Memorandum of Understanding TOS-MCT/2002/316/In/CL.012

Université Blaise Pascal LGCB
Laboratoire de Génie Chimique et Biochimique

TECHNICAL NOTE: 55.1

MODELLING OF *RHODOSPIRILLUM RUBRUM* GROWTH IN CYLINDRICAL PHOTOBIOREACTOR.

STABILITY ANALYSIS OF CONTINUOUS CULTURES AND OPERATING DOMAIN DEFINITION

prepared by/*préparé par* J.-F. Cornet, L. Favier-Teodorescu, C.G. Dussap

reference/*réference* MOU TOS-MCT/2002/316/In/CL.012
issue/*édition* 1
revision/*révision* 0
date of issue/*date d'édition*
status/*état* Final version
Document type/*type de document* Technical Note
Distribution/*distribution* MELiSSA all

A P P R O V A L

Title *titre*	Modelling of *Rhodospirillum rubrum* growth in cylindrical photobioreactor. Stability analysis of continuous cultures and operating domain definition	issue 1 *issue*	revision 0 *revision*

author *auteur*	J.-F. Cornet, L. Favier-Teodorescu, C.G. Dussap	date *date*	Nov.2005

approved by *approuvé par*		date *date*

C H A N G E L O G

reason for change /*raison du changement*	issue/*issue*	revision/*revision*	date/*date*

C H A N G E R E C O R D

Issue: 1 Revision: 0

reason for change/*raison du changement*	page(s)/*page(s)*	paragraph(s)/*paragraph(s)*

TABLE OF CONTENTS

1 SCOPE AND OBJECTIVES

During the past years 2000-04, many continuous cultures of the purple non-sulphur bacterium *Rhodospirillum rubrum* were achieved at LGCB in a cylindrical photobioreactor and in photoheterotrophic conditions, using acetate as carbon source. The experimental work was divided in two parts:

- First, experimental tests were performed at constant incident light flux q_R = 100 W m^{-2} but varying the residence time in the PBR leading to different values of biomass concentrations at steady state (Favier-Teodorescu *et al*., 2003 - TN 49.2 -);
- Second, the incident light flux was varied keeping constant the biomass concentration by acting on the residence time in the PBR (Cornet *et al*., 2005 – TN 49.3 -).

At the same time, the basis for a kinetic model describing the main steady state results obtained for productivities at constant light flux were sketched in the previous reported TN 49.2. Taking now into account all the experimental results presented in TN 49.2 and 49.3 and paying special attention to the different regimes obtained, this technical note presents a more general first knowledge model for the growth of *Rhodospirillum rubrum*, cultivated in a cylindrical radially-illuminated photobioreactor and operating in continuous and photoheterotrophic modes with acetate as carbon source.

The approach is however limited to experimental conditions which have been identified as satisfactory, i.e. a correct mixing corresponding to rotation speeds equal or higher than 400 rpm, and no photoinhibition corresponding to incident light fluxes lower than 300 W m^{-2}.

Even if further additional theoretical work will be necessary in the future to reach the same level of description as the fourth compartment of MELiSSA (*Arthrospira platensis*), the approach described here is predictive and innovative in that:

- A generalized two-flux method is used to solve the RTE in the PBR with exact optical radiative properties for *Rs. rubrum*, theoretically calculated by the classical Lorenz-Mie theory (Van de Hulst, 1981; Bohren and Huffman, 1983) and taking into account by a first preliminary semi-empirical relation the changes in pigment content;

- Energetic yields are used to formulate the coupling between the available light energy and kinetic rates (Cornet *et al*., 2001), their theoretical values being calculated by thermodynamic analysis and metabolic flux approaches;
- A zone model is presented and a working illuminated volume is defined in the PBR, reconciling local cellular energetic and mean kinetic rates (Cornet *et al*., 1992 and 1998);
- A stoichiometric approach takes into account the PHB storage in the cells for any condition of PBR illumination.

The obtained knowledge model, requiring only one experimental coefficient determination for rate calculation, is then used to explain the wide variety of PBR regimes observed by varying incident light flux and liquid flow rate of the feed in continuous mode. Special attention has been paid to the analysis of the stability conditions for the PBR function, and to the definition of an operating domain for the main process variables.

Without any ambiguity, it is clear that the main part of the work described hereafter was previously published (Cornet *et al*., 2003) because the corresponding TN was not written in time (2002 or 2003). Nevertheless, important precisions regarding the MELiSSA purposes have been added (new statements about light transfer model, description of a Fortran code as a tool in defining the operating domain for the PBR from the present state of the art…). Furthermore, important modifications regarding the predictive modelling of the light transfer model with actual particle shapes in cylindrical geometries are expected from the contractual MELiSSA work planned for 2005-2006.

2 MATERIALS AND METHODS

All the experimental aspects involve in this work have been extensively described in the preceding technical notes (TN 49.2 and 49.3). Particularly, the analytical techniques and the description of the pilot photobioreactor used are detailed in the TN 49.2 (Favier-Teodorescu *et al.*, 2003). The aim of this short paragraph is just to briefly summarise the culture conditions and the analytical procedures.

2.1 Culture conditions

Rhodospirillum rubrum ATCC 25903 was cultivated in the basal salt medium of Segers & Verstraete (1983) as described by Suhaimi et al. (1987) with acetate and ammonium chloride as C and N sources, and biotin as the only vitamin. Acetate and NH_4^+ were adjusted to avoid C and N limitation in the PBR, keeping a C/N ratio of 3. A phosphate buffer was used (0.49 g L^{-1} KH_2PO_4 and 0.52 g L^{-1} K_2HPO_4), and pH was adjusted to 6.9.

Rs. rubrum was grown in a stirred, cylindrical, radially illuminated photobioreactor (radius 0.08 m) containing 5 L of culture media (see Figure 1). A 10% (vol.) inoculum was used. Temperature and pH were maintained automatically at 30°C and 7 using respectively a coiled heat exchanger and a 2 mol L^{-1} H_2SO_4 solution. Stirring was set at 400 rpm using two rushton impellers of 6 cm diameter, and argon was introduced at 4 L h^{-1} to maintain anoxygenic conditions. A continuous artificial illumination was provided by 55 halogen lamps (Sylvania professional 25 BAB 38°, 12V, 20W) arranged around the reactor. Illumination of the culture was controlled by adjusting the power supplied to the lamps. Light incident fluxes were calibrated from a method described elsewhere (Cornet *et al*., 1997).

All the experiments were carried out in continuous mode with different residence times, and the incident light flux q_R was varied between 50 and 400 W m^{-2} [350-950 nm]. Samples were taken periodically from the output flow to analyze the changes in the system until a steady state was obtained. At steady state, dry weight, acetate and acetoacetate concentrations, pigment, protein, total carbohydrate and poly-β-hydroxybutyric acid (PHB) contents were determined. All the experimental results given at steady state were averaged over at least six residence times in the PBR. The CO_2 mole fraction in the output gas phase of the reactor was continuously analyzed using a CO_2 infrared analyzer (ADC, England). The dissolved CO_2 was quantified by applying the gas mass balance on the reactor from the knowledge of the volumetric CO_2 gas-liquid mass transfer coefficient preliminary determined.

Figure 1: Photograph of the 5 L – working volume cylindrical photobioreactor radially illuminated used in this study in chemostat mode.

2.2 Analytical procedures

Acetate and acetoacetate in the culture medium were assayed by HPLC analysis. The chromatograph (Agilent 1100, Agilent Technologies, Palo Alto, CA, USA) was fitted with two ion exclusion columns (Resex ROA 300 x 7.8 mm, Phenomenex, Torrance, CA, USA) mounted in series. Bacteriochlorophyll *a* and carotenoids in the biomass were estimated from the absorbances at 880, 515 and 720 nm (Vernon and Garcia, 1967) of a sonicated cell suspension after applying a correction to take into account light scattering by cells in the sample. The PHB content was determined by GC using the method developed by Braunegg et al. (1978). Analyses were performed on a polar supelco 2-4084 (Supelco. Inc., Bellefonte, PA) capillary column (30 m x 0.32 mm), with a flame ionisation detector. Nitrogen was used as carrier gas (1.7 mL min^{-1}) and caproic acid as internal standard. Total protein content was

determined by the method of Lowry after hydrolysis of samples in NaOH. Total carbohydrate content was determined according to the phenol method of Herbert et al. (1971).

The elemental analysis of biomass (C, H, O, N, S, P) was carried out at CNRS Vernaison (France) for each run at steady state.

For all the experimental results reported and discussed in this paper, the carbon recovery percentage (CRP) defined as the mass ratio of total carbon in the output flows of the reactor over the total carbon in the input flows of the reactor was 100 $\pm$ 10%.

3 THE UNCHANGED LIGHT TRANSFER AND KINETIC MODELS

The main aspects interesting the light transfer and kinetic models have already been presented in the Technical Note 49.2 (Favier-Teodorescu *et al*., 2003). The aim of this third paragraph is just to summarise the main assumptions and limits of the light transfer model and to describe the method used in formulating the coupling with local kinetic rates at different incident energy inputs from a metabolic fluxes analysis.

3.1 Radiant Light Transfer

The quasi-steady-state one-dimensional equation of radiative transfer for a non-emitting participating medium (with a and s respectively the volumetric absorption and scattering coefficients) in cylindrical coordinates:

$$\cos\theta \frac{dI(r,\theta)}{dr} = -(a+s)\, I(r,\theta) + \frac{s}{2}\int_0^{\pi} I(r,\theta)\; p(\theta,\theta')\sin\theta' d\theta' \quad (1)$$

over a given interval of wavelength, may be solved applying the generalized two-flux method. The assumption is made that a semi-isotropic intensity distribution exists (and not necessarily

an isotropic phase function as for the Schuster-Schwarzschild approach (Schuster, 1905)), allowing the integral in Equation (1) to be solved for azimuthally symmetric radiation. Performing the integration on each hemisphere thus defining the hemispherical flux densities $q_r^{\pm}$ and combining with the local radiant energy balance:

$$\frac{1}{r}\frac{d(rq_r)}{dr} = -aG_r \quad (2)$$

gives rise to a complicated problem in finding general analytical solutions for the radial profiles in radiative flux density $\mathbf{q} = \iint_{4\pi} I\cos\theta\, d\omega$, and in irradiance $G = \iint_{4\pi} I\, d\omega$ (we introduced here a new notation *G* for irradiance because it is now a quasi-normalised notation in the physicists community) respectively defined by the integral over all the directions (solid angle ω). In all cases, the two-flux approach requires also to define hemispherical quantities characterising absorption $\hat{a}$ and scattering $\hat{s}$ of light by particles such as:

$$\begin{aligned} \hat{a} &= \Gamma a = \Gamma EaC_X \\ \hat{s} &= \Gamma bs = \Gamma bEsC_X \end{aligned} \quad (3)$$

in which the back-scattered fraction *b* is evaluated (giving $\mu = \cos\theta$) by the integral of the phase function *p* (Brewster and Tien, 1982; Koenigsdorff *et al*., 1991):

$$b = \int_{-1}^{0} p(\mu,\mu')\, d\mu' \quad (4)$$

and Γ is a constant equalled to 1 if the radiation field is collimated and to 2 if it is diffused. These mean optical radiative properties for *Rhodospirillum rubrum* (Eq. 3-4) were calculated in the range of wavelength [350-950 nm], and are given in Table 1. For this purpose, we assumed that *Rs. rubrum* was equivalent to a sphere with a log-normal size distribution law and we used the classical predictive Lorenz-Mie theory (Van de Hulst, 1981; Bohren and Huffman, 1983).

The resolution of the system of Equations (1-4) with the appropriate boundary conditions corresponding to a mean homogeneous radial incident flux at R, $q_R^- = q_R$, may be done in assuming a non translucent medium (i.e. considering that the medium is not too dilute). In this case, a good approximation for the radial irradiance profile was already established (Cornet *et al.*, 1995), giving:

$$\frac{G_r}{q_R} \cong \frac{R}{r} \frac{2\Gamma \cosh(\delta r)}{\cosh(\delta R) + \alpha \sinh(\delta R)} \quad (5)$$

knowing:

$$\alpha = \sqrt{\frac{\hat{a}}{\hat{a} + 2\hat{s}}}$$

$$\delta = \sqrt{\hat{a}(\hat{a} + 2\hat{s})}$$

This simplified analytical approach enables to easily characterise the light transfer problem in the complicated cylindrical geometry, but it must be kept in mind that eq. (5) is not available in case of very dilute medium (low biomass concentration) or approaching the limit r = 0. For these last cases, a more tedious mathematical analysis is required. Nevertheless, eq. (5) is a useful relation applicable in many practical conditions for cylindrical PBR.

$Ea = 1.5\ 10^4\ w_{PIG}$ m^2 kg^{-1} where w_{PIG} is given by Equation (12) in text
Es = 1900 m^2 kg^{-1} (mean constant PHB content)
b = 0.011 [dimensionless]
$\overline{\psi}$ = 2.6 10^{-8} kg J^{-1}
ρ_M = 0.706 [dimensionless]
K = 25 W m^{-2}
G_{min} = 0.6 W m^{-2}

Table 1: Numerical values of the coefficients for the mathematical knowledge model. The considered spectrum is [350 – 950 nm].

3.2 Coupling Light Transfer with Local and Spatial Kinetic Rates

3.2.1 LOCAL COUPLING

The local volumetric growth rate r_X is easily deduced from the Local Volumetric Rate of radiant Energy Absorbed $\mathcal{A}$ (LVREA) applying two conversion yields:

$$r_X = \rho \overline{\psi} \mathcal{A} = \rho \overline{\psi} a G_r \quad (6)$$

First, the mean mass stoichiometric quantum yield $\overline{\psi}$ (i.e., calculated from a mean wavelength in the considered range [350-950 nm] and for a given emission spectrum of the lamps) appears quasi-constant in regard to radiant light energy changes. The bar indicates a time-averaged value on the metabolism so as to be able to apply the relations of the thermodynamics of irreversible processes (TIP) in the yield calculation (Cornet *et al*., 1998), or a metabolic flux network approach (Favier-Teodorescu *et al.,* 1999 – TN 45.4-). Considering the mixing time in the PBR as a few seconds (corresponding to the mean time for cells to gather in all the existing local intensities in the PBR, or a cycle time), it is clear that the environmental relaxation time (some milliseconds) is much smaller than the considered relaxation time for applying TIP (a minute, Dussap, 1988), and hence, the metabolism inside the cells can be safely considered as frozen with respect to the dynamics of change in radiant light energy conditions. Consequently, these latter mechanisms can be removed from the dynamic description of the system (Roels, 1983) justifying the choice of the thermodynamic relaxation time in averaging $\overline{\psi}$. Also, if we compare the TIP relaxation time with the characteristic dynamics for growth (a few hours), it is clear that the main reactions involved in the thermodynamic analysis of photosynthesis can be considered as operating at pseudo-steady-state, justifying this approach *a posteriori*. Finally, because we are not able at present time to formulate a TIP approach for *Rs rubrum* in photoheterotrophic conditions, we used in this work a mean value of $\overline{\psi}$ calculated from the number of quanta $\overline{N}_{h\upsilon}$ involved in the synthesis of one C-mole of biomass

obtained from a metabolic flux approach (Favier-Teodorescu et al., 1999 – TN 45.4; Favier-Teodorescu, 2004).

Conversely, the energetic yield ρ roughly corresponding to the primary efficiency of electron transfer in photosynthetic antennas is strongly dependent on the total available radiant light energy G, and so appears as a local quantity, giving a physical basis to the definition of a local kinetic rate r_X. The maximum value of this yield ρ_M appears at the compensation point for photosynthesis and may be calculated from a thermodynamic treatment of the radiant energy conversion process, which is still a problem under debate (Bejan, 1988; Badescu, 2000). However, a theoretical calculation of the law for changes with available radiant light energy is today an unrealistic challenge, despite tentative analysis (Paillotin, 1974), and one can postulate a law in the form:

$$\rho = \rho_M \frac{K}{K+G} \quad (7)$$

giving with Equation (6) a well-known hyperbolic behaviour for growth:

$$r_X = \rho_M \overline{\psi} \, Ea C_X K \frac{G}{K+G} \quad (8)$$

The calculated coefficients for the local volumetric biomass growth rate are given in Table 1; the sole coefficient experimentally identified is then the half saturation constant for light K.

3.2.2 SPATIAL COUPLING

The calculation of the mean spatial growth rate $<r_X>$ is obtained in accordance with our previous analysis (Cornet and Albiol, 2000). This approach postulates that there may exist an efficient dark zone in the PBR in which, nevertheless, the rate is set by the physical light driven process occurring inside the working illuminated volume. This assumption is supported at the metabolic level by the existence of the reverse electron transfer (RET) mechanism, enabling the synthesis of reducing power $NAD(P)H_2$ for short dark residence time. In the

general case (physical limitation by light transfer), the total volume of the reactor is then divided into three parts:

- a dark volume V_1 in which no growth occurs when the residence time is not too long to allow that a new metabolism to take place ($r_{X1} = 0$);

- a volume V_2 (β fraction) corresponding to the dark operative zone in the PBR with a mean rate set by the illuminated zone;

- a volume V_3 (γ fraction) corresponding to the working illuminated volume in which the local rates r_{X3} are given by Equation (8).

The mean growth rate is then given by:

$$< r_X >= (1-\beta-\gamma)\frac{1}{V_1}\iiint_{V_1} r_{X1}dV + \beta\frac{1}{V_2}\iiint_{V_2} r_{X2}dV + \gamma\frac{1}{V_3}\iiint_{V_3} r_{X3}dV$$

with $r_{X1} = 0$. From the previous hypothesis on the dark operative volume, $\frac{1}{V_2}\iiint_{V_2} r_{X2}dV = \frac{1}{V_3}\iiint_{V_3} r_{X3}dV$, and taking into account the possibility of having only an illuminated surface fraction f_I on the PBR, one obtains:

$$< r_X >= f_I\,(\beta+\gamma)\,\frac{1}{V_3}\iiint_{V_3} r_{X3}dV \qquad (9)$$

Comparisons of Equation (9) with different experimental results in cylindrical PBR led to the simple (and expected?) result that it was necessary to choose $\beta = \gamma$. The final formula giving the mean growth rate is then:

$$< r_X >= f_I\,2\gamma\frac{1}{V_3}\iiint_{V_3} r_{X3}dV \qquad (10)$$

This formula is correct only if $\gamma \le 0.5$ (physical limitation by light transfer); otherwise, Equation (9) applies with $\beta + \gamma = 1$ ($\beta \le 0.5$), showing that in physical limitation, the PBR is twice more efficient as in the kinetic regime ($\gamma = 1$; $V_3 = V_T$).

In both cases, the working illuminated volume V_3 is defined considering the minimal radiant light energy available for photosynthesis G_{min} (Table 1), independently determined from the appearance of the linear behaviour of batch cultures (Cornet and Albiol, 2000). In the case of one-dimensional approximation for the radiant light transfer (Eq. 5), the volume integral in Eq. (9-10) reduces to a simple integral in which the working illuminated volume is only determined from the calculation of the radial illuminated part of the PBR (Cornet *et al*., 1995; Cornet *et al*., 1998).

Importantly, it must be noticed that the previous hypothesis taking $\beta = \gamma$ is not independent of the hydrodynamics, or roughly speaking, of the mixing time in the PBR. Clearly, the cycle time or the light/dark exposure frequency in the completely stirred tank reactor may affect the biomass productivity, especially if a dark efficient zone exists (the β fraction) in heterotrophic conditions. This point has been investigated showing that the light-limited productivities were impaired to some extent if a minimum rotation speed (i.e. a maximum mixing time) was not respected in the PBR, invalidating in these cases the $\beta = \gamma$ assumption. Because this discussion will require further lengthy developments and additional experimental work, we limited this previous analysis to results for which the best mixing conditions were satisfied, i.e. as previously stated, to experiments with a rotation speed of at least 400 rpm.

4 ADDITIONAL LAWS FOR METABOLIC CHANGES

At the present time, it is not possible to perform a complete thermodynamic analysis for the photosystem of *Rs. rubrum* as it was done for *Arthrospira* (Cornet *et al*., 1998). Consequently, we are unable to provide a general predictive law for the theoretical calculation of the $P/2e^-$ ratio in the cell, or for the calculation of the key energetic balanced synthesized metabolite versus the incident light flux (Cornet *et al.*, 1998). For *Rs. rubrum*, this key metabolite is clearly the poly-β-hydroxybutyrate which accumulates inside the cells (Cornet *et al*., 2005; TN 49.3) when the incident light flux q_R is varied. By analogy with the theoretical analysis performed for *Arthrospira* and according to the previous experimental results obtained with

Rs. rubrum (Cornet *et al*., 2005; TN 49.3), we propose the following relationship to take into account changes in the mole fraction of PHB versus the incident light flux:

$$x_{PHB} = 0.3 \ln\left(1 + \frac{q_R}{100}\right) \quad (11)$$

The choice in relating the mole fraction to a general spatial information item (the incident radiant flux q_R) rather than a local variable in the PBR relies on the assumptions already discussed to apply the thermodynamics or the flux methods to the metabolism of the micro-organism. This approach is validated by the numerous experimental results obtained for the exopolysaccharide content in *Arthrospira platensis* cultivation (Cornet *et al.*, 1998). Also, the general form of the law (11) has a physical basis in defining light averaged spatial values in the PBR (not yet published). This makes it possible to calculate the PHB content in the total biomass and then establish the global stoichiometry as presented in a following section. This stoichiometry defines the yields for substrates and products, and then their respective molar rates, in particular for the CO_2 evolution.

At the same time, because there exists a very complex regulation of the *Rhodospirillum* photosystem by the pigment content, we need to be able to take into account the metabolic deviations occurring, especially in the bacteriochlorophyll *a* content, when the degree of light limitation (the working illuminated fraction γ) is varied. These changes have no effect on global stoichiometry, but the pigment content is strongly involved in the definition of the absorption coefficient for light (Equation 3), and therefore in the radiative transfer description. From the experimental results obtained varying q_R and the biomass concentration C_X in a broad domain, we can suppose that for a low value of the illuminated fraction γ in the PBR (strong light limitation), the pigment content remains constant in the active biomass, and so it decreases with the incident light flux increasing the PHB content (Eq. 11). This is gradually modulated as γ approaches 1 (in a kinetic regime) for which the pigment content appears independent of q_R and constant at its minimal value (1%). The law for the mass pigment fraction in the cells w_{PIG} then takes the following form (for $0 < q_R < 400$ W m^{-2}):

$$w_{PIG} = \frac{2.4(1 - w_{PHB}) - (1.4 - 2.4\, w_{PHB})\ \gamma}{100} \quad (12)$$

where w_{PHB} is the mass fraction for PHB in the total biomass, calculated from Equation (11). The illuminated fraction γ appears here as the main lumped parameter to formulate the coupling between the mass pigment content and the radiant light transfer. The value of the mass absorption coefficient *Ea* versus the incident light flux onto the PBR q_R is then easily calculated by direct proportionality from Eqs. (11-12) (Table 1).

This relation shows that there is no dependence for *Ea* when the PBR is entirely illuminated (i.e. γ= 1; w = 0.01), as experimentally confirmed in the domain [50-400 W m^{-2}], and that the pigment content is also minimum at very high light flux (400 W m^{-2}) for any value of γ.

5 SPATIAL MASS BALANCE FOR BIOMASS

When the kinetic model enabling the calculation of the mean volumetric growth rate $<r_X>$ has been established, the dynamic or steady-state behaviour of the total biomass concentration is then calculated applying the overall mass balance on the PBR (considered as a completely stirred tank reactor with a residence time τ) and assuming that there is no biomass in the feed:

$$\frac{d\,C_X}{d\,t} = < r_X > - \frac{C_X}{\tau} \quad (13)$$

A Fortran code was developed to find the steady state solutions of Eq. (13), with Equations (3-12) for the calculation of the mean biomass volumetric growth rate, and to study their stability (see paragraph 8 below). It must be pointed out that the described kinetic model presents a high degree of non-linearity, and so finding the numerical solutions of Eq. (13) near the steady state is arduous, especially for high biomass concentrations, and may become CPU time consuming.

6 GENERALISING STOICHIOMETRIC MODELLING ON ACETATE

From elemental analysis on biomass obtained in precultures, the elemental formula for active biomass of *Rhodospirillum rubrum* ATCC 25903 (i.e. without intracellular PHB) was established as:

$$C H_{1.75} O_{0.34} N_{0.188} S_{0.006} P_{0.014}$$

Consequently, in regular culture conditions (i.e. without excretion of acetoacetate nor polyphosphate accumulation in the cells), the stoichiometric equation for the growth of *Rs rubrum* in any condition of intracellular PHB content (mole fraction *x*) is:

$$C_2H_4O_2 + a\ H_2SO_4 + b\ H_3PO_4 + c\ NH_3 \xrightarrow{<r_X>} \underbrace{2(1-x)(1-y)\ \underbrace{CH_{1,75}O_{0,34}N_{0,188}S_{0,006}P_{0,014}}_{\text{Active Biomass}} + 2x(1-y)\ \underbrace{CH_{1,51}O_{0,50}}_{\text{Polyhydroxybutyrate}}}_{\text{Total Biomass}} + 2y\ CO_2 + d\ H_2O \quad (14)$$

y corresponds to the mole fraction of produced carbon which is released as CO_2; *x* is the mole fraction of PHB in the total biomass, depending only of the radiant incident light flux onto the PBR as mentioned above (Eq. 11).

If the PHB content *x* is known, the coefficients reduction of Eq. (14) with no degree of freedom is straightforward:

$$y = \frac{0.612 - 0.102x}{4.612 - 0.102x}$$

a = 2(1-*x*)(1-*y*) 0.006

b = 2(1-*x*)(1-*y*) 0.014

c = 2(1-*x*)(1-*y*) 0.188

d = 2 – 0.52(1-*x*)(1-*y*) – 4*y* – *x*(1-*y*)

enabling to calculate the total biomass elemental formula from the values of x and y and giving the final stoichiometry. For example, with a PHB content $x = 0.2$, one has:

$$C_2H_4O_2 + 0.00836\ H_2SO_4 + 0.0195\ H_3PO_4 + 0.262\ NH_3 \xrightarrow{<r_x>} 1.7423\ CH_{1.702}O_{0.37}N_{0.15}S_{0.0048}P_{0.01} + 0.2577\ CO_2 + 0.948\ H_2O \quad (15)$$

Finally, the mass conversion yield for acetate $Y_{X/S}$ is also obtained by:

$$Y_{X/S} = [2(1-x)(1-y) + 2x(1-y)]\frac{[12 + Z_H + 16Z_O + 14Z_N + 32Z_S + 31Z_P]}{60} \quad (16)$$

where the *Zi*'s are the stoichiometric coefficients for each element *i* in the total biomass. This analysis allows the calculation of the rates of consumption of substrates or the rates of product evolution providing $<r_X>$ is known from the kinetic part of the model. It will be extensively used for comparison with the experimental results described below.

7 RESULTS AND DISCUSSION

7.1 Analysis of the first critical mode

In continuous mode and at constant incident light flux q_R, the degree of light limitation (the γ fraction) is easily modified in the PBR by varying the residence time τ (Eq. 13). At short residence times, the biomass concentration and the productivity are low and the PBR operates in kinetic mode, i.e., the incident light is in excess and the whole culture volume is illuminated ($\gamma = 1$). The PBR functioning then displays stable behaviour which is clearly represented on Figure 2 by model calculation at an incident light flux q_R of 100 W m^{-2} with a residence time τ ranging between 4 h (washing out limit) and 9 h.

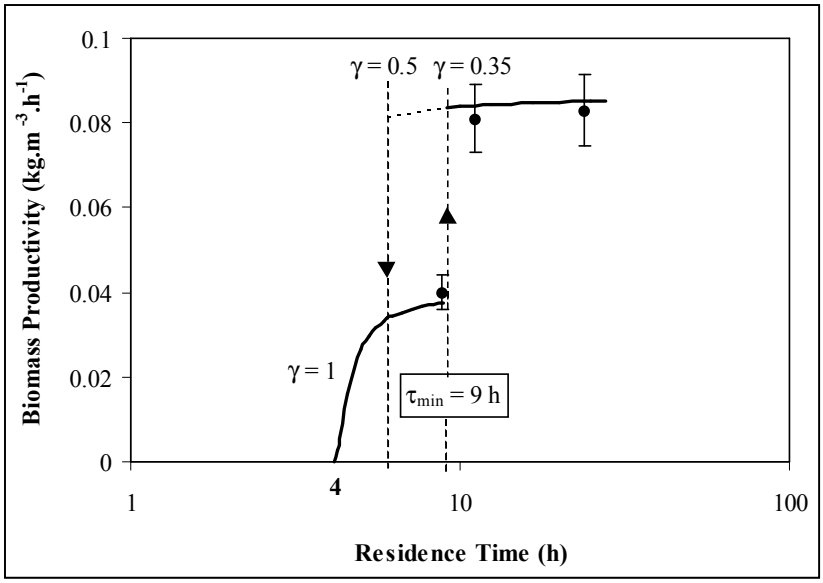


Figure 2: Comparison between experimental and model biomass productivities obtained in a photobioreactor illuminated at q_R = 100 W m^{-2} and operating in continuous mode with ranging residence time (logarithmic scale). The critical residence time τ_{min} between kinetic and light-limited regimes is indicated, together with the particular values for the illuminated fraction γ in the PBR, corresponding to the hysteresis effect for the unstable domain (sub-critical bifurcation).

(———) Stable functioning for the PBR; (– – –) Unstable domain; (•) Experiment

This stable branch is confirmed by the experimental result obtained at steady state for τ = 8.8 h (Fig. 2). At a critical residence time value τ_{min} (τ_{min} = 9 h) or higher, the steady state biomass concentration increases, so that γ decreases, and the kinetic regime is no longer satisfied in the PBR. The model calculations (solid line) show a twofold increase in productivity (Fig. 2). This corresponds to the appearance of a physical limitation by light energy transfer rate, only characterized by the incident light flux q_R and the specific illuminated area of the PBR. In these cases, the illuminated fraction γ is lower than 0.5 and the reactor productivity is given by Eq. (10). This functioning is stable as confirmed by the experimental values obtained at τ = 11.2 h and 23.8 h respectively (Fig. 2). This is an illustration of the efficiency of the

intermediate dark zone (β fraction, Eq. 9) enabling highest productivities only in conditions of physical limitation by light energy transfer. As previously discussed in the model section, the gain in productivity, depending of the importance of the dark efficient zone (β fraction) is strongly related to the mixing time in the PBR. Moreover, mixing time values have been invoked as a justification in formulating the coupling between light transfer and kinetic rates. Independent experiments were conducted for hydrodynamic characterization of the used PBR (Cornet, 1992) showing a very good agreement with the correlation proposed by Nagata (1975), then enabling to use the relation $N.t_m = 25$ to calculate the mixing time t_m in any condition of rotation speed N with a confidence interval of ten percents. As preliminary explained, all the experiments reported in this paper were led at 400 rpm in order to satisfy the assumption $\beta = \gamma$ (the highest value for β) for the dark efficient zone in the PBR. In this case, the mixing time is found to about four seconds, corresponding to a cycle time for cells and enabling to calculate the mean residence time of cells in light and darkness during a cycle, or the light/dark exposure frequency. These results are easily obtained indeed from the calculation of the working illuminated fraction γ in the PBR at a given steady state concentration and incident light flux. Table 2 summarizes the results, showing that, at the opposite of the conclusions recently published for an autotrophic air-lift PBR (Barbosa *et al*., 2003), the frequency of light exposure has no effect on the productivity in light-limited conditions if a sufficient short threshold cycle time is respected by mixing.

Finally, between 6 h and 9 h of residence time, model calculations show, surprisingly, that two regimes may coexist (two different numerical solutions can be obtained by Eq. 13); a low productivity in kinetic regime with $\gamma = 1$, and a high productivity in light-limited conditions with $\gamma \leq 0.5$. For this latter case, the first point solution of Eq. (13) appears at a critical value of $\tau'_{\min}$ = 6 h for the residence time, corresponding exactly to a value for the illuminated fraction γ of 0.5. This demonstrates that it is not possible to obtain steady state solutions in continuous cultures with values of γ ranging between 0.5 and 1, whereas these conditions are generally encountered in batch culture conditions. Moreover, experimental results have demonstrated that this branch (dotted line in Fig. 2) was unstable and led rapidly to the washing out of the PBR.

	Kinetic Regime (low productivity)	**Physical limitation by light (maximum productivity with $\beta = \gamma$)**			
	$\gamma = 1$	$\gamma = 0.276$	$\gamma = 0.249$	$\gamma = 0.195$	$\gamma = 0.123$
	q_R = 100-250 Wm^{-2} C_X = 0.4-0.5 kg m^{-3}	q_R = 100 Wm^{-2} C_X = 0.9 kg m^{-3}	q_R = 250 Wm^{-2} C_X = 1.5 kg m^{-3}	q_R = 50 Wm^{-2} C_X = 1.2 kg m^{-3}	q_R = 100 Wm^{-2} C_X = 2.0 kg m^{-3}
Cycle time t_c (s)	3.8				
Light exposure time t_l (s)	3.8	1.1	1.0	0.8	0.5
Dark inefficient exposure time t_d (s)	0	1.6	1.8	2.2	2.8

Table 2: Assessment of light exposure times (t_l) and dark inefficient exposure times (t_d) during a cycle time in the photobioreactor at 400 rpm. The different conditions for incident light flux q_R and biomass concentration C_X are respectively indicated for each experiment.

This behaviour may be clarified by the so-called nonlinear dynamics theory. At the critical residence time τ_{min}, the appearance of a limit cycle with a finite amplitude (a trivial steady-state solution of Eq. 13 exists with $C_X = \langle r_X \rangle = 0$); no marginal stability at the bifurcation point, and a hysteresis phenomenon as previously explained between $\tau'_{\min} = 6$ h and $\tau_{min} = 9$ h of residence time, correspond to a typical sub-critical bifurcation (Bergé *et al.*, 1988; Thompson and Stewart, 1991). The instability observed in the range $[\tau'_{\min}; \tau_{min}]$ of residence time could then result in an oscillating stable limit cycle leading to the washing out of the PBR (trivial solution with $\langle r_X \rangle = 0$).

The general behaviour depicted in Fig. 2 at $q_R = 100$ W m^{-2} was confirmed by experiments at different incident light fluxes (Cornet *et al.*, 2005 – TN 49.3). For example, at $q_R = 50$ W m^{-2}, the calculated critical residence time τ_{min} was 10.5 h, and so a cultivation with $\tau = 17$ h (higher residence time) gave a stable steady state at high productivity in accordance with the model calculation (Table 3). Moreover, a dynamic experiment was performed at $q_R = 250$ W m^{-2} with a calculated critical residence time $\tau_{min} = 11.5$ h. The residence time was initially kept at a low

value affording a stable steady state in kinetic mode (γ= 1, Fig. 3). It was then increased above the critical value (τ= 12.5 h), leading to an increase in productivity up to a maximum value, in close agreement with the model calculations (solid line, Fig. 3). Finally, the residence time was decreased under the critical value (τ= 11.1 h) to operate in the non-stable branch, leading to a rapid washing out of the PBR.

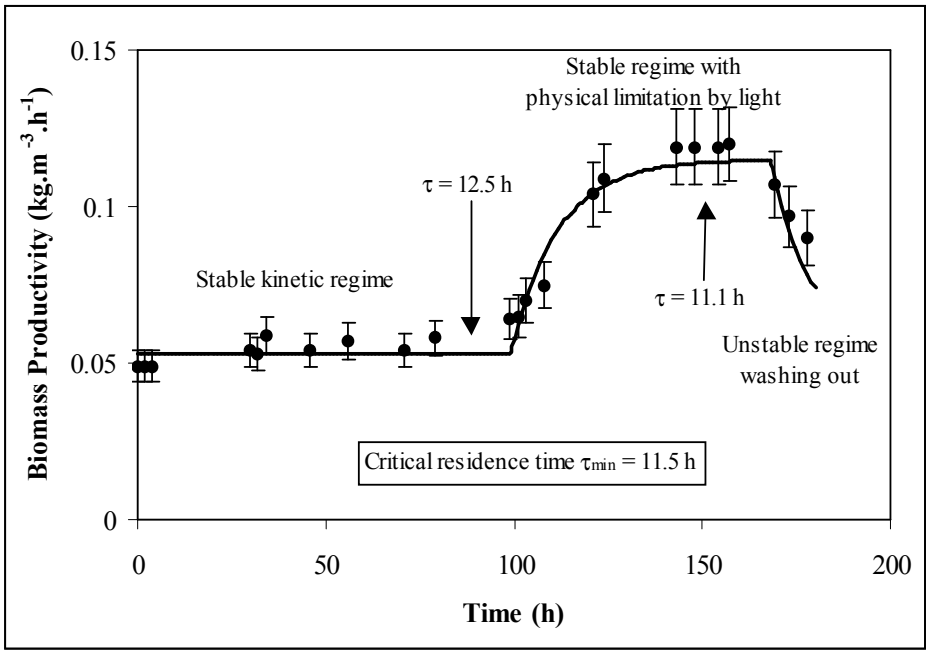


Figure 3: Comparison between model and experimental data for the biomass productivity obtained in a continuous photobioreactor illuminated at q_R = 250 W m^{-2}. The residence time was slightly varied around the calculated critical value τ_{min} = 11.5 h. From a stable kinetic regime, a higher value (τ = 12.5 h) provides the maximum productivity in light-limited condition, whereas a lower value (τ= 11.1 h) leads to the unstable domain, and to the washing out of the reactor.

All the stable and steady state results for biomass productivities obtained at different non-photoinhibiting incident light fluxes and summarised in Table 3 can be presented in a synthetic

manner, enlightening the sub-critical bifurcation phenomenon for the PBR functioning in continuous mode.

q_R (W m^{-2})	**Stable kinetic regime (γ= 1)**		**Stable regime with physical limitation by light (γ< 0.5)**	
	Experimental productivity [kg m^{-3} h^{-1}]	Model productivity [kg m^{-3} h^{-1}]	Experimental productivity [kg m^{-3} h^{-1}]	Model productivity [kg m^{-3} h^{-1}]
50	n d		0.068 ± 0.007	0.062
			τ= 17 h (γ= 0.195)	
100	0.042 ± 0.005	0.038	0.082 ± 0.009	0.083
	τ= 8.8 h		τ= 11.2 (γ= 0.276) and 23.8 h (γ= 0.123)	
250	0.054 ± 0.006	0.052	0.115 ± 0.01	0.113
	τ= 11 h		τ= 12.5 h (γ= 0.249)	

Table 3: Comparison between model and experimental productivities in stable, steady-state continuous mode for different radiant incident fluxes q_R and different residence times τ in the photobioreactor. The values of the working illuminated fraction γ for experiments in physical limitation by light are respectively indicated.

In order to obtain a general overview of this problem, it is necessary to define reduced quantities which appears independent of a given value for the incident light flux q_R. Taking for the definition of the reduced residence time τ^* the value corresponding to the critical residence time $\tau^* = \tau/\tau_{\min}$, it is then easy to summarise the results in term of reduced productivity $P^* = P_X/(P_X)_{\max}$ on Figure 4. The results thus obtained show that the value of the sub-critical residence time $\tau^{*\prime}$ was roughly equal to 2/3 in any case and that the model was proved in very good agreement for any input of light energy flux, confirmed by the experimental values in kinetic and physical limitation regimes very close to the critical residence time τ^* = 1 (Fig. 4).

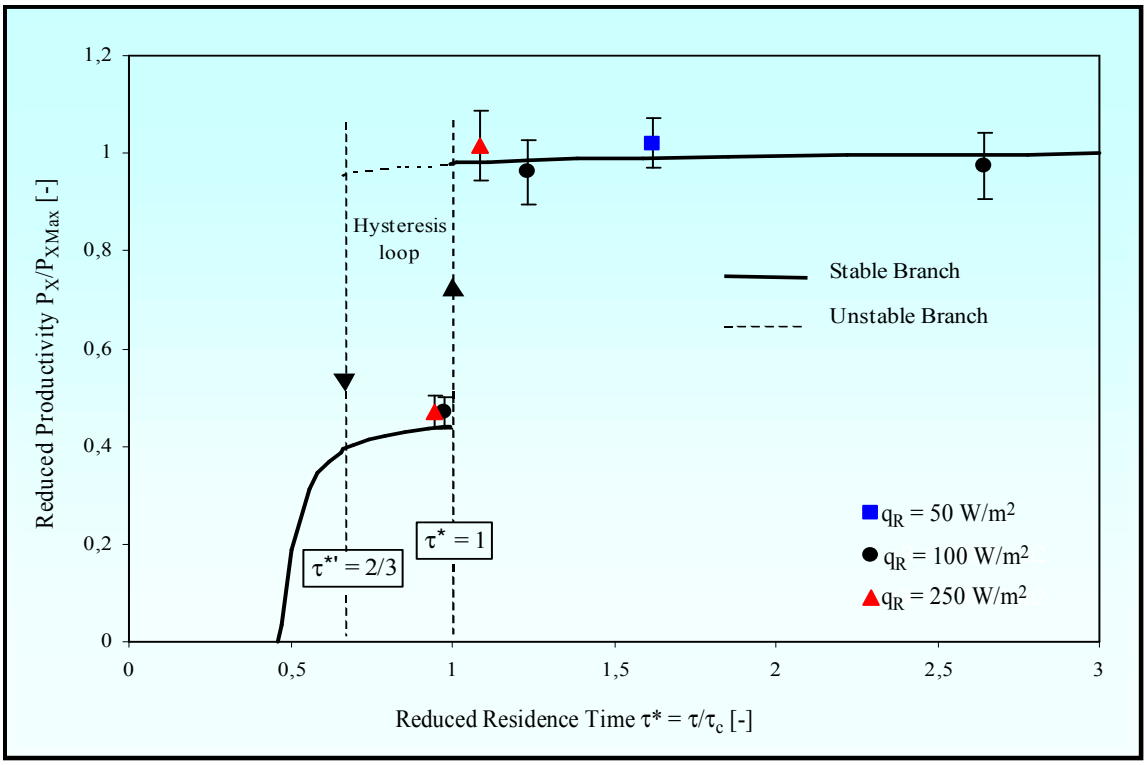


Figure 4: Generalised reduced representation of the sub-critical bifurcation characterised by the doubling of the biomass productivity and a hysteresis loop occurring when cultivating *Rs. rubrum* in continuous PBR. The model calculation is represented by the continuous line (for stable domain) and the dotted line (for unstable domain) and compared to the experimental points corresponding to Table 3 for different incident light fluxes (symbols depicted on the figure). The reduced abscissa is defined from the critical residence time $\tau^* = \tau/\tau_{min}$ for any incident light flux.

From a metabolic point of view, it must be noted that the decrease in biomass growth rate is accompanied by the appearance of acetoacetate in the output flow of the PBR. This implies that this metabolite plays a crucial role in the metabolic deviations occurring on the non-stable branch of the stability diagram (Fig. 2). In all cases, it must be pointed out that experimental results are well predicted by model calculations, both for kinetic results (Table 3 and Fig. 4)

and for stoichiometric results at stable steady state conditions under light limitation (Eqs. 11, 14 and Table 4). In addition, Table 4 clearly shows that the global stoichiometry is not sensitive to the PHB content changes since both experimental results and model calculations gave constant mass and molar conversion yields for acetate and CO_2.

q_R [W m^{-2}]	Global C-formula		PHB mass fraction [%]		Biomass/Acetate mass conversion yield $Y_{X/S}$ [kg/kg]		Mole percentage of evolved CO_2 in the total produced carbon [%]	
	Experimental	Model	Experimental	Model	Experimental	Model	Experimental	Model
50	$CH_{1.71}O_{0.39}N_{0.17}S_{0.004}P_{0.010}$	$CH_{1.72}O_{0.36}N_{0.17}S_{0.005}P_{0.012}$	15 ± 2	12	0.65 ± 0.06	0.647	14 ± 2	13.0
100	$CH_{1.71}O_{0.38}N_{0.16}S_{0.003}P_{0.009}$	$CH_{1.70}O_{0.37}N_{0.15}S_{0.005}P_{0.011}$	20 ± 2	20	0.64 ± 0.06	0.646	13 ± 2	12.9
250	$CH_{1.73}O_{0.36}N_{0.15}S_{0.003}P_{0.008}$	$CH_{1.66}O_{0.40}N_{0.12}S_{0.004}P_{0.009}$	34 ± 4	36	0.64 ± 0.06	0.644	13 ± 2	12.5
400	n.d.	$CH_{1.63}O_{0.42}N_{0.10}S_{0.003}P_{0.007}$	49 ± 5	47	0.63 ± 0.06	0.643	12 ± 2	12.3

Table 4: Main stoichiometric results obtained in stable steady state continuous cultures at different radial incident light flux q_R onto the photobioreactor (these are mean values, except at q_R = 50 W m^{-2}). All the results hold for cultures under limitation by light energy transfer rate ($\gamma < 0.5$) corresponding to the domain of validity for the stoichiometric analysis (Eq. 14 in text).

7.2 Analysis of the second critical mode

The stable behaviour at high productivity under light transfer limitation is not observed at any residence time higher than the critical value τ_{min}. It has been experimentally observed in fact that a second critical value τ_{max} exists from which the PBR productivity begins to oscillate (Favier-Teodorescu *et al.*, 2003 – TN 49.2). The oscillating behaviour has been typically observed on the biomass concentration in the output flow of the reactor, obtained at an incident

radiant light flux q_R of 100 W m^{-2} and with a constant residence time (Fig. 5). It appears at high biomass concentration, and then for low values of γ (< 0.1). Because the biomass productivity oscillates between the maximum value calculated by the model and the half of this maximum, one can postulate that this corresponds to an alternate inactivation of the dark operative volume (β fraction), probably linked to a photosystem regulation mechanism. This regulation also involves the acetoacetate as an intermediate metabolite, the oscillations of its concentration displaying a phase shift with the biomass concentration in the output of the PBR (data not shown). Thus it seems that as previously seen for the non-stable branch at low residence times, the acetoacetate production was concomitant with the inactivation of the dark operative volume in the PBR. However, for high biomass concentration and residence times, this volume was periodically restored, enabling the reactor productivity to oscillate. These metabolic changes were also accompanied by morphological changes of the cells because the trichome division was stopped, leading to very long filaments, and the motility of cells was lost. Similar sustained oscillations in cells concentration for continuous photoautotrophic cultures of *Chlorella vulgaris* were already reported (Javanmardian and Palsson, 1992) with also as a consequence, important changes in the division cycle of cells.

Practically, from Eqs. (9) and (13), it is easy to show that the alternate inactivation of the β fraction can lead to periodic oscillations of the biomass concentration C_X at constant residence time between the two boundary values:

$$\begin{aligned} C_{X\max} &= \frac{1}{2}\tau < r_X > (1+\zeta) \\ C_{X\min} &= \frac{1}{2}\tau < r_X > (2-\zeta) \end{aligned} \qquad (17)$$

where:

$$\zeta = [1 - \exp(-\frac{T}{\tau})] \qquad (18)$$

is a damped factor to take into account that the steady state $C_X^{\infty} = \tau < r_X >$ is never reached because of the existence of the semi-period T for oscillating behaviour. Likewise, noting that $\gamma = V_3/V_T$, one can rewrite Eq. (10) in light limitation as:

$$< r_X >= f_I \frac{2}{V_T} \iiint_{V_3} r_{X3}\, dV = f_I \frac{2}{V_T} \mathcal{I} \quad (19)$$

in which the integral $\mathcal{I}$ does not depend on the biomass concentration C_X or on the time t, and is only a function of the incident light flux q_R and the reactor geometry, i.e., a constant.

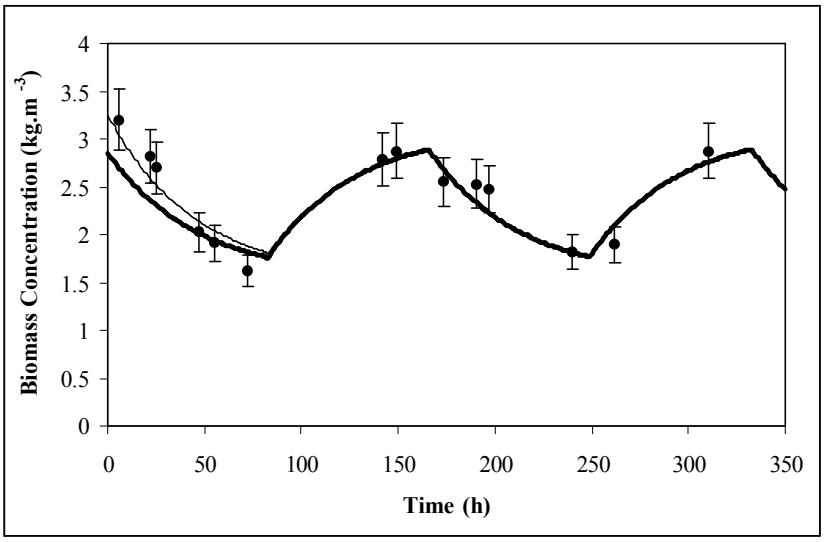


Figure 5: Comparison between experimental data and model for typical oscillations of the biomass concentration in the output flow of the photobioreactor. The conditions for the continuous culture were an incident light flux q_R = 100 W m^{-2} and a constant residence time τ = 43.5 h.

(———) Actual model calculation from a given initial condition

(———) Asymptotic oscillating behaviour given by Equation (20)

This makes it possible to integrate Eq. (13) over a semi-period of time T, and to combine with Eqs. (17-19) to obtain the general relation giving the asymptotic periodic time course for the biomass concentration:

$$C_X(t) = f_I \frac{\tau}{V_T} \mathcal{I} \left\{ \frac{3}{2} + (-1)^{\left\lceil \frac{t}{T} \right\rceil - 1} \left[\zeta e^{-\frac{1}{\tau}\left[t - \left(\left\lceil \frac{t}{T} \right\rceil - 1 \right) T \right]} - \frac{1}{2} \right] \right\} \quad (20)$$

in which $\lceil x \rceil$ is the so-called ceiling function rounding the value of x to the first higher integer, and where $(-1)^{\lceil x \rceil - 1}$ is the square wave function.

Equation 20 has been used on Figure 5 with a semi-period T = 83 h (thick line) and then ζ = 0.85, to display the asymptotic periodic behaviour of the biomass concentration in the output flow of the reactor, whereas the complete model (Eq. 13) was used to take into account the initial condition at a higher value of concentration when the residence time was changed (thin line). These results show first that the asymptotic behaviour is rapidly reached as early as the first period, and second that the model calculations are again in close agreement with the experimental data (Fig. 5). Moreover, if the value of ζ is assumed to be a constant, then Eq. (18) allows the calculation of the semi-period T for any residence time τ in the PBR; however this needs to be verified by further experiments.

7.3 The pigment content regulation as a possible explanation

As previously mentioned in the model descriptions, the coupling between light transfer, stoichiometry and kinetic rates depends strongly on the photosystem functioning in photoheterotrophic conditions. This functioning is intimately linked to a caught light regulation mechanism by cells, which vary their pigment content, i.e., the number and size of photosynthetic antennas.

Using Eq. (5) for light transfer, it is possible to obtain the variation in pigment content versus the biomass concentration in the PBR instead of the illuminated fraction γ, by model calculation (Eq. 12). This was done as an example for an incident light flux q_R of 100 W m^{-2} in Figure 6 showing a close agreement between experimental data and model calculations.

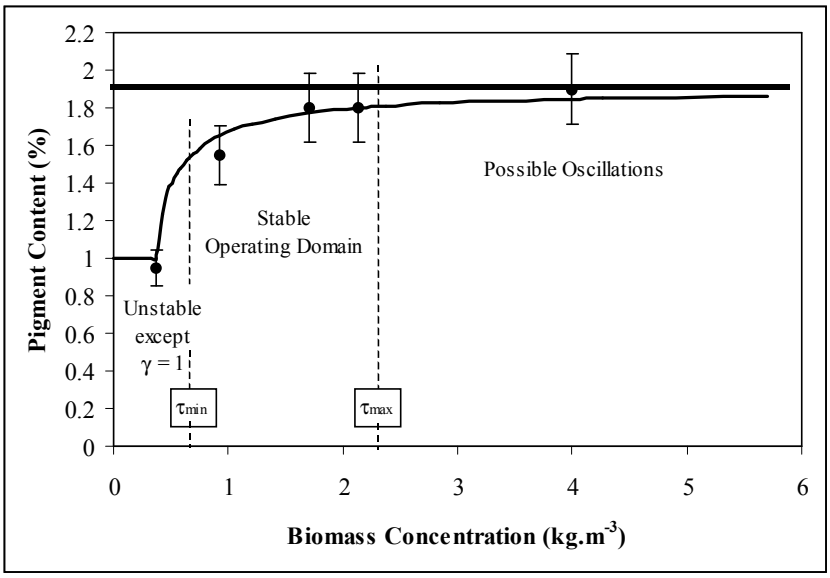


Figure 6: Comparison between model calculation and experimental data for the pigment content versus the biomass concentration in the photobioreactor. The results were obtained at a constant incident light flux q_R = 100 W m^{-2}, and varying the residence time. The experimental points correspond to Figures 2 and 7, and the two critical residence times are represented.

This figure gives some insight on the stability behaviour in the PBR and could serve as a qualitative explanation of the pigment regulation mechanism. The first dashed line corresponds to the minimum critical residence time τ_{min}, i.e. it appears that for biomass concentrations higher than 0.7 kg m^{-3} at this incident light flux, the productivity is stable and maximum because the PBR operates in light transfer limitation (Fig. 2). Likewise, the second dashed line at high concentration corresponds roughly to the appearance of the oscillating behaviour for the productivity in the PBR. These two lines then define the three domains of functioning for the PBR. At low biomass concentration (below τ_{min}), the relation giving the pigment content is very sensitive and it is clear that a very small change in biomass concentration in the PBR involves a marked pigment content regulation mechanism for the photosystem, probably affecting the biomass growth rate. Moreover, in these conditions, the residence time in the

PBR is short, which amplifies the effect of a decrease in growth rate, leading to a rapid decrease in biomass concentration, which in turn emphasizes the pigment content regulation that slackens the growth rate, and so on… Such a mechanism is probably responsible for the washing out of the PBR observed when operating in the unstable branch domain of Figure 2. Conversely, for biomass concentrations higher than 2.4 kg m^{-3} (τ_{max}), the relation between the pigment content and the biomass concentration in the PBR is not sensitive and is nearly a constant. For this reason the above adaptation mechanism becomes impossible, involving greater metabolic and morphologic changes in the cells as an oscillating growth rate. Consequently, the appearance of oscillating behaviour in the PBR is defined in our model when the pigment content approaches its maximum asymptotic value, allowing the calculation of the value of the maximum critical residence time τ_{max} leading to a stable maximum productivity for the PBR.

The model was then used to draw a complete diagram for the general behaviour of a cylindrical PBR illuminated at a mean constant incident light flux of 100 W m^{-2} (Fig. 7). This includes a possible two-points biomass concentration (given by Eq. 17) oscillating behaviour from a calculated critical residence time τ_{max} = 28 h. Experimental results obtained in each different set of conditions are in close agreement with these calculations but this needs to be confirmed by further experiments at other incident light fluxes in the operating domain (1 – 300 W m^{-2}).

7.4 The operating domain and control of the PBR

For any engineering application using continuous cultures in PBR with *Rs. rubrum* in photoheterotrophic conditions, it is obviously desirable to define a stable domain with the highest productivity. From the analysis presented in this paper and for a given incident light flux, this operating domain ranges clearly between the two critical residence times τ_{min} and τ_{max} defined above (see Fig. 7).

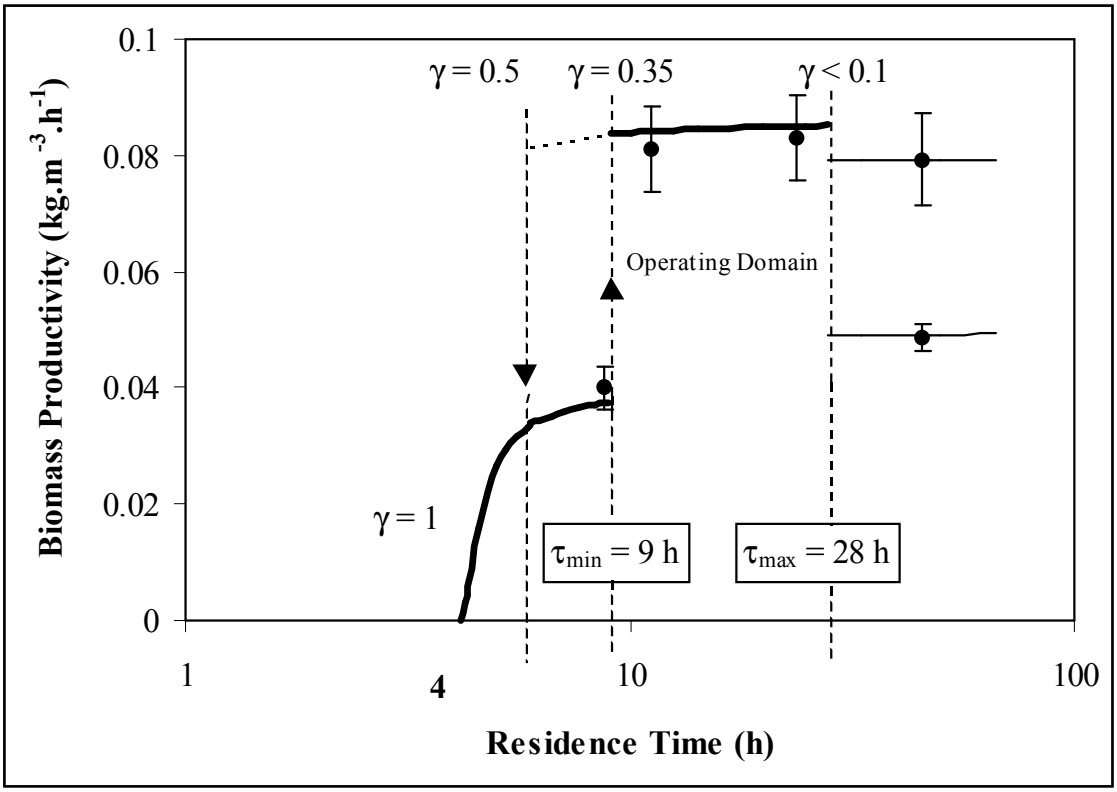


Figure 7: Complete stability diagram with the same conditions as Figure 2 (q_R = 100 W m^{-2}), but showing the appearance of the second critical residence time τ_{max} with oscillating behaviour. The comparison is made between experimental productivities and model calculations in this condition. The engineering operating domain (corresponding to a factor 3 in which the liquid flow rate may be chosen) between the two critical values is indicated.
(———) Stable functioning for the PBR; (- - - - -) Unstable domain; (———) Oscillating behaviour

Controlling the PBR by incident light flux is not a trivial procedure because operating in a chemostat can lead to biomass concentration changes and then to washing out or oscillating behaviour. Likewise, operating in a turbidostat can lead to residence time changes with the

same consequences. The best option is to work at constant working illuminated fraction γ, i.e. to operate in a "luminostat".

Because the numerical values of τ_{min} and τ_{max} strongly depend on the incident light flux q_R, the model was used with q_R ranging between 1 and 300 W m^{-2} (operating domain without photoinhibition) in order to define the corresponding common values of γ available at any incident flux onto the PBR. The results are very surprising and show a very narrow range for which γ must remain at around 0.2 $\pm$ 20% if it is desired to control the productivity over the total range of incident light flux q_R. Practically, this implies accurately measuring the mean incident light flux and the biomass concentration in the PBR, and then adapting the residence time in order to maintain the value of γ at around 0.2 in all cases. This, of course, is only feasible by model-based predictive control (Cornet *et al.*, 2001), and requires having an accurate and sound knowledge description of the radiant light transfer in the PBR for γ calculation.

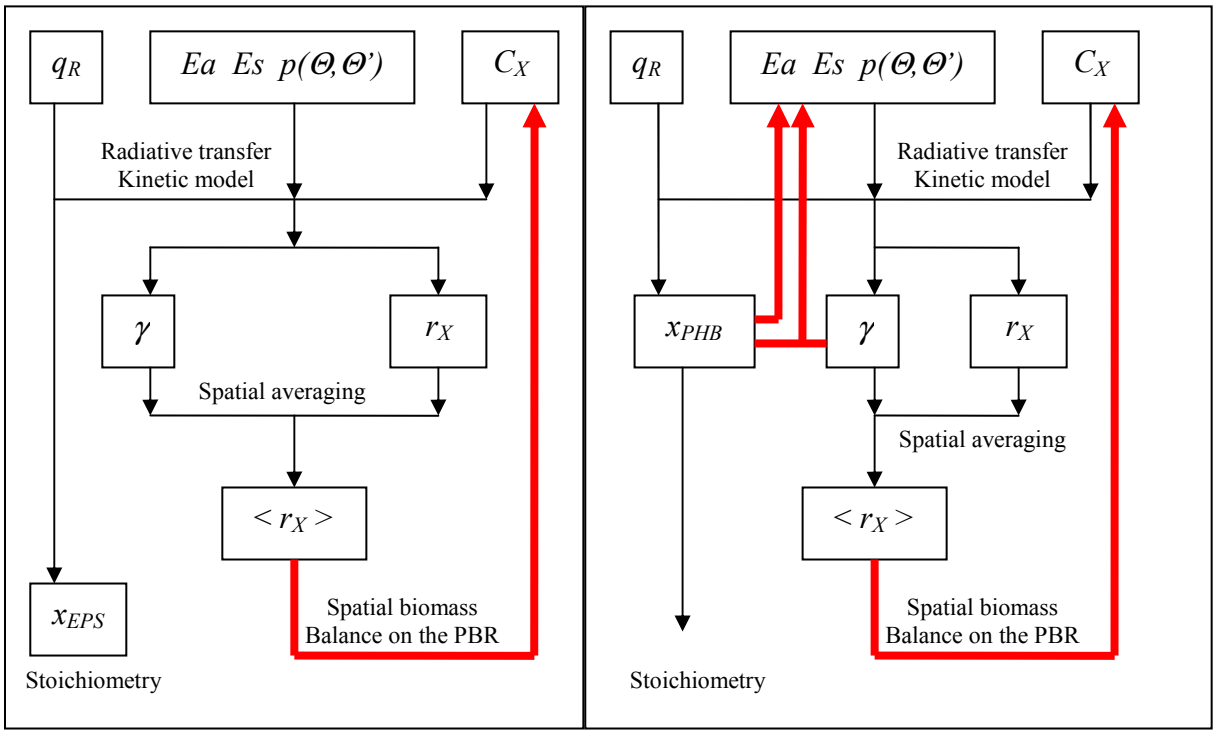
8 THE Fortran CODE RHODOCONTCYL

All the main aspects of the model presented above have been used to write a Fortran code for the cultivation of *Rs rubrum* in a radially illuminated, cylindrical and continuous PBR. This program is mainly devoted to the research of steady state solutions for biomass productivity (and named for this reason Rhodocontcyl) using eq. (13) taking the accumulation term $d/dt = 0$, and provides all the kinetic and stoichiometric information available from the model calculation (Figure 8).

```
*****************************************************

Productivite (g.l-1.h-1) =      0.08295913

Concentration en Biomasse (g/l) =      0.921768111

Fraction volumique eclairee =      0.279763835

Fraction volumique efficace =     0.559527669

Fraction massique en Pigments =      0.0166101506

Fraction massique en PHB =      0.200926954

Rendement massique en Acetate Ys/x =      1.54725217

Concentration en Acetate (g/l) =      2.57379229

Pourcentage molaire de CO2 produit =     0.128690211

Valeur de ALPHA =      0.925065173

Formule Elementaire de la Biomasse Totale
_____________________________________

C    1
H    1.7000934
O    0.373271067
N    0.148906494
S    0.00475233512
P    0.0110887822

*****************************************************
```

Figure 8: Example of the output obtained when using the rhodocontcyl software for simulation in stable steady state conditions. The case study corresponds to an incident light flux of 100 W m^{-2} and a residence time of 11.2 hours, in very good agreement with the experimental results given on Figure 2 and Table 3.

It must be emphasised that the numerical convergence may be, in some cases, very difficult to achieve because of the high non-linearity of the problem in comparison with the well-known case for *Arthrospira platensis* (see scheme 1).



a- *Arthrospira* **b**- *Rhodospirillum*

Scheme 1: The non-linearities in finding the steady state solution for the biomass productivity in a PBR cultivating (**a**) *Arthrospira platensis* and (**b**) *Rhodospirillum rubrum*.

When the proposed conditions (incident light flux and residence time) could lead to the appearance of an oscillating behaviour for the biomass productivity in the PBR, this possibility is analysed (from the preliminary approach described in 7.3) and the code returns a message as described in Figure 9. This code may then be used to calculate the stable steady states in biomass productivity in kinetic or light-limited regimes and may be considered as a first tool to define the operating engineering domain for a given geometry of PBR and incident energy input.

```
*****************************************************

 !   COMPORTEMENT PERIODIQUE   !

DEMI-PERIODE =   94.9999988 heures

Productivite maximale (g.l-1.h-1) =     0.0782903035
Productivite minimale (g.l-1.h-1) =     0.0486373488

Concentration en Biomasse maximale (g/l) =     3.91451517
Concentration en Biomasse minimale (g/l) =     2.43186744

Formule Elementaire de la Biomasse Totale
_____________________________________

C   1
H   1.7000934
O   0.373271067
N   0.148906494
S   0.00475233512
P   0.0110887822

*****************************************************
```

Figure 9: Example of the output produced when using the rhodocontcyl software for simulation in which a possible oscillating behaviour was identified. The case study corresponds to an incident light flux of 100 W m^{-2} and a residence time of 43.5 hours, in good agreement with the experimental results given on Figure 7.

9 CONCLUSIONS

The internal structure of the first knowledge model presented in this paper appears well adapted to predict and simulate the apparently surprising regimes obtained for biomass productivity when the photoheterotrophic bacterium *Rs. rubrum* is cultivated in a continuous photobioreactor. In particular, its ability to calculate the minimum residence time corresponding to a sub-critical bifurcation for productivity, and splitting into stable and unstable domains the high productivity obtained in physical light transfer limitation has been proved. In the same manner, a method for the calculation of the maximum residence time

leading to an oscillating behaviour of the PBR is proposed and discussed, and the ability of the model to simulate the oscillations in biomass concentration has been checked. Stoichiometric aspects have also been investigated and a law proposed for the calculation of the PHB content inside the cells when the incident light flux onto the PBR is varied.

However, further experiments are necessary to confirm the validity of the calculated critical residence times in many different conditions of light fluxes, flow rates and PBR geometries, especially for the appearance of the oscillating behaviour. Additionally, even if the proposed model appears structurally correct, an important additional work also needs to be done to increase the accuracy of the light transfer model, including a predictive calculation of the radiative properties of the cells from their actual form and varying the pigment and PHB contents, and also establishing an exact expression for eq. 5 in cylindrical coordinates. Finally a more thorough knowledge of the pigment content regulation mechanism, together with a prediction of the PHB content changes from $P/2e^-$ ratio calculations could be achieved, applying the thermodynamics of irreversible processes to the *Rs. rubrum* photosystem functioning in photoheterotrophic mode.

In its present form, however, the model can be used to set operating conditions for the main process variables (incident light flux, residence time…, using for example the Fortran code Rhodocontcyl), affording high and stable biomass productivities in a PBR. In the near future, it will be assessed in dynamic conditions as a tool for model-based predictive control of heterotrophic photobioreactors operating in continuous mode.

Notations

a	Mean volumetric absorption coefficient for the considered domain of wavelength [m^{-1}]
$\mathcal{A}$	Local Volumetric Rate of radiant Energy Absorbed (LVREA) [W m^{-3}]
b	Fraction of light back-scattered by micro-organisms [dimensionless]
C_X	Biomass concentration [kg m^{-3}]
Ea	Mean mass absorption coefficient for the considered domain of wavelength [m^2 kg^{-1}]
Es	Mean mass scattering coefficient for the considered domain of wavelength [m^2 kg^{-1}]
f_I	Illuminated surface fraction of the photobioreactor [dimensionless]
I	Specific radiant light intensity for the considered domain of wavelength [W m^{-2}]
G	Irradiance for the considered domain of wavelength [W m^{-2}]
G_{min}	Minimal irradiance available by photosynthesis in photoheterotrophic conditions [W m^{-2}]
K	Half saturation constant for light energetic yield conversion [W m^{-2}]
N	Rotation speed [s^{-1}]
p	Normalized phase function for scattering [dimensionless]
$\mathbf{q}$	Radiant light energy flux for the considered domain of wavelength [W m^{-2}]
q_R	Radial incident light flux on the reactor for the considered domain of wavelength [W m^{-2}]
r	Radius [m]
R	Radius of the photobioreactor [m]
r_X	Volumetric biomass growth rate [kg m^{-3} h^{-1}]
s	Mean volumetric scattering coefficient for the considered domain of wavelength [m^{-1}]
t	Time [h]
t_c	Cycle time for cells [s]
t_d	Dark inefficient residence time for cells [s]
t_l	Light residence time for cells [s]
t_m	Mixing time in the reactor [s]
T	Semi-period for oscillations [h]
V	Volume [m^3]
V_T	Total volume of the photobioreactor [m^3]
w_{PHB}	Mass fraction for PHB in total biomass [dimensionless]

w_{PIG}	Mass fraction for pigments in total biomass [dimensionless]
x_{PHB}	Mole fraction for PHB in total biomass [dimensionless]
$Y_{X/S}$	Biomass/Acetate mass conversion yield [$kg_{biomass}\ kg_{acetate}^{-1}$]

Greek Letters

α	Lumped parameter for optical radiative properties of the medium [dimensionless]
β	Fraction for dark operative volume in the photobioreactor [dimensionless]
γ	Fraction for working illuminated volume in the photobioreactor [dimensionless]
δ	Length constant characterizing the light transfer [m^{-1}]
θ	Angle [rad]
ζ	Damped factor [dimensionless]
ρ	Energetic yield for photon conversion [dimensionless]
ρ_M	Maximum energetic yield for photon conversion [dimensionless]
τ	Residence time [h]
τ_{min}	Critical minimum residence time [h]
$\tau'_{\min}$	Sub-critical minimum residence time [h]
τ_{max}	Critical maximum residence time [h]
$\overline{\psi}$	Mean mass stoichiometric quantum yield for the considered domain of wavelength [$kg\ J^{-1}$]
ω	Solid angle [sr]

Averaged Quantities

$\hat{x} = 2\pi \int_0^{\frac{\pi}{2}} x \sin\theta \, d\theta$ or $2\pi \int_{\frac{\pi}{2}}^{\pi} x \sin\theta \, d\theta$ hemispherical averaging

$\bar{x} = \frac{1}{\Delta t} \int_{\Delta t} x \, dt$ time averaging

$< x >= \frac{1}{V} \iiint_V x \, dV$ volumetric averaging

References

BADESCU, V. 2000. Thermodynamics of Solar Energy Conversion into Work. In *Thermodynamics of Energy Conversion and Transport*; Seniutycz, S., De Vos, A., Eds.; Springer Verlag: New York; pp 14-48.

BARBOSA, M. J.; JANSSEN, M.; HAM, N.; TRAMPER, J.; WIJFFELS, R. H. 2003. Microalgae cultivation in air-lift reactors: modeling biomass yield and growth rate as a function of mixing frequency. Biotechnol. Bioeng., **82**: 170-179.

BEJAN, A. **1988**. *Advanced Engineering Thermodynamics*. John Wiley and Sons Inc., New York.

BERGE, P.; POMEAU, Y.; VIDAL, C. **1988**. *L'ordre dans le chaos*. Hermann, Paris, 2nd ed.

BOHREN, C. F.; HUFFMAN, D. R. **1983**. *Absorption and scattering of light by small particles*. John Wiley and Sons Inc., New York.

BREWSTER, M. Q.; TIEN, C. L. 1982. Examination of the two-flux model for radiative transfer in particular systems. *Int. J. Heat Mass Transfer*. **25**: 1905-1907.

CORNET, J.-F. 1992. *Etude cinétique et énergetique d'un photobioreacteur*. Thèse de Doctorat. Université Paris XI Orsay, France, n° 1989.

CORNET, J-F.; ALBIOL, J. **2000.** Modeling photoheterotrophic growth kinetics of *Rhodospirillum rubrum* in rectangular photobioreactors. *Biotechnol. Prog*., **16**: 199-207.

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. 1998. Kinetics and energetics of photosynthetic micro-organisms in photobioreactors. *Adv. Biochem. Eng./Biotechnol*., **59**: 153-224.

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.

CORNET, J-F.; DUSSAP, C-G.; LECLERCQ, J-J. 2001. Simulation, design and model based predictive control of photobioreactors. In Focus on Biotechnology; Hofman, H., Thonart, P., Eds.; Kluwer academic publishers: Dordrecht; **Vol**. **4**, pp 227-238.

CORNET, J.-F.; FAVIER, L.; DUSSAP, C. G. 2003. Modeling stability of photoheterotrophic continuous cultures in photobioreactors. *Biotechnol. Prog*. **19**: 1216-1227.

CORNET, J.-F.; FAVIER-TEODORESCU, L.; DUSSAP, C. G. 2005. Kinetic and stoichiometric analysis of *Rhodospirillum rubrum* growth in cylindrical photobioreactor at different light fluxes – Experimental aspects – Technical Note 49.3, ESA/ESTEC contract 12924/98/NL/MV.

CORNET, J-F.; MARTY, A.; GROS, J-B. 1997. Revised technique for the determination of mean incident light fluxes on photobioreactors. *Biotechnol. Prog*., **13**: 408-415.

DUSSAP C-G. 1998. *Etude thermodynamique et cinétique de la production de polysaccharides microbiens par fermentation en limitation par le transfert d'oxygène. Modèle structuré de la production de xanthane*. Thèse de Doctorat ès Sciences Physiques, Université Blaise Pascal, Clermont-Ferrand, France, n° 409.

FAVIER-TEODORESCU, L. 2004. *Etude cinétique et stoechiométrique de la croissance de* Rhodospirillum rubrum *en photobioréacteur*. PhD Thesis, Université Blaise Pascal, n° DU 1538; n° ordre 411.

FAVIER-TEODORESCU, L.; CORNET, J.-F.; DUSSAP, C. G. 2003. Kinetic and stoichiometric analysis of *Rhodospirillum rubrum* growth in cylindrical photobioreactor at constant incident light flux – Experimental aspects and preliminary modelling – Technical Note 49.2, ESA/ESTEC contract 12924/98/NL/MV.

FAVIER-TEODORESCU, L.; PONS, A.; POUGHON, L. 1999. Stoichiometric analysis of *Rhodospirillum rubrum* growth on different carbon substrates. Technical Note 45.4, ESA/ESTEC Contract 13323/98/NL/MV.

HERBERT, D.; PHIPSS, P. J.; STRANGE, R. E. 1971. *Chemical analysis of Microbial Cell*. In : Methods in microbiology. Norris J.R. and Ribbons D.W. (Ed.), Academic Press New York.

JAVANMARDIAN, M.; PALSSON, B. O. 1992. Continuous photoautotrophic cultures of the eukaryotic alga *Chlorella vulgaris* can exhibit stable oscillatory dynamics. *Biotechnol. Bioeng*., **39**: 487-497.

KOENIGSDORFF, R.; MILLER, F.; ZIEGLER, R. 1991. Calculation of scattering fractions for use in radiative flux models. *Int. J. Heat Mass Transfer*, **34**: 2673-2676.

NAGATA, S. 1975. *Mixing, principles and applications*. John Wiley and Sons Inc., New York.

PAILLOTIN, G. 1974. *Etude théorique des modes de création, de transport et d'utilisation de l'énergie d'excitation électronique chez les plantes supérieures*. Thèse de Doctorat ès Sciences, Université Paris XI – Orsay, France, n° 1380.

ROELS, J. A. 1983. *Energetics and Kinetics in Biotechnology*. Elsevier biomedical press, Amsterdam.

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

SEGERS, L.; VERSTRAETE, W. 1983. Conversion of organic acids to H_2 by *Rhodospirillaceae* to grown with glutamate or dinitrogen as nitrogen source. *Biotech. Bioeng*., **25**: 2843-2853.

SUHAIMI, M.; LIESSENS, J.; VERSTRAETE, W. 1987. NH_4^+-N assimilation by *Rhodobacter capsulatus* ATCC 23782 grown axenically and non-axenically in N and C rich media. *J. Appl. Bacteriol*., **62**: 53-64.

THOMPSON, J. M. T.; STEWART, H. B. 1991. *Nonlinear dynamics and chaos*. John Wiley and Sons Ltd, New York, 7th ed.

VAN DE HULST, H. C. 1981. *Light scattering by small particles*. Dover Publications Inc., 2nd ed.

VERNON, L. P.; GARCIA, A. F. 1967. Pigment-Protein complexes derived from *Rhodospirillum rubrum* chromatophores by enzymatic digestion. *Biochim. Biophys. Acta*, **143**: 144-153.