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Kinetic Modeling of *Rhodospirillum rubrum* Growth in Rectangular Photobioreactors

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Technical Note 45.1

Kinetic Modeling of *Rhodospirillum rubrum* Growth in Rectangular Photobioreactors

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Content

Introduction and Objectives

For the mathematical modeling of photobioreactors (PBRs) it is necessary to understand and formulate the coupling between the metabolism of micro-organisms and the physical phenomenon of light transfer inside the culture medium. PBRs are governed by radiant light energy availability, which is highly heterogeneous within the culture volume (Aiba 1982; Cornet *et al*., 1992; Cassano *et al*., 1995; Acien Fernandez *et al*., 1997). This spatial heterogeneity causes varying local reaction rates, which makes it necessary to derive local equations and calculate the mean volumetric growth rate by integration over the working illuminated volume in the reactor (Cornet *et al*., 1992; Cornet *et al*., 1995; Cornet *et al*., 1998).

Whatever the level of complexity chosen to describe the radiant light energy transfer in the culture medium, the resulting model presents a degree of generality, when applied to different photosynthetic micro-organisms, because it is only necessary to determine the light absorption and scattering coefficients for each species. This is not the case for the problem of coupling radiant light energy transfer and growth kinetics because of the high diversity in the metabolism of photosynthetic micro-organisms (algae, cyanobacteria, purple bacteria, etc.). The greatest differences are between photo-autotrophic and photo-heterotrophic metabolisms.

The authors have already proposed a simple monodimensional mathematical model for describing light transfer in PBRs with different shapes (Cornet *et al*., 1995); the coupling with growth kinetics for the photo-autotrophic cyanobacterium *Spirulina platensis* were formulated and validated in batch and continuous cultures (Cornet *et al*., 1992; Cornet *et al*., 1995; Cornet *et al*., 1998).

This technical note shows how this approach can be extended to the photo-heterotrophic purple bacterium *Rhodospirillum rubrum*. Here, two problems are addressed:

(i) The experimental determination of the mass absorption and scattering coefficients *Ea*, *Es*, for unicellular and small-size micro-organisms, in the range [350-950 nm].

(ii) The formulation of coupling between light transfer and growth kinetics for photo-heterotrophic micro-organisms.

Five carbon sources, which are the most representative of the output of the liquefying compartment, were used (acetate, propionate, butyrate, isovalerate, isobutyrate) to determine the effects of different substrates on growth kinetics.

This work relies on the experimental tests performed at UAB and reported in TN 25.7 (Albiol and Godia, 1996) and in TNs 37.81-82 (Lenguaza *et al*., 1998*a* and *b*).

1- Model Developments

1.1- Radiant light transfer model

To account independently for light scattering by whole cells, and light absorption by pigments, it has been established that the simplest model for radiant light transfer in a culture medium derives from the assumptions of Schuster (Schuster, 1905; Cornet *et al*., 1992; Cornet *et al*., 1995) the main points of which can be summarized as follows:

- Only the main direction for propagation of light is considered, i.e., a monodimensional approximation.
- The phase function for scattering of light inside the medium is assumed to be isotropic, i.e., the field of radiation is the same in all directions.
- The angular distribution of the incident light flux is neglected.

Hence the profile of the mean radiant light energy available I_S over the considered spectrum ($I_\Sigma = \int_{l} \int_{2p} \int_{p} I(q, f, l) \sin q \, dq \, df \, dl$) in rectangular reactors illuminated on one side is given by (Cornet *et al*., 1992; Cornet *et al*., 1995):

$$\frac{I_\Sigma}{F_0} = 2 \frac{(1+a)e^{-d(Z-1)} - (1-a)e^{d(Z-1)}}{(1+a)^2 e^{d} - (1-a)^2 e^{-d}} \qquad (1)$$

where $Z = z/L$ is the dimensionless abscissa, L the optical thickness of the medium, F_0 the incident radiant light flux and a and d are given by:

$$a = \left(\frac{Ea}{Ea + Es} \right)^{1/2} \quad (2)$$

$$d = (Ea + Es) a \, C_X \, L \quad (3)$$

Ea and *Es* are respectively the mean Schuster mass absorption and scattering coefficients of light for the medium, averaged over the considered spectrum (that is *Ea = 2a* and *Es = 2s*, *a* and *s* being the mean actual absorption and scattering coefficients). These two parameters have of course to be determined for each micro-organism. C_X is the biomass concentration in the culture medium.

It must be emphasized that the accuracy of this model depends mainly on the correct determination of the mean incident flux F_0 and on the coefficients *Ea* and *Es*. The methods for the determination of F_0 are given elsewhere (Cornet *et al.*, 1997; Cornet *et al*., 1998); the calculation of the coefficients *Ea* and *Es* will be briefly presented further on.

1.2- Coupling light transfer and growth kinetics

The coupling of light energy transfer and local growth kinetics has to be completely understood for it to be possible to calculate the mean volumetric growth rates, which are the sole macroscopic quantities experimentally measurable. For this purpose, it is necessary to have a kinetic law for the considered micro-organism, that is a relation between the specific growth rate *m* and the mean local available radiant light energy I_S. For the purple bacterium *Rhodospirillum rubrum*, the considered range of wavelengths must be 350 - 950 nm because of the absorption of bacteriochlorophylls in the near infra-red. The experimental determination of specific growth rates for different incident fluxes in this range was already performed with acetate as carbon source (Albiol, 1994), and postulating a Monod law, the following equation was established (Figure 1):

$$m = m_M \frac{I_\Sigma}{K_J + I_\Sigma} \quad (4)$$

with $m_M = 0.15$ h^{-1} and $K_J = 15$ $W.m^{-2}$.

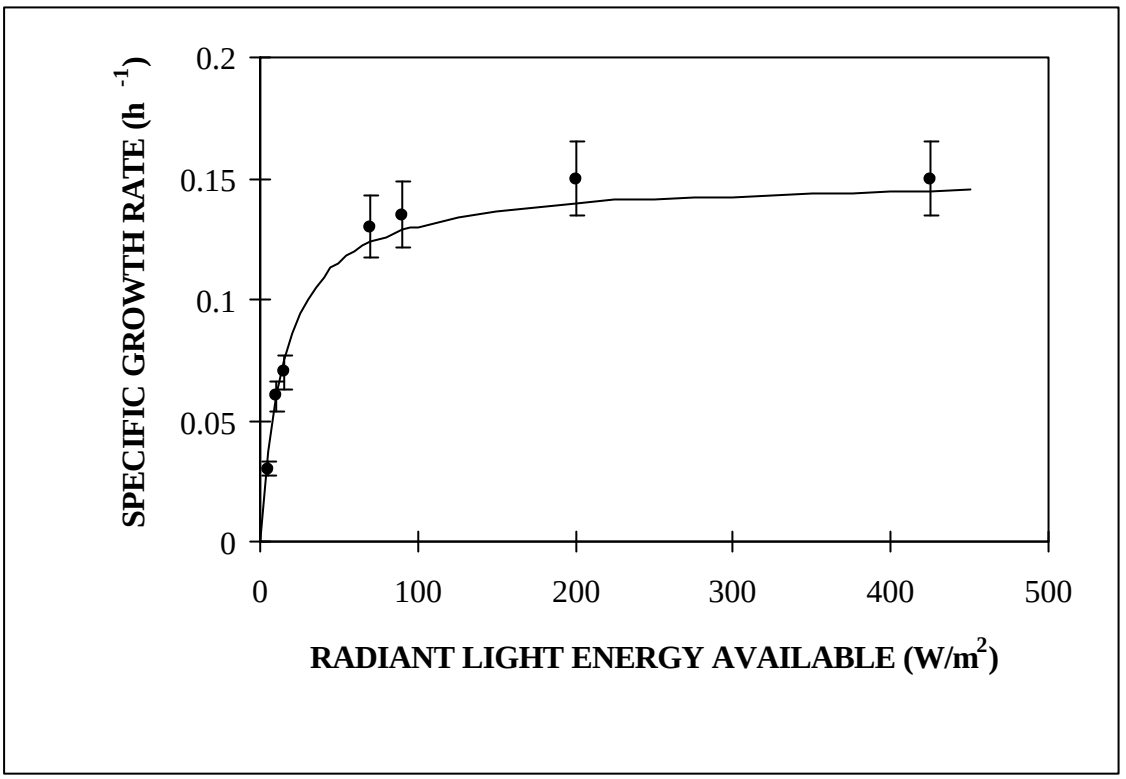

Figure 1: Specific growth rate (m_M) versus radiant light energy available (I_S). Experimental determination in small tubes with dilute cultures considered at the same light energy throughout the volume (Albiol, 1994).

The constant K_J depends on pigment and antenna composition for each micro-organism, i.e., it is a characteristic of the quantum yield for a given micro-organism, and it is independent of substrate. The mean specific growth rate m_M is obviously dependent on the carbon source and will be related later to the number of carbons for the substrate.

Thus the mean volumetric growth rate in the reactor is given by:

$$< r_X >= \frac{1}{V}\iiint_V r_X dV = \frac{1}{V}\int_V\int\int m_M C_X \frac{I_\Sigma}{K_J + I_\Sigma} dV \qquad (5)$$

The main problem arising in the calculation of this integral term is that the kinetic parameters of the model m_M and K_J are defined only in those parts of the reactor where a metabolic activity occurs. For photo-autotrophic micro-organisms, the authors postulated defining a dark zone with no metabolic activity and an illuminated zone in which growth exclusively occurs. This approach is necessary to have constant kinetic parameters over a wide range of incident fluxes, providing fully predictive models (Cornet *et al*., 1992; Cornet *et al*., 1995).

From preliminary experimental results obtained in rectangular PBR, it appeared that this assumption was invalid for photo-heterotrophic micro-organisms such as *Rs. rubrum*, because growth could occur even in a dark zone if the residence time of the cells in the dark volume was sufficiently short. This effect is easily explained in Figure 2, which shows electron transfer with associated ATP synthesis for photosynthesis of photo-heterotrophic micro-organisms. In photo-autotrophic conditions, the classical Z scheme with two photosystems shows clearly that light quanta are necessary both for ATP and reducing power synthesis. Conversely, the scheme for photo-heterotrophic (Fig. 2) functioning, with one photosystem, shows that the possibility exists of accumulating reducing power in the dark using the transmembrane potential to generate a reverse electron transfer (RET) from succinate to a final acceptor ($NADH_2$). From this RET, no additional light energy is necessary for the synthesis of storage intermediates such as polyhydroxybutyrate (PHB) or glycogen. However, in this case, ATP synthesis is not possible and such mechanism can operate only for short dark residence times of cells. It is obvious from Fig. 2 that for higher dark residence times of cells, all metabolic activity is stopped if no proper electron acceptor is available, or if the residence time of cells remains to short to avoid appearance of a fermentative metabolism.

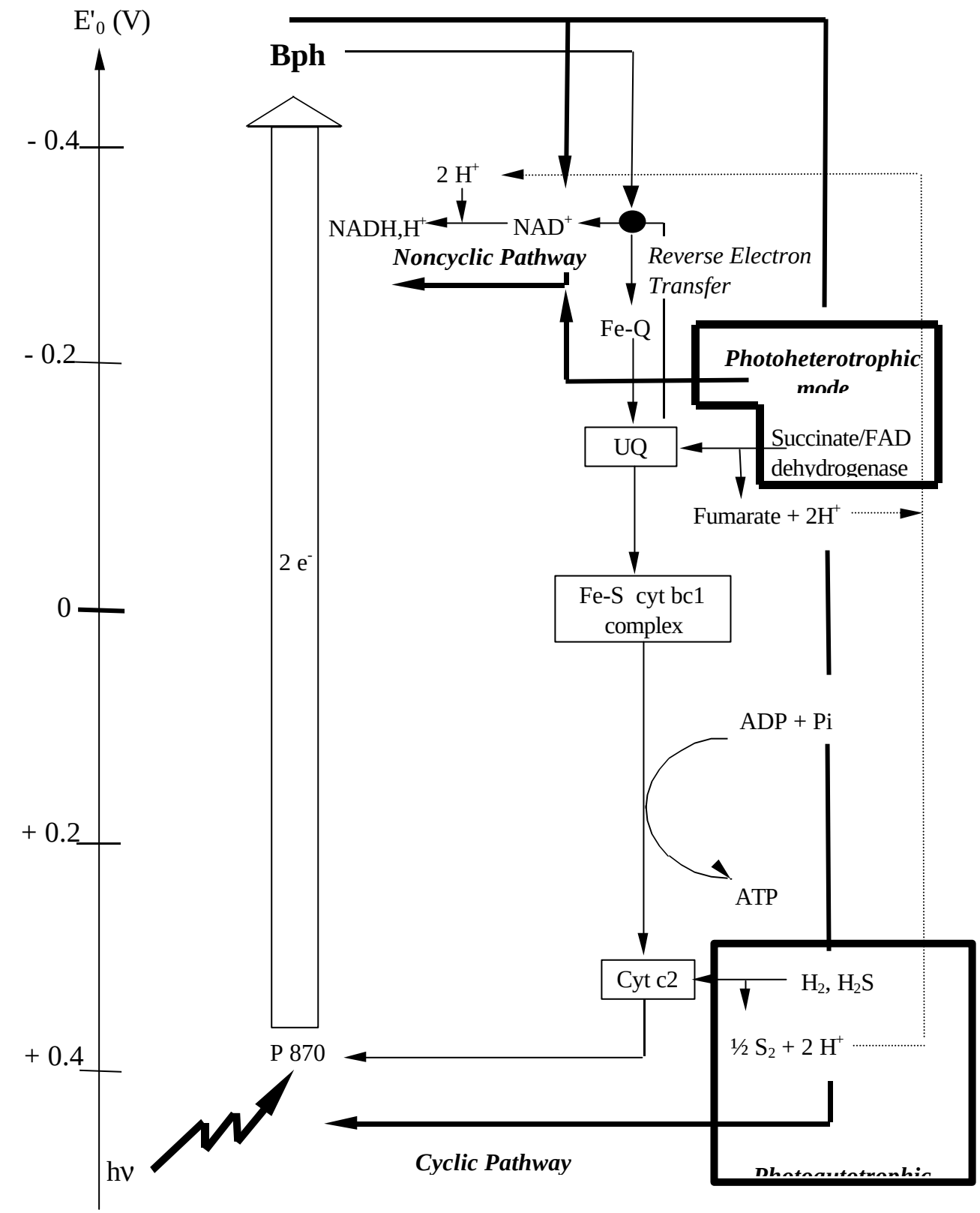

Figure 2: Electron transfer chain and electric potential of the main redox systems for photosynthesis in photo-heterotrophic purple bacteria with Photosystem I: Bph, bacteriopheophytin; Fe-Q, iron-ubiquinone complex; UQ, ubiquinone; Fe-S cyt *bc1*,

iron-sulfur protein cytochrome *bc1* complex; Cyt *c2*, cytochrome *c2*.

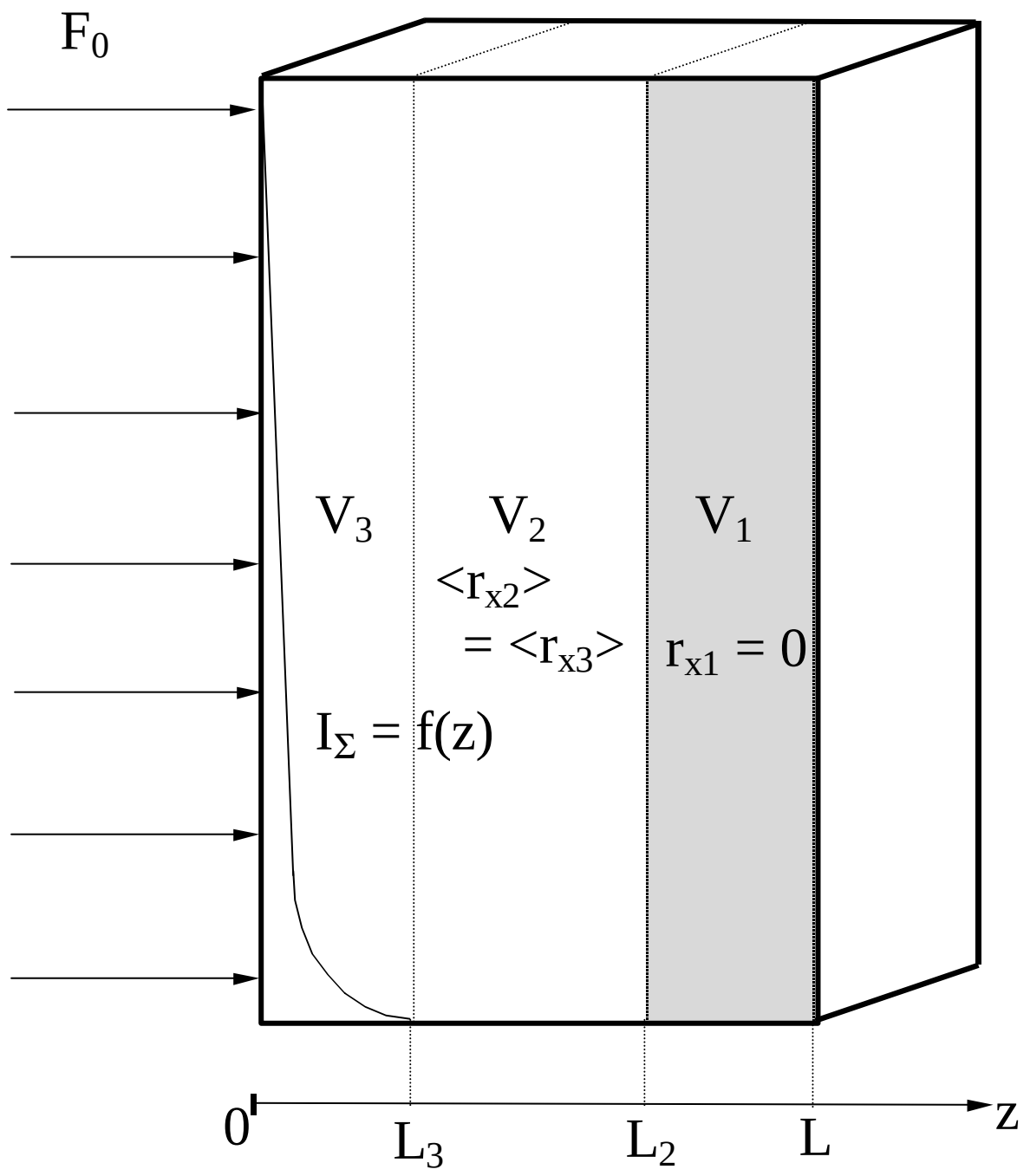

Figure 3: Definition of the three zones with different metabolic activity in modeling photo-heterotrophic growth kinetics in rectangular photobioreactors illuminated from one side (incident flux F_0). The working illuminated volume is defined by the length L_3 and the volume V_3. The intermediate zone with storage of reducing power in the dark is defined by the volume V_2 and the length (L_2 - L_3). In the residual zone (L - L_2), at the rear of the reactor, the volumetric biomass growth rate is zero.

Consequently, it is necessary to divide the total volume of the reactor into three different metabolic zones to calculate the integral (5), for a good resulting predictability. Equation (5) is then rewritten as:

$$< r_x >= (1 - b - g)\frac{1}{V_1}\iiint_{V_1} r_{X_1} dV + b\frac{1}{V_2}\iiint_{V_2} r_{X_2} dV + g\frac{1}{V_3}\iiint_{V_3} r_{X_3} dV \quad (6)$$

The volume V_3 is an illuminated zone in which the growth rate profile is given by coupling equations (1) and (4); The volume V_2 is a dark zone in which the residence time of the cells remains sufficiently short for metabolic activity to continue; and the volume V_1 is a dark zone where no significant biomass growth takes place, i.e. $r_{X1} = 0$. For reactors illuminated on one side, the monodimensional approximation holds true and the volumetric integrals can be replaced by simple integrals in the z-direction and so the volumetric fractions β and γ are given by (Figure 3):

$$\gamma = V_3/V = L_3/L \text{ and } \beta = V_2/V = (L_2 - L_3)/L \quad (7)$$

The lengths L_2 and L_3 have to be determined to calculate the mean volumetric growth rate $<r_X>$.

First, the length L_3, called the working illuminated volume, can be calculated in the same way as for *Spirulina*, from the knowledge of the mean efficient intensity E_J by taking the root of the equation (Cornet *et al*., 1992; Cornet *et al*., 1995):

$$2\frac{(1+\alpha)e^{-d(Z_3-1)} - (1-\alpha)e^{d(Z_3-1)}}{(1+\alpha)^2 e^{d} - (1-\alpha)^2 e^{-d}} - \frac{E_J}{F_0} = 0 \quad (8)$$

where $Z_3 = \gamma = L_3/L$.

For photoautotrophic micro-organisms, the mean efficient intensity E_J has been shown to correspond to the compensation point for photosynthesis, because below this point, respiration is more important than photosynthesis (Cornet *et al*. 1992; Cornet *et al*., 1995). For photo-heterotrophic bacteria, of course, this is not so, and the mean efficient intensity actually corresponds to the minimal radiant light energy at which photosynthesis remains efficient. Such a value can be obtained by independent experiments or by identification from kinetic results. For example, a value of 10^{-2} W.m^{-2} has been

obtained for *Spirulina* (Cornet, 1992) with a Haxo-Blinks (1950) type electrode, that is probably a quite general.

Second, the length L_2 can also be easily established because it splits the reactor volume into a metabolically active zone and an inefficient one. Consequently, it must be defined from the appearance of linear mean growth rate in batch experiments.

The last point is then to define the growth rate in the intermediate zone r_{X2}. Here again one can postulate that the simplest way is to define the mean volumetric integral $\frac{1}{V_2}\iiint_{V_2} r_{X_2} dV$ directly. Because the volume is defined from a short dark residence time of cells, it is assumed that the mean volumetric growth rate in the volume V_2 is not affected and is the same as in the illuminated one V_3. Therefore:

$$\frac{1}{V_2}\iiint_{V_2} r_{X_2} dV = \frac{1}{V_3}\iiint_{V_3} r_{X_3} dV \qquad (9)$$

This assumes that the mean volumetric growth rate in the dark zone is controlled by the volumetric growth rate in the working illuminated volume, i.e. that the intermediate zone corresponds roughly to a relaxation mechanism i.e. a decay of the transmembrane potential insuring the RET. This is an *a posteriori* definition of the volume V_2.

Equation (6) with equation (7) and (9) then becomes, for monodimensional approximation:

$$< r_X >= f_I\,(b+g)\frac{1}{L_3}\int_0^{L_3} r_{X_3} dV \qquad (10)$$

The illuminated surface fraction f_I has been introduced into equation (10) to describe cases in which only part of the photoreactor surface is illuminated.

Equation (10) with equation (4) will be used for simulations given in the Results and Discussion section below.

2- Materials and Methods

2.1- Determination of absorption and scattering coefficients *Ea*, *Es*

In previous work (Cornet *et al*., 1992; Cornet *et al*., 1994), the coefficients $Ea(\lambda)$ and $Es(\lambda)$ were spectrophotometrically determined from the measurement of the true transmission of a sample and the application of the empirical relation of Shibata (Shibata, 1958) to calculate the reflection of the sample. In this method, originally proposed by Aiba (1982), the coefficients $Ea(\lambda)$ and $Es(\lambda)$ were calculated from the solution of a non-linear system of equations, giving the values of transmission and reflection from the Schuster hypotheses.

However, the relation of Shibata is only satisfied for biological material or micro-organisms having a characteristic size greater than 100 µm. Because the diameter of *Rs. rubrum* is rather small (1 - 1.5 µm), the preceding method was not applicable. Accordingly a new procedure was proposed for the calculation of these coefficients (Cornet, 1996). The empirical relation of Shibata is replaced by a theoretical relation given by the Lorentz-Mie theory (Van de Hulst, 1957), which depends on the characteristic size of the micro-organism.

The true transmission of the sample was measured spectrophotometrically by the opalescent plate method (Shibata, 1958) in a SHIMATZU UV-160A spectrophotometer. The mass absorption and scattering coefficients $Ea(\lambda)$ and $Es(\lambda)$ were then calculated by solving the non-linear system of two equations (Cornet, 1996):

$$T(\lambda) = \frac{4a}{(1+a)^2 e^{\delta} - (1-a)^2 e^{-\delta}} \qquad (11)$$

$$\frac{\delta}{a} = \frac{N_p p D^2 L f(\lambda)}{4}\left[2 - \frac{4}{\rho}\sin\rho + \frac{4}{\rho^2}(1-\cos\rho)\right] \qquad (12)$$

the parameters a and δ are defined by equations (2) and (3); D is the mean diameter of the micro-organism considered (Sauter diameter of 1.17 µm); N_p is the number of micro-organisms per unit volume determined using a Malassez cell. The term $f(\lambda)$ is a transfer function to obtain results according to the assumptions of Schuster, which is an isotropic phase function for scattering; how to

determine $f(\lambda)$ is described elsewhere. This can be obtained experimentally (Cornet, 1996) or from a lengthy theoretical treatment. Finally, ρ is the size parameter given by:

$$\rho = 2\pi \frac{4D}{3\lambda}(\bar{n} - 1) \quad (13)$$

where $\bar{n}$ is the ratio of the refractive index for micro-organism over the refractive index of the culture medium ($\bar{n}$ = 1.034).

Once the wavelength-dependent coefficients $Ea(\lambda)$ and $Es(\lambda)$ have been determined using equations (11-13), the mean coefficients *Ea* and *Es* can be averaged over the considered range of wavelength, i.e., 350 - 950 nm in this study.

2.2- Batch cultures in rectangular reactors

Experimental batch cultures were performed in rectangular photobioreactors (Roux flasks) of 1.1 L working volume. Cultures were grown in a set-up, enclosed in a dark chamber with black surfaces, arranged as depicted in Figure 4.

Illumination was arranged in monodimensional conditions. The lamps used were Sylvania professional 25 BAB 38°, 12V 20W. Different incident light fluxes were obtained either by varying the length between the lamps and the reactor, or by using a different numbers of 12 V lamps (modifying the illuminated surface fraction f_I) or one lamp at a lower voltage.

Mean incident fluxes of photosynthetically active radiation (PAR) were measured using a quantum sensor, (Licor Li-190SA), attached to an LI-189 portable meter. The sensor gives the photosynthetic photon flux density (PPFD) in $\mu mols.s^{-1}.m^{-2}$. Conversion of quantum units to radiometric units in the range 350-950 nm was carried out using a constant factor of 0.425 obtained from the emission spectrum of the lamps. These measurements were confirmed, using Reinecke salt as actinometer, to calculate the mean incident irradiation on the illuminated surface (Cornet *et al.*, 1997). Practically, for f_I = 1, i.e., the whole of the surface illuminated, we used a high value for the incident flux of 420 $W.m^{-2}$, then a low value of 45 $W.m^{-2}$. To verify the flexibility of the model, a value of 300 $W.m^{-2}$ with decreasing values of f_I (respectively 1, 2/3, 1/6) and a value of 125 $W.m^{-2}$ with f_I = 1/6 were also tested. All the model parameters were determined in the experiments at 45 $W.m^{-2}$, and then used as predictive values for simulations in the other illuminating conditions.

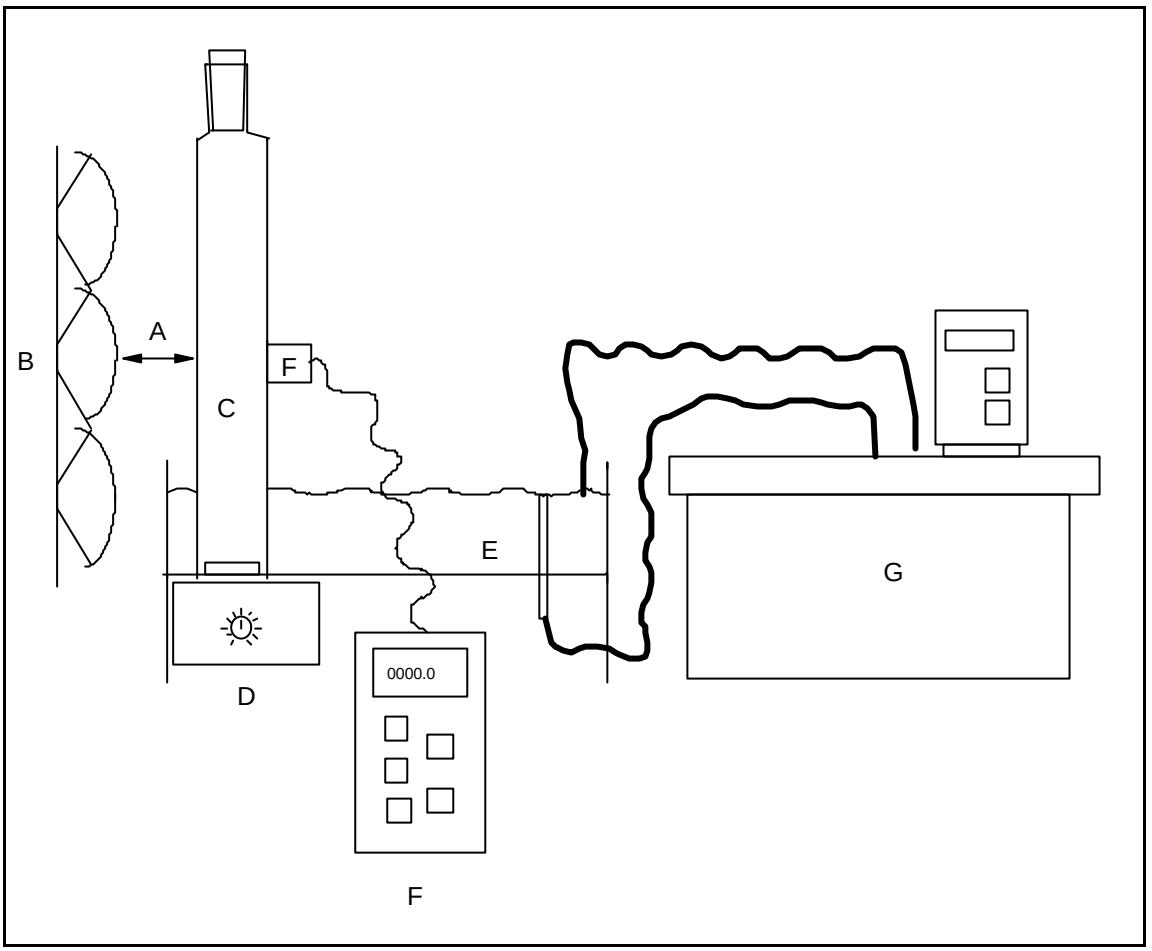

Figure 4: Experimental set-up; A: lamp-bioreactor distance. B: Lamp support. C: Bioreactor. D: magnetic stirrer. E: water bath. F: light sensor. G: thermostatic bath

Biomass dry weight was calculated from the measured absorbency of a sample (A_{700}) and its value interpolated on a calibration curve taken from previous determinations (Albiol 1994).

The bacterial strain *Rhodospirillum rubrum* (ATCC 25903) was obtained from the American Type Culture Collection. It was revived and subcultured for maintenance using the supplier's recommended media.

Experimental culture media were based on the basal salts mixture of Segers & Verstraete as described by Suhaimi (Suhaimi *et al.* 1987), using different carbon sources and biotin as the only

vitamin. To maintain the pH of the culture media and to decrease medium culture precipitation, which could affect the measurements, the following modifications were made. Phosphate concentration was decreased to the following levels: KH_2PO_4 0.2 g/l K_2HPO_4 0.3 g/l. Buffer capacity to maintain the culture pH was obtained using 3-morpholino propane sulphuric acid (MES) 21 g/l. Phosphate was autoclaved separately. The pH was adjusted to 6.9. At the end of the culture the pH was found to be 7.4.

The carbon sources used in these experiments were acetic acid (2.5 g/l), butyric acid (1.84 g/l), propionic acid (2.06 g/l), isovaleric acid (1.7 g/l) and isobutyric acid (1.84 g/l). The concentrations used correspond to 1 gC/l for all cases. Sodium carbonate concentrations for each case were respectively 0.25 g/l, 1.35 g/l, 0.67 g/l, 1.23 g/l, 1.35 g/l. In each experiment, carbon source was analyzed by a GPC method to make sure that it was never limiting.

Temperature (30^{o}C) was maintained using a water bath with heating and cooling capacities. The culture was kept homogeneous with a magnetic stirrer.

3- Results and Discussion

3.1- Results for absorption and scattering coefficients *Ea*, *Es*

Wavelength-dependent absorption and scattering coefficients $Ea(l)$ and $Es(l)$ for *Rs. rubrum* were determined with opalescent plates according to equations (11-13). The wavelength range used was 350 -950 nm. The results obtained are given in Figure 5. The absorption spectrum obtained is the same as in literature data (Göbel, 1978).

The final values for the parameters obtained were (mean values of wavelength):

$$Ea = 270\ m^2 \cdot kg^{-1} \text{ of biomass}$$

$$Es = 370\ m^2 \cdot kg^{-1} \text{ of biomass}$$

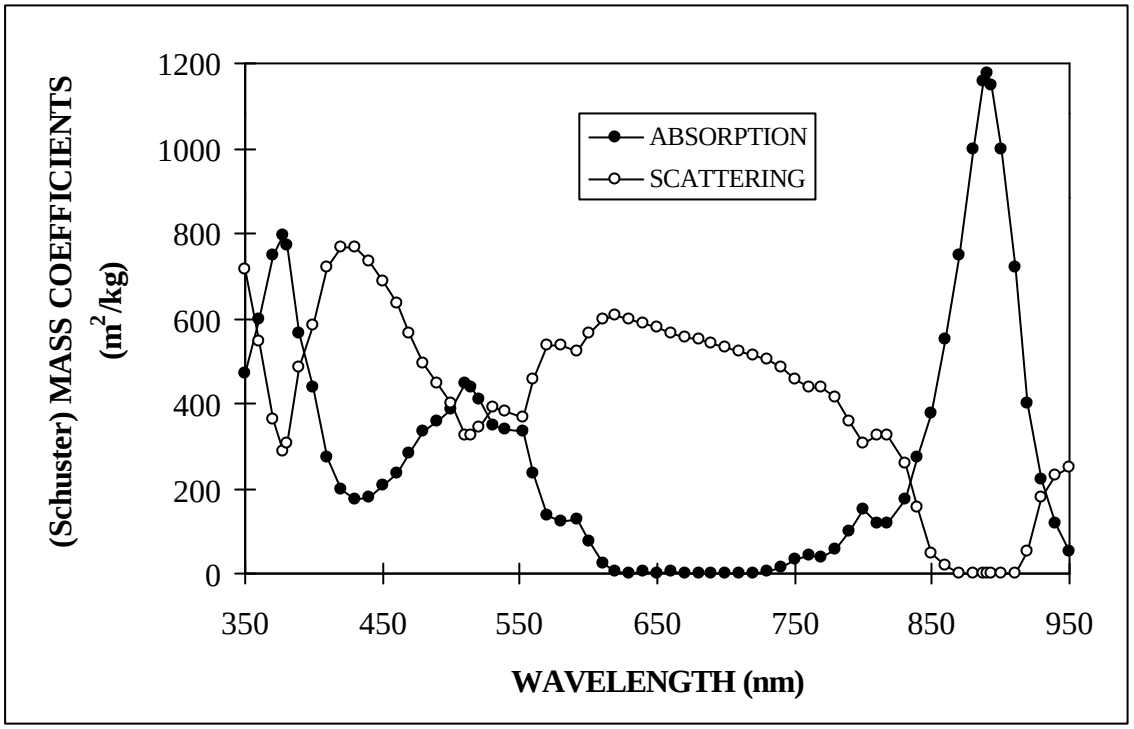

Figure 5: Mass absorption and scattering coefficients spectrum *Ea(l)* and *Es(l)* for *Rs. rubrum* determined spectrophotometrically as explained in text. These results were used to calculate the mean values over the total spectrum (350-950 nm), *Ea* = 270 $m^2.kg^{-1}$ and *Es* = 370 $m^2.kg^{-1}$.

3.2- Kinetic results

In some experiments, the biomass time course for batch cultures shows a lag phase. This effect has already been reported in the literature (Sawada and Rogers, 1977) for batch cultures of *Rs. rubrum* and these authors showed that this lag phase corresponded to a pigment adaptation of the cells.

Because the kinetic model proposed in this paper is unable to allow for these modifications, which are also highly non-reproducible, we present and discuss only simulations obtained on growth phases for each experiment. This is not a problem because the main aim of this study was to obtain a

robust model for continuous cultures of *Rs. Rubrum*, which will not be affected by lag phase phenomena.

For each independent experiment, simulations were performed from the following mass balance for batch culture:

$$\frac{dC_X}{dt} = < r_X > \quad (14)$$

The model parameters were first determined from cultures at incident light flux of 45 W.m^{-2} for each substrate. At this low flux, linear phases appear, enabling to determine a critical biomass concentration leading to the calculation of the intermediate zone of volume V_2. The kinetic model involves only four parameters K_J, m_M, ε_J corresponding to the definition of working illuminated volume V_3, and a proportionality constant $q = L_2/L_3$ (q>1, see Fig. 3) corresponding to the definition of the intermediate zone V_2. We have then from eq. (7):

$$b = \frac{L_2 - L_3}{L} = \frac{L_3 (q-1)}{L} \quad (15)$$

$$b + g = \frac{L_3 + (L_2 - L_3)}{L} = \frac{L_2}{L} = q \frac{L_3}{L} \quad (16)$$

The parameter K_J has been already mentioned as a constant for the micro-organism considered and independently determined at 15 W.m^{-2}. The mean incident efficiency ε_J has been taken as some characteristics for photosynthesis, and the same value as for *S. platensis* (10^{-2} W.m^{-2}) has been postulated. Because of the high sensitivity of the model to this parameter, it has proved to be a very good choice from all simulations. It is of course a key parameter because it determines the length L_3, i.e., the working illuminated volume and so the mean growth rates in zones 3 and 2.

From the appearance of the linearity on each experiment, a mean value of the length L_2 was determined giving $q = 3.2 \pm 0.2$ corresponding to an intermediate volume $V_2 = L_2 - L_3$ slightly higher than twice V_3. This is an important result showing that cells can continue their metabolic activity without apparent modification, during a residence time of about twice the residence time in the light. This suggests that the RET is possible from the transmembrane potential generated in the illuminated phase.

For each carbon source, only one parameter remained to be identified, namely the specific growth rate m_M. The identification was performed using a Gauss-Newton algorithm for the minimization of a quadratic criterion. The results of the procedure for experiments at F_0 = 45 W.m^{-2} are as follows:

Acetate (2 C): m_M = 0.15 h^{-1}

Propionate (3 C): m_M = 0.13 h^{-1}

Butyrate (4 C): m_M = 0.115 h^{-1}

Isovalerate (5 C): m_M = 0.07 h^{-1}

Isobutyrate (4 C): m_M = 0.07 h^{-1}

The mean standard deviation for all the values was lower than 5%.

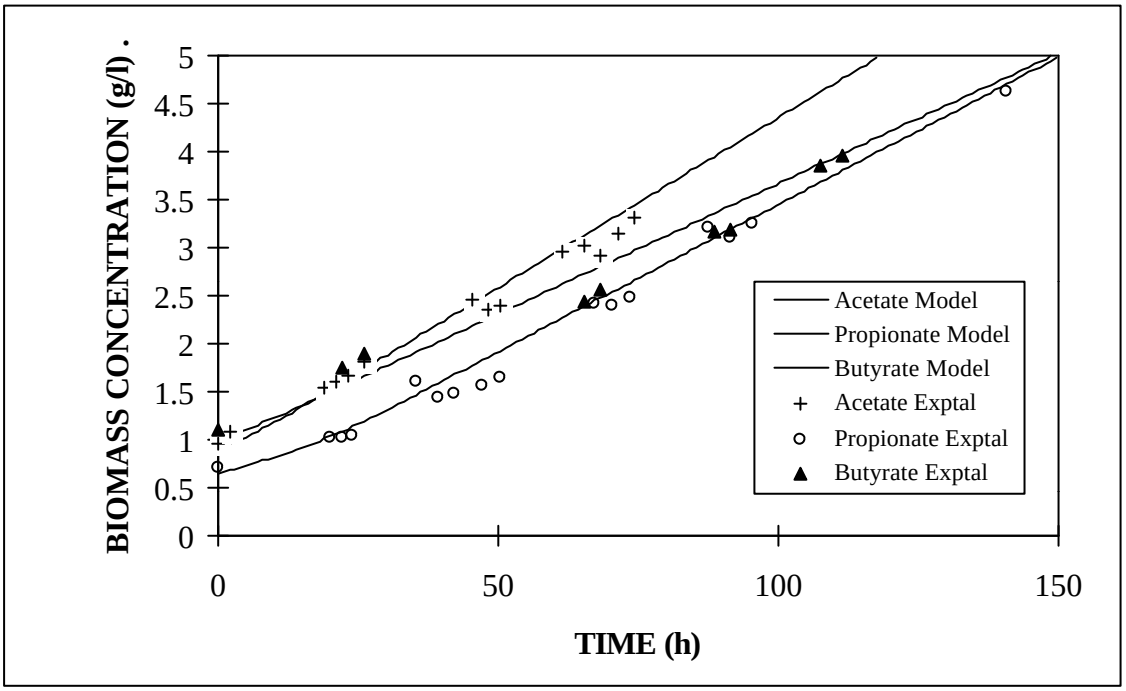

Figure 6a: Experimental results and model simulations for batch cultures in rectangular photobioreactor at 45 W.m^{-2} and for three different carbon substrates: Acetate, Propionate, Butyrate.

The value for acetate confirms the value previously obtained on independent experiments (Albiol, 1994) and presented above (eq. 4). The results obtained show a linear dependence of the specific growth rate increasing the number of carbons for the volatile fatty acid (VFA), except for branched VFAs which present lower but the same growth rate. This is justified because the preferred form for the metabolism is acetate and because the branched VFAs have be split to give linear VFAs before assimilation. Comparisons between experimental results for each substrate and model simulations are given in Figure 6 and show a close agreement (maximum deviation 10%) taking into account the generality of the model.

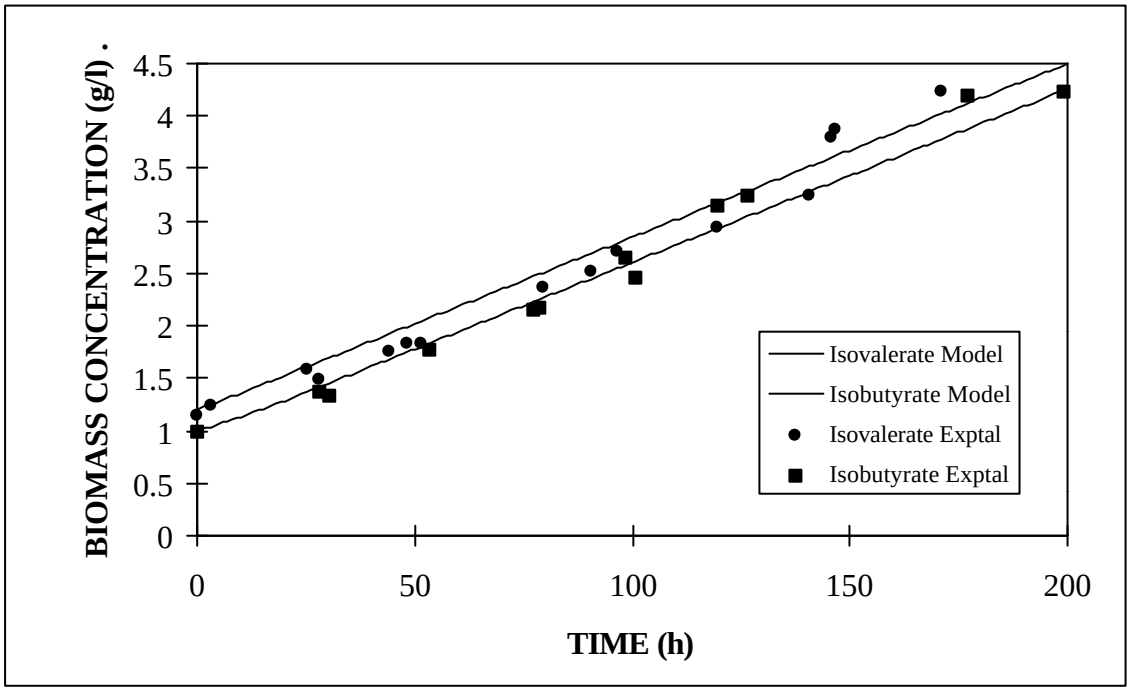

Figure 6b: Experimental results and model simulations for batch cultures in rectangular photobioreactor at 45 $W.m^{-2}$ and for two different carbon substrates: Isovalerate and Isobutyrate.

Figure 7 corresponds to the model validation for the same substrates (except for isobutyrate which leads to a photo-inhibition phenomenon at high flux) at an incident light flux of 420 $W.m^{-2}$ and with the previous values of the parameters. The maximum deviation is still lower than 10 % showing the ability of the model to simulate kinetic behavior of *Rs. rubrum* at very high incident fluxes (10 times higher than those used for parameter identification).

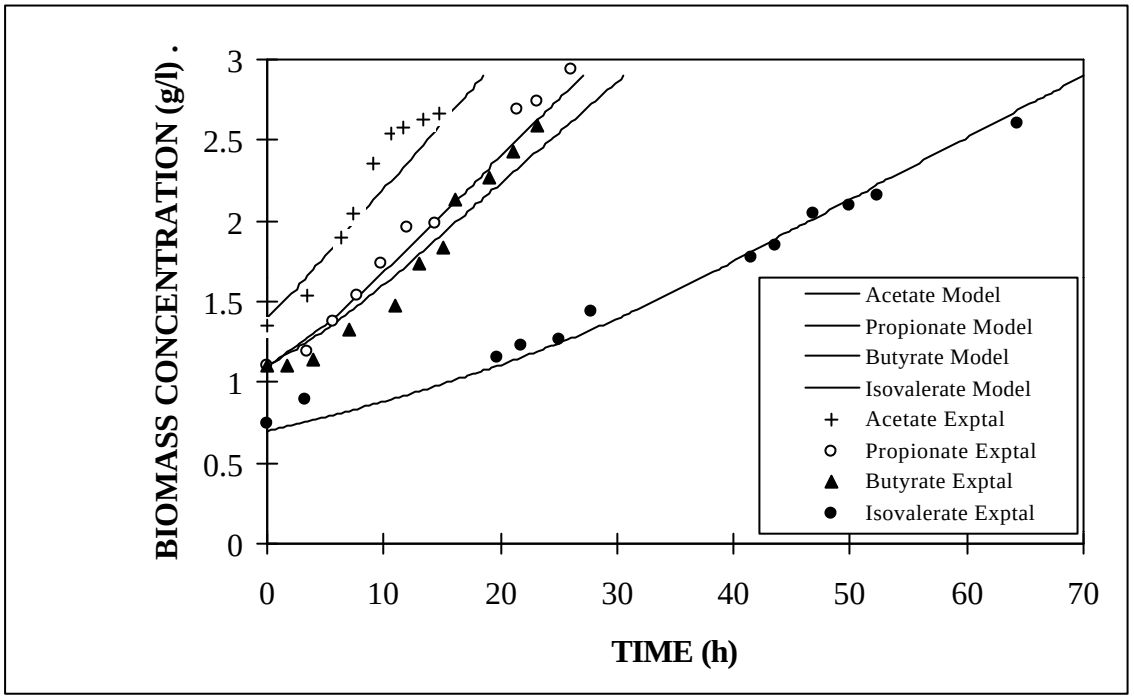

Figure 7: Experimental results and model simulations for batch cultures in rectangular photobioreactor at 420 $W.m^{-2}$ and for four different carbon substrates: Acetate, Propionate, Butyrate, Isovalerate.

Finally, we evaluated the flexibility and the robustness of the model in cases where the photoreactor was partially illuminated, i.e., when the illuminated surface fraction f_I was lower than 1. This feature frequently appears for pilot or industrial scale PBRs. To do this, with an incident flux of

300 W.m^{-2}, the total number of lamps was decreased from 6 (f_I = 1) to 4 (f_I = 2/3), then 1 (f_I = 1/6) and then, only one lamp was used with an incident flux of 125 W.m^{-2}. The results of simulations are given on Figure 8 and show close agreement with experimental values.

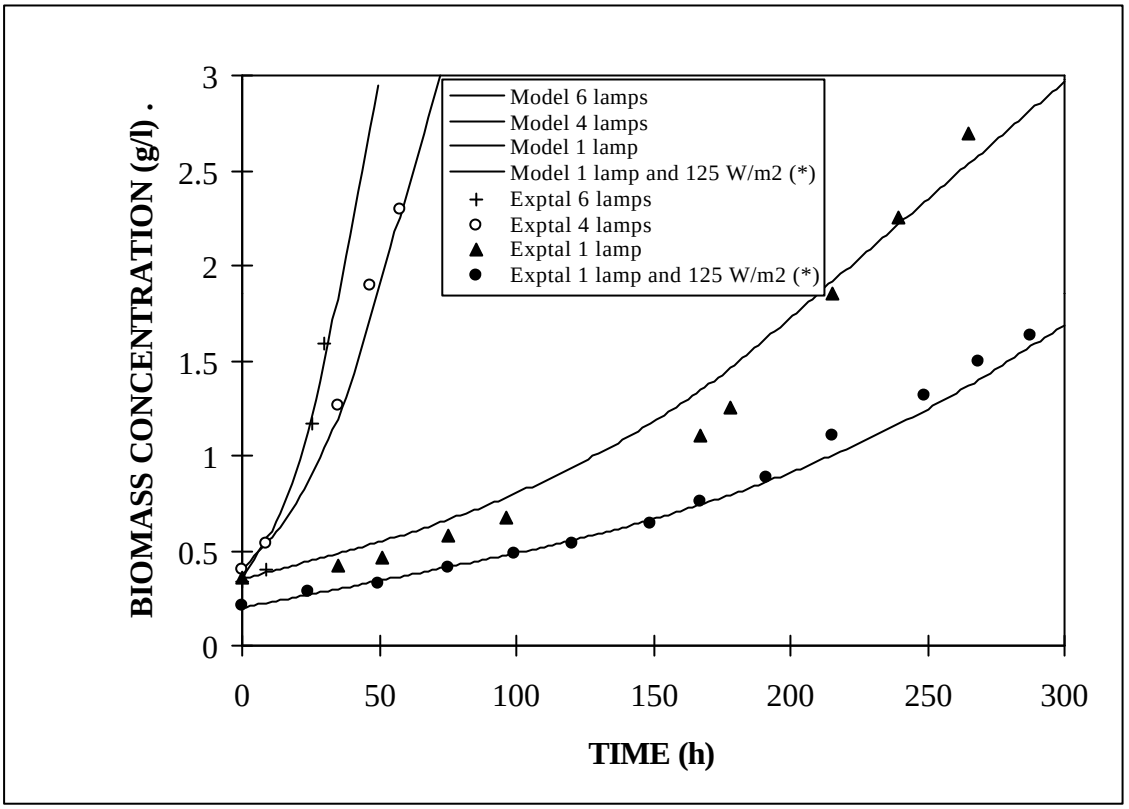

Figure 8: Experimental results and model simulations for batch cultures in rectangular photobioreactor at 300 W.m^{-2} (except *) and with different illuminated surface fractions f_I . (+) 6 lamps (f_I = 1); (O) 4 lamps (f_I =2/3); (▲) 1 lamp (f_I = 1/6); (●) 1 lamp and F_0 = 125 W.m^{-2} (f_I = 1/6).

The good predictability and robustness of the proposed knowledge model on five different carbon sources and incident fluxes varying by a factor of 10, mainly relies on the correct description of the physical limiting transfer of radiant light energy and the correct formulation of the coupling with

local rates and metabolism. First the radiant light transfer is described with a simple and tractable monodimensional model requiring only three parameters F_0, *Ea* and *Es* (Cornet *et al*., 1998). These parameters can be easily determined experimentally from independent experiments as previously stated. Second, the local kinetic law is postulated, requiring two parameters m_M and K_J, this later being a general characteristics of the micro-organism, and m_M being related to a given substrate. Thus, in this case, independent experiments can allow the assessment of these parameters assessment (Albiol, 1994). Finally, writing the coupling between light transfer and local kinetics then requires two additional parameters to be identified, ε_J and q defining the two active metabolic zones in the PBR. This identification, from experiments in which a high biomass concentration was reached, is easily performed when a constant mean growth rate $<r_X>$ is observed on batch cultures.

Stoichiometrically, it is important to note that knowledge of the two metabolically active zones 3 and 2 is a powerful tool in obtaining mean stoichiometries for produced biomass, from two different stoichiometries for biomass growth, one in the working illuminated volume, and one (for storage of PHB) in the dark operative volume. The calculation of a dark volume being twice the illuminated one in strongly light limited cultures, could explain the high percentage of PHB (up to 70%) in the cells reported in the literature (Merrick, 1978).

From an engineering standpoint, the preliminary results given in this paper in rectangular reactors, can be now generalized to different reactor geometries: cylindrical, tubular, annular, etc. as previously done for *Spirulina* culture (Cornet et al., 1995). In these cases only the reformulation of radiant light transfer is necessary and is already available in the above stated geometries (Cornet *et al*., 1995, Cornet, 1998). If the coupling with local kinetics has been correctly established, as assumed given the variety of experimental conditions tested, the proposed model is fully predictive in any kind of PBR in which the problem of radiant light transfer can be formulated.

Conclusions and Perspectives

A knowledge model to understand the kinetic behavior of PBR has been proved to rely mainly on the correct description of two points. First, the radiative transfer for light in complex absorbing and scattering media must be accurately formulated. This is a prerequisite in using consistent local variables that can be related to physical characteristics for micro-organism photosynthesis, obtained on independent experiments at the cellular scale. Second, the coupling with local kinetics involves understanding transient metabolisms occurring when the cells move from illuminated to dark zones in the PBR.

In this technical note, a simple and tractable 2-flux method is used in describing the radiative transfer problem, already proved to be of sufficient accuracy. Due to the inappropriateness of the application of the Aiba-Shibata method for the determination of light absorption and scattering coefficients to *Rs. rubrum*, a new and powerful method, based on the Lorentz-Mie theory, has been applied. The equations used in this paper for rectangular geometries are also available in other geometries for the PBR, with different boundary conditions for the incident flux.

Compared with photo-autotrophic bacteria, the coupling with local metabolism was more difficult to formulate with photo-heterotrophic micro-organisms, and a dark intermediate zone with some metabolic activity was defined and introduced. This approach affords a correct calculation of the mean volumetric growth rates observed. The extreme sensitivity of the simulations to the model parameters, together with their independent measurements (only the maximum specific growth rate m_M was identified from experimental results) and their physical interpretation, demonstrates the reliability of the proposed values.

The robustness of the proposed knowledge model is thus guaranteed by the small number of parameters used and their physical consistency, avoiding coupling phenomena in the identification procedure and empirical fitting. The close agreements obtained with experimental results in a rectangular photoreactor with a wide range of operating conditions allow the use of this model as a tool for simulation and design of batch or continuous PBR cultivating photo-heterotrophic micro-organisms. Its use for model-based predictive control of PBRs with ADERSA is also planned. However, the kinetic behavior of cultures with mixed carbon sources remains to be investigated.

Special attention must be paid to the fact that this technical note deals with biomass growth kinetics only. A crucial problem remains the formulation of correct associated stoichiometric equations in order to calculate the C-substrate/biomass conversion yields (strongly variable from preliminary results of TNs 37.81-82), and consequently to predict the C-substrate consumption rates. This is a prerequisite step before investigating the carbon limited growth of *Rs. rubrum*.

At present time, the proposed kinetic model indicates that, for each carbon substrate, two stoichiometric equations have to be established. The first one being only valid in the working illuminated volume (neglecting in a first approximation minor metabolic deviations resulting in the mean available intensity gradient), and the second one only valid in the dark efficient zone, corresponding to the relaxation phenomenon and reducing power storage in the cells. The challenge about this last point arises from the fact that such a stoichiometric behavior, corresponding to short residence time of cells at obscurity (order of magnitude of a second), will never be observed nor measured as a whole !

Notations

a Mean actual mass absorption coefficient ($m^2.kg^{-1}$)

C_X Total biomass concentration ($kg.m^{-3}$ or $g.l^{-1}$)

D Characteristic size for a given micro-organism (e.g. its mean diameter, m)

Ea Mean Schuster mass absorption coefficient ($m^2.kg^{-1}$)

E_J Mean efficient intensity ($W.m^{-2}$)

Es Mean Schuster mass scattering coefficient ($m^2.kg^{-1}$)

f_I Illuminated surface fraction for the PBR (dimensionless)

F_0 Mean incident light flux ($W.m^{-2}$)

I_Σ Mean local available radiant light energy ($W.m^{-2}$)

K_J Half saturation constant for kinetic law ($W.m^{-2}$)

L Length of the photoreactor (m)

$\overline{n}$ Ratio of the mean refractive index of the micro-organism over the refractive index for the culture medium (dimensionless)

N_p Number of micro-organisms per unit volume (m^{-3})

$q=L_2/L_3$ Proportionality constant between illuminated and dark operative volumes (dimensionless)

r_X Local volumetric biomass growth rate ($kg.m^{-3}.h^{-1}$)

s Mean actual mass scattering coefficient ($m^2.kg^{-1}$)

t Time (h)

V Volume (m^3)

z optical thickness (m)

Z=z/L Dimensionless optical thickness (dimensionless)

$\langle\rangle = \frac{1}{V}\iiint_V dV$ Mean volumetric integral quantity (dimensionless)

Greek letters

β Dark operative volume fraction (dimensionless)

γ	Working illuminated volume fraction (dimensionless)
λ	Wavelength (m)
μ	Specific growth rate (h^{-1})
μ_M	Maximum specific growth rate (h^{-1})
ρ	Size parameter (see text, dimensionless)

Subscripts

1	For dark zone with no metabolic activity in the PBR
2	For dark operative intermediate zone in the PBR
3	For working illuminated zone in the PBR

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