) and other non-spherical shapes (like *Rs. rubrum* and *A. platensis* considered as cylinders) for only one wavelength. These results were obtained with optimised compiled Matlab® routines on the faster computer of the lab (bi-processor Xeon 2.8 GHz).

As a detailed proposal, it is necessary to treat separately the cases of *A. platensis* and *Rs. rubrum*. In any way, it should be decided in the MELiSSA team if we want to build only a data bank of mean radiative properties in the PAR or a spectral data bank. For this last case indeed, the computing work could become considerable taking into account the 300 (or 500) considered wavelengths, except if the wavelength interval is increased to 5 or 10 nanometres (for example). In all cases, these computations could be performed at ESA, on a computer being one or two orders of magnitude faster than the equipment of LGCB. In addition of the basic shape and size information, this bank should contain at least the optical properties, the mass absorption and scattering coefficients, ten thousands of angular values for the phase function, the averaged back-scattered fraction b_2 and the matrices P(N,N) for the d-S_N methods (with N = 32 or 96).

5.2.1. *Arthrospira platensis*

Except in case of mineral limitations, the process parameters affecting mainly the basic characteristics of the *Arthrospira* cells are the mixing conditions and the incident light flux onto the PBR (which controls the radiation field for a given biomass concentration).

The **mixing conditions** (mainly mechanical mixing with turbines) modify indeed the mean length of the filaments and then the size distribution. It must be noticed that this factor does not affect the more sensitive size parameter related to the radius of the cylinder. Clearly, the more important changes are obtained in varying the **incident light flux**, as it is well established that a higher light flux leads to an increase in EPS content and in a lesser extent to a decrease in the relative phycocyanin content (PS II) in order to equilibrate the imposed ratio $P/2e^-$. The mixing conditions and the mean incident light flux should then be considered as the two main variables in building the data bank of radiative properties. For this last variable, a predictive kinetic and stoichiometric model has been established (Cornet, 2007) applying the thermodynamics of irreversible processes on the photosynthetic apparatus, enabling then to calculate all the physiological modifications linked to the $P/2e^-$ changes imposed by physical light limitation. Fortunately, one can consider that important changes in EPS content with light flux may have only reduced effect on the radiative properties of *A. platensis* because this corresponds to a case of coated particle, but with a refractive index of the EPS likely very close to the mean refractive index of the active biomass. In these conditions, the main effect could be linked only to a slight increase of the mean size parameter of the cells. Finally, cases where the typical concentrations of the culture medium (i.e. Zarrouk medium) for *Arthrospira* would be modified, led to a third important variable because it is also well-known that the **ionic strength** strongly influences the pigment content of photosystem II (Cornet, 1992).

5.2.2. *Rhodospirillum rubrum*

At the opposite of the previous micro-organism, we have always considered that the size distribution of *Rs. rubrum* was independent of the mixing conditions and there is no reason today to reject this assumption. Unfortunately, the behaviour of the cells in response to an imposed $P/2e^-$ ratio by light limitation is more complicated than that of *Arthrospira* (Favier-Teodorescu *et al.*, 2003, TN 49.2). First, the effect of the **incident light flux** is known to increase the intracellular content in PHB (Favier-Teodorescu *et al.*, 2003 - TN 49.2 and Cornet *et al.*, 2005 - TN 55.1), perhaps associated to an increase in the size parameter of the cells. These PHB granules, considered as inclusions and having a refractive index (between 1.45 and 1.50) higher than that of the cells (considered as a whole) is supposed to strongly modify the radiative properties of *Rs. rubrum* (eq. 12 of TN 83.2). Second, we also observed that the pigment content varied dramatically (a factor two) with the radiation field inside the reactor. Until now, we consider as a semi-predictive model that this pigment content could be related both to the incident light flux and to the **lightening illuminated fraction inside the reactor** γ (Cornet *et al.*, 2003; Cornet *et al.*, 2005 - TN 55.1). Clearly, these two main variables should be considered in building the data base for *Rs. rubrum* with a strong influence on the radiative properties and then to the kinetic behaviour of the PBR (performances, bifurcations, stability domain…).

6. Conclusions and Perspectives

This Technical Note was devoted to the experimental validations of the theoretical methods developed in a companion TN 83.2 (Cornet *et al.*, 2006) enabling to theoretically calculate, from very basic information (shape, size distribution and pigment content) both the optical and radiative properties of a given micro-organism, and then to use them in quasi-exact numerical methods (or less accurate assumptions) in solving the RTE in rectangular and curvilinear one-dimensional coordinates.

In a first part, using a high accuracy optical bench with an integrating sphere and operating in rectangular coordinates, we obtained experimental true transmittances for samples of *A. platensis* and *Rs. rubrum* suspensions of different concentrations. Using then the highest order method (d-S_{96}) considered as an exact method to treat these data proved an excellent agreement with experimental results validating the proposed method and the data bank of *in vivo* absorption spectra for pure pigments used.

In a second part, these experimental results enable to discuss the validity of different assumptions, either for the order of the numerical method used in solving the RTE (d-S_{96}, d-S_{32}, d-S_2) or for the approximation of actual shape by an equivalent sphere. This last method always used in the literature has been proved to give generally catastrophic results whereas the simple analytical two-flux method using true radiative properties revealed correct results for optical thicknesses lower than 5. These important conclusions regarding the accuracy in the radiation field calculation in PBR are available for the first time in the MELiSSA team (and to our knowledge, in the international community). They authorise to choose *a priori* the more suitable assumption depending of the final given application as for example the use of simple analytical two-flux method for control laws (saving the computation time) or the use of d-S_{32} numerical method for the stability analysis of *Rs. rubrum* continuous cultures.
In any way, as already mentioned in conclusions of TN 83.2, we can consider now that the predictive character and the robustness that we have reached in the knowledge model describing the radiation field inside PBR of any given geometry, corresponds to the last possible level of sophistication that we need in the MELiSSA project. It should serve as a basic knowledge in the future as long as simulations, scale-up procedures, control laws or new designs (even requiring a new photosynthetic micro-organism) are concerned.

Finally, because it cannot be envisaged to integrate the radiative properties calculations in the whole numerical kinetic and stoichiometric model (taking into account important changes in the biomass composition) regarding the very important computing times needed, we have proposed to develop a data base for these properties. It should be built taking into account the main process variables which are known to affect the biomass

composition or characteristic size as mixing, incident light flux, lightening illuminated fraction and ionic strength, with a discretisation sufficiently closed to authorise a faster estimate by interpolation. If this work is envisaged in the future, we will have to decide if we construct a base with only averaged wavelength radiative properties, or a spectral base. This latter case indeed, involving to multiply the number of calculations by 30 to 300 times for *A. platensis*, and 50 to 500 times for *Rs. rubrum*, but increasing the accuracy in the LVREA calculation inside the PBR corresponds to a considerable amount of computational work. In any case, considering the reduced computational possibilities of LGCB, a solution using a vectorial supercomputer would be sought and negotiated with ESA.

NOTATIONS

Roman Letters

a	Minor semi-axis of the particle (radius for a sphere) [m]
$A_R = b/a$	Asphericity ratio [0]
b_2	Backward scattered fraction [0]
Ea_λ, Ea	Mass absorption coefficient (spectral or mean) [$m^2.kg^{-1}$]
Es_λ, Es	Mass scattering coefficient (spectral or mean) [$m^2.kg^{-1}$]
g	Asymmetry factor [0]
n_λ, n	Real part of the refractive index (spectral or mean) [0]
n_r	Relative real refractive index [0]
n_m	Real refractive index for the surrounding medium [0]
$n(r, x)$	Size distribution law [0]
N	Quadrature order [0]
N_p	Volume density of particles [m^{-3}]
$\mathbf{P} = P(N,N)$	Matrix of the phase function for d-S_N method of order N [0]
$q_\cap$	Hemispherical incident light flux [$W.m^{-2}$]
$Q^\bullet$	Mean correction factor [0]
r	Radius [m]
$\bar{r}$	Mean radius [m]
x, x_a	Size parameter = $2 \pi a n_m / \lambda$ [0]

Greek Letters

α_λ	Linear scattering modulus [0]
γ	Lightening illuminated volume fraction inside a PBR [0]
κ_λ, κ	Imaginary part of the refractive index (spectral or mean) [0]
λ	Wavelength [m]
σ	Standard deviation for the log-normal law [m]
τ_L	Optical thickness [0]
ϖ	Albedo of single scattering [0]

Abbreviations

d-S_N	Differential discrete ordinate method of order N
EPS	Exopolysaccharide
LVREA	Local volumetric rate of radiant energy absorbed
MOPS	3-(N-Morpholino)-propanesulfonic acid
PAR	Photosynthetically active radiation
PBR	Photobioreactor
PHB	Polyhydroxybutyrate
PS II	Photosystem II
RTE	Radiative transfer equation

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