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MODELING OF PHYSICAL LIMITATIONS IN PHOTOBIOREACTORS.

APPLICATIONS TO SIMULATION AND CONTROL OF THE *Spirulina* COMPARTMENT OF THE MELiSSA ARTIFICIAL ECOSYSTEM.

TECHNICAL NOTE 19.2.

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MODELING OF PHYSICAL LIMITATIONS IN PHOTOBIOREACTORS.

TN.19.2: Modeling of exopolysaccharide synthesis in cultures of *Spirulina platensis.*

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The cyanobacterium *Spirulina platensis* cultivated in photobioreactor produces an associated exopolysaccharide in different quantities depending on the culture conditions. Preliminary results have shown that the percentage of exopolysaccharide in the total biomass produced varied with the limitation occuring in the photobioreactor:

- under physical limitation by light, this percentage is increased with the radiant light energy available in the photobioreactor;

- under mineral limitation by nitrate or sulfate, *S. platensis* synthesizes only carbohydrates such as intracellular glycogen and exopolysaccharide (CORNET *et al.*, 1992b).

These modifications in the metabolic pathways of the microorganisms lead to important changes in the global formula of the produced biomass and the conversion yields of substrates in products which are often considered as constant may vary with the cultures conditions. This invalidates classical stoichiometric approach for modeling growth rates in photobioreactors.

Based on a single stoichiometric equation, with constant conversion yields, the model proposed in the Technical Note 19.1 was unable to take into account such metabolic deviations because the exopolysaccharide was supposed to be produced with a constant rate of 20% of the total biomass whatever the value of the light radiant incident flux, even though this percentage is only valid for low radiant incident energy. Moreover, under mineral limitation, only the intracellular accumulation of glycogen was considered and the exopolysaccharide synthesis was not taken into account (CORNET *et al.*, 1992b).

The purpose of this Technical Note is to establish an independent kinetic equation for the rate of exopolysaccharide synthesis, associated with an independent stoichiometric equation providing a good accuracy in the determination of global formulae for the total biomass produced in a large range of operating conditions. This problem is particularly crucial for the physical limitation by light which is the general culture condition observed in photoreactors and represents the main objective of this work.

Two different approaches may be considered in modeling the rate of exopolysaccharide synthesis and the advantages or disadvantages of these two ways will be dicussed later in the text. The first one is based on the determination of an equation providing the ratio $P/2e^-$ versus the volumetric rate of radiant energy absorbed. This ratio accounts for the rate of ATP over the rate of reduced cofactor $NADPH,H^+$ produced by photosynthesis, which imposes the rate of exopolysaccharide synthesis if the growth rate of biomass is known (see paragraphs 3 and 4 in this TN). This approach is the first step toward a biochemically structured model for growth of *Spirulina platensis*. The second one consists in the establishement of an independent equation for the rate of exopolysaccharide synthesis in the same form as for active biomass growth, i.e. from the local available radiant light energy $4\pi J$ at each point of the reactor (see paragraph 5 in this TN).

Note that the background for the comprehension of this Technical Note is included in the preceding Technical Note (TN 19.1) which dealt with the adaptation of the light energy transfer model to cylindrical geometries and with the light and mineral limitations in photoreactors. The nomenclature used in this TN is the same as in TN 19.1.

1- Results on continuous cultures of *S. platensis* in photobioreactor under different incident radiant light fluxes.

Continuous cultures in a 5 L cylindrical photobioreactor have been performed under different incident light fluxes and different dilution rates in CNRS of Gif sur Yvette (for all details on this study, see final report of Centre National d'Etudes Spatiales, France, n° 91/CNES/0411). The main results reported here (Table 1) give the overall composition in biomass obtained under different culture conditions, especially the exopolysaccharide, which allow to develop models for the rate of exopolysaccharide synthesis later in this TN.

Table 1: Changes in overall composition of *S. platensis* produced in continuous cultures under different light limiting conditions.

Mean radiant incident flux F_0 (W/m^2)	**Volumetric rate of radiant energy absorbed $<A>$ (W/m^3)**	**Overall composition of biomass (%)**
10	80	Proteins: 65 Fat: 10 Carbohydrates: 15 Exopolysaccharide: 10
100	720	Proteins: 56 Fat: 10 Carbohydrates: 14 Exopolysaccharide: 20
160	1200	Proteins: 48 Fat: 10 Carbohydrates: 15 Exopolysaccharide: 27
230	1600	Proteins: 42 Fat: 10 Carbohydrates: 14 Exopolysaccharide: 34

Table 1 clearly shows the modifications in the ratio proteins/exopolysaccharide in the produced biomass when the incident and/or absorbed radiant energy is increased in the reactor. Thus, the global formula of total biomass changes significantly with the illuminating conditions of the reactor, i.e. under physical limitation by radiant light transfer. This justifies the use of at least two independent stoichiometric and kinetic equations for active biomass and for exopolysaccharide synthesis, these two rates being related differently to the rate of radiant light energy available.

The use of two equations corresponds to the assumption that the active biomass remains unchanged in elemental formula (macromolecule composition) under different light limiting conditions. This hypothesis seems justified in regard with the experimental determinations given above and keeping in mind that the accuracy of such analysis by classical experimental methods ranges between 10 to 20% (BLIGH and DYER, 1959; Pierce kit; HERBERT *et al.*, 1971...).

2- Obtention of the volumetric rate of radiant energy absorbed <A> in cylindrical monodimensional approximations.

The determination of the volumetric rate of radiant energy absorbed in the photoreactor <A> is preliminary required in order to establish an equation giving the ratio P/2e$^-$ versus <A>.

As previously mentionned in TN 19.1, the relation between the radiant energy flux **F** and the local volumetric rate of radiant energy absorbed by any biomass C_X is as follows (CORNET *et al.*, 1992a):

$$-\nabla.\mathbf{F} = EaC_X 4\pi J = A \quad (1)$$

and in cylindrical coordinates:

$$-\frac{1}{r}\frac{\partial(rF_r)}{\partial r} = EaC_X 4\pi J_r = \alpha\delta 4\pi J_r = A \quad (2)$$

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

By integration on the total volume of the reactor, one obtains:

$$<A> = \frac{1}{V}\int_V A dV \quad (3)$$

Finally, from equations (2) and (3), in cylindrical coordinates, the integral is:

$$<A> = <4\pi J_r> \alpha\delta = \frac{\alpha\delta}{\pi R^2}\int_0^R 2\pi r(4\pi J_r)dr \quad (4)$$

Remembering the expression of $4\pi J_r$ in TN 19.1:

$$\frac{4\pi J_r}{F_R} = \frac{R}{r}\frac{2\cosh\delta r}{\cosh\delta R + \alpha\sinh\delta R} \quad (5)$$

equation (4) gives:

$$\boxed{\frac{<A>}{2F_R/R} = \frac{2\alpha\sinh\delta R}{\cosh\delta R + \alpha\sinh\delta R}} \quad (6)$$

This relation enables to calculate the volumetric rate of radiant energy absorbed in a cylindrical reactor <A>. Note that the ratio <A>/($2F_R$/R) asymptotically reaches a maximum value as the biomass quantity increases, which confirms that the reactor performance may be limited by light energy transfer when biomass concentrates in the medium. It is easily established that the asymptotic value of $<A>/(2F_R/R)$ is given by:

$$\boxed{\lim_{C_X,R\to\infty}\left[\frac{<A>}{2F_R/R}\right]=\frac{2\alpha}{1+\alpha}} \quad (7)$$

Equation (6) and (7) will be used in paragraph 4 in order to provide a relation between the ratio $P/2e^-$ and $<A>$.

3- Stoichiometric equations. Definition of the ratio $P/2e^-$.

From a detailed analysis of the biochemical pathways in *S. platensis*, it is possible to write two stoichiometric equations, involving energetic aspects such as the required quantities in ATP and reduced cofactor $NADPH,H^+$ to achieve a C-mole of active biomass or exopolysaccharide (CORNET, 1992).

For the active biomass, the composition is supposed to be constant and equal to 70% proteins, 16% carbohydrates, 10% fats and 4% nucleic acids. Thus, the stoichiometric equation obtained is as follows (CORNET, 1992):

$$CO_2 + 1.3794\ H_2O + 0.1921\ HNO_3 + 0.0052\ H_2SO_4 + 3.568\ ATP + 2.874\ [NADPH,H^+]$$

$$\xrightarrow{<J_{XA}>} CH_{1.566}O_{0.405}N_{0.192}S_{0.0052}P_{0.0063} + 3.5616\ Pi + 3.568\ ADP + 2.874\ NADP^+ \quad (8)$$

The C-molar mass of such a biomass is 23.096 kg/C-kmole.
$<J_i>$ is the molar volumetric rate of compound i, related to the mass volumetric rate $<r_i>$ by means of the C-molar mass of compound i (CMM_i): $<J_i> = <r_i>/CMM_i$. Thus, for the active biomass XA, we have:

$$<J_{XA}> = \frac{<r_{XA}>}{23.096} \quad (9)$$

The two equations for photosynthesis are then:

$$2.874\ NADP^+ + 2.874\ H_2O \xrightarrow{<J_N>} 2.874\ [NADPH,H^+] + 1.437\ O_2 \quad (10)$$

$$3.568\ (ADP + Pi) \xrightarrow{<J_P>} 3.568\ ATP + 3.568\ H_2O \quad (11)$$

and by summation of equations (8), (10), (11) we find a non-structured classical stoichiometric equation in the same form as in TN 19.1:

$$CO_2 + 1.3794\ H_2O + 0.1921\ HNO_3 + 0.0052\ H_2SO_4 + 0.0063\ Pi$$

$$\xrightarrow{<J_{XA}>} CH_{1.566}O_{0.405}N_{0.192}S_{0.0052}P_{0.0063} + 1.437\ O_2 \quad (12)$$

Taking into account pH modifications during growth and using an oxydo-reduction balance, equation (12) may be rewritten as:

$$HCO_3^- + 1.3794\ H_2O + 0.1921\ NO_3^- + 0.0052\ SO_4^{2-} + 0.0063\ HPO_4^{2-} + 0.2151\ H^+$$

$$\xrightarrow{<J_{XA}>} CH_{1.566}O_{0.405}N_{0.192}S_{0.0052}P_{0.0063} + 1.437\ O_2 + OH^- \quad (13)$$

This equation enables some stoichiometric informations to be defined:

- the conversion yield of nitrate into active biomass is 0.516 kg NO_3^-/kg XA;
- the conversion yield of sulfate into active biomass is 0.022 kg SO_4^{2-}/kg XA.

The ratio J_P/J_N is defined as the rate of photophosphorylation over the rate of production in reduced cofactors by photosynthesis and it is named P/2e⁻ ratio. One can observe from equation (8) that this ratio is equal to 1.24 for active biomass.

From the same analysis, and knowing the composition and global formula for the exopolysaccharide (EPS), the following stoichiometric equation may be provided (CORNET, 1992):

$$CO_2 + 2.22\ H_2O + 0.015\ H_2SO_4 + 3.33\ ATP + 1.920\ [NADPH,H^+]$$

$$\xrightarrow{<J_{EPS}>} CH_{1.650}O_{0.950}S_{0.015} + 3.33\ Pi + 3.33\ ADP + 1.920\ NADP^+ \quad (14)$$

The C-molar formula of such an exopolysaccharide is 29.33 kg/C-kmole, and the volumetric rates are given by:

$$<J_{EPS}> = \frac{<r_{EPS}>}{29.33} \quad (15)$$

Equations for photosynthesis lead to:

$$1.920\ NADP^+ + 1.920\ H_2O \xrightarrow{<J_N>} 1.920\ [NADPH,H^+] + 0.96\ O_2 \quad (16)$$

$$3.33\ (ADP + Pi) \xrightarrow{<J_P>} 3.33\ ATP + 3.33\ H_2O \quad (17)$$

and by summation, we have:

$$CO_2 + 2.22\ H_2O + 0.015\ H_2SO_4 \xrightarrow{<J_{EPS}>} CH_{1.650}O_{0.950}S_{0.015} + 0.96\ O_2 \quad (18)$$

From an oxydo-reduction balance, equation (18) becomes:

$$HCO_3^- + 2.22\ H_2O + 0.015\ SO_4^{2-} + 0.03\ H^+$$

$$\xrightarrow{<J_{EPS}>} CH_{1.650}O_{0.950}S_{0.015} + 0.96\ O_2 + OH^- \quad (19)$$

The conversion yield of sulfate into exopolysaccharide is equal to 0.049 kg SO_4^{2-}/kg EPS. From equation (14), the P/2e⁻ ratio for exopolysaccharide is equal to 1.73.

All these stoichiometric considerations will be used in the two next paragraphs which report the calculation of the mass volumetric rates in exopolysaccharide $<r_{EPS}>$. It must be noticed that the mass volumetric rate in active biomass is given by the same equations that in TN 19.1.

4- Obtention of $<r_{EPS}>$ from the volumetric rate of radiant energy absorbed <A> and from the ratio $P/2e^-$. The biochemically structured approach.

The mean volumetric rate of radiant energy absorbed <A> depends on the way to illuminate the reactor and on the geometry of the reactor; so it is a specific data of a given photobioreactor. Nevertheless, when the monodimensional approximation is expected, the equations governing this volumetric rate such as equation (6) remain valid as long as the cylindrical or rectangular monodimentional approximations may be assumed.

For these reasons, it is possible to calculate a conversion factor relating, for a given incident radiant energy flux, the mean volumetric rate of radiant energy absorbed <A> in the photoreactor of Gif-sur-Yvette and the value of <A> in the radially illuminated reactor of ESTEC. This calculation is necessary in order to establish a relation of the form $P/2e^- = f <A>$, because the data presented in Table 1 have been obtained in the reactor of Gif which is non-radially illuminated (cylindrical monodimensional approximation unjustified) and have to be extended to the reactor of ESTEC.

From preliminary data of CCN5 (see appendix) about the reactor of ESTEC and for given incident radiant energy fluxes in the range 20-200 W/m^2, a conversion factor for <A> has been determined. For this calculation, the mean volumetric rates of radiant energy absorbed <A> on the reactor of Gif have been obtained from a complex tridimensional gridding algorithm (CORNET *et al.*, 1993), and <A> on the reactor of ESTEC has been obtained by means of equation (6). The results (details are not reported in this TN) give a constant conversion factor of 5.4 with a mean standard deviation of 10%.

From the different compositions in total biomass given in Table 1, and knowing the $P/2e^-$ ratio for each major component in biomass as in equation (8) and (14), the $P/2e^-$ ratio for the total biomass produced at different level of <A> in Table 1 have been determined. This enables to establish the required equation $P/2e^- = f <A>$ from the following results presented in Table 2.

Table 2: $P/2e^-$ ratio obtained for the total biomass produced under different volumetric rates of radiant energy absorbed.

Mean radiant incident flux F_0 or F_R (W/m^2)	**Volumetric rate of radiant energy absorbed in the reactor of Gif (W/m^3)**	**Volumetric rate of radiant energy absorbed in the reactor of ESTEC (W/m^3)**	**Ratio $P/2e^-$ of the total biomass produced (dimensionless)**
10	80	430	1.274
100	720	3890	1.312
160	1200	6480	1.346
230	1600	8640	1.374

Then, the relation obtained is:

$$\boxed{P/2e^- = <J_P>/<J_N> = 1.222\ 10^{-5} <A> + 1.267} \quad (20)$$

This allows to calculate the volumetric rate of exopolysaccharide synthesis $<r_{EPS}>$ if the volumetric rate of active biomass $<r_{XA}>$ is known, by the way that will be discussed now. Equation (20) remains valid in the range 0-250 W/m^2 for incident fluxes i.e. up to 9 kW/m^3 for the volumetric rate of radiant energy absobed <A> in the cylindrical reactor of ESTEC.

Finally, from stoichiometric equations (8) to (13) and (14) to (19) given in paragraph 3, two additional energy balances have to be established, in order to calculate the rate of exopolysaccharide synthesis $<r_{EPS}>$. These two balances are obtained from the assumption that there is no accumulation in ATP and reduced cofactors NADPH,H$^+$ in the cells. This assumption corresponds to the pseudo steady state hypothesis. Thus, from stoichiometric equations (8),(10),(11) and (14),(16),(17), we have the following balances:

-ATP balance:

$$3.568 <J_{XA}> + 3.33 <J_{EPS}> = <J_P> \quad (21)$$

- Cofactor balance:

$$2.874 <J_{XA}> + 1.920 <J_{EPS}> = <J_N>' \quad (22)$$

In this case, seven variables are unknown, $<r_{XA}>$, $<J_{XA}>$, $<r_{EPS}>$, $<J_{EPS}>$, $<J_P>$, $<J_N>$, <A>, and remembering that the ratio P/2e$^-$ is equal to $<J_P>/<J_N>$, and that the volumetric rate in active biomass $<r_{XA}>$ is given by the model in TN 19.1, we need six equations to solve the problem. These are equations (9) and (15), giving a relation between molar and mass volumetric rates, equation (20) giving the P/2e$^-$ ratio, energy balances (21) and (22) and equation (6) giving the volumetric rate in radiant energy absorbed.

So, the mass volumetric rate of exopolysaccharide synthesis is given by:

$$\boxed{< r_{EPS} >= 29.33\frac{\left(\frac{< J_P >}{< J_N >}2.874 - 3.568\right)\frac{< r_{XA} >}{23.096}}{\left(3.33 - \frac{< J_P >}{< J_N >}1.92\right)}} \quad (23)$$

This method presents the advantage to be a first step toward a general biochemically structured approach allowing to provide independent equations of rates for each biomass component and so to foresee metabolic deviations and yield modifications occuring when the microorganisms are cultivated under physical or biological stresses.

At the opposite, equation (6) giving the volumetric rate of radiant energy absorbed is only valid for cylindrical monodimensional approximation i.e. for radially illuminated reactors. Consequently, equation (20) becomes unefficient in the case of other geometries such as rectangular reactors and a new relation should be provided. Moreover, it has been established (CORNET *et al.*, 1993) that equation (6) was inappropriate for low biomass concentrations (<0.2 kg/m^3) and that only the asymptotic value given by equation (7) for high biomass concentrations was sufficiently accurate to assess the volumetric rate of radiant energy absorbed.

Examples:

Two examples are given here from the preliminary results of CCN5 (see appendix) obtained on the cylindrical reactor of ESTEC, for high and low incident radiant light fluxes.

In the first example, the incident flux on the reactor is 195 W/m^2, so the model of TN 19.1 gives a value of $<r_{XA}> = 3.6\ 10^{-2}\ kg.m^{-3}.h^{-1}$ which is in good agreement with the experimental value (5% of deviation). This volumetric rate must be corrected by the 20% of constant exopolysaccharide fraction supposed to be produced at the same rate which gives:

$$<r_{XA}> = 3.0\ 10^{-2}\ kg.m^{-3}.h^{-1}$$

Equations (6) or (7) lead to the value of the volumetric rate of radiant energy absorbed $<A> = 6860\ W/m^3$ and equation (20) leads to $<J_P>/<J_N> = 1.351$. Thus, equation (23) provides the value of the volumetric rate of exopolysaccharide synthesis:

$$<r_{EPS}> = 1.63\ 10^{-2}\ kg.m^{-3}h^{-1}$$

The percentage of exopolysaccharide in the total biomass is then 35%, which is in good agreement with the experimental determinations for high radiant light fluxes in Table 1.

In the second example, the incident radiant flux is taken at 21 W/m^2 and the equations of model in TN 19.1 lead to the following corrected value for active biomass (10% of deviation in regard with experimental value):

$$<r_{XA}> = 1.21\ 10^{-2}\ kg.m^{-3}.h^{-1}$$

Equations (6) and (7) give $<A> = 750\ W/m^3$, and equation (20) gives $<J_P>/<J_N> = 1.276$. So equation (23) enables to calculate:

$$<r_{EPS}> = 1.73\ 10^{-3}\ kg.m^{-3}.h^{-1}$$

The percentage of exopolysaccharide in the total biomass is then 12% which is still in good agreement with the values in Table 1.

5- Obtention of $<r_{EPS}>$ from the profiles of local radiant energy available $4\pi J$. The non-structured approach.

In TN 19.1, the mean biomass volumetric rate $<r_{XA}>$ was obtained from the volumetric integral of a local rate r_{XA} which follows a Monod type law in regard with the available radiant light energy $4\pi J$ (CORNET *et al.*, 1992a):

$$< r_{XA} >= \mu_M C_{XA} \frac{1}{V} \int_{V_2} \frac{4\pi J}{K_J + 4\pi J} dV \quad (24)$$

with $\mu_M = 0.073\ h^{-1}$ and $K_J = 20\ W/m^2$.

This local rate involved two parameters μ_M and K_J which have been identified from several experiments with the concept of working illuminated volume V_2 and remain valid as long as the monodimensional approximation is expected. This means that the parameters values remain identical in cylindrical or rectangular coordinates. So, a similar equation may be established for the volumetric rate of exopolysaccharide synthesis $<r_{EPS}>$, leading to:

$$\boxed{< r_{EPS} >= \mu_M^{EPS} C_{XA} \frac{1}{V} \int_{V_2} \frac{4\pi J}{K_J^{EPS} + 4\pi J} dV} \qquad (25)$$

From data given in Tables 1 and 2 and for each mean incident radiant energy flux, the expressions of profiles $4\pi J$ in rectangular or cylindrical coordinates have been used (TN 19.1) in order to identify the parameters $\mu_M{}^{EPS}$ and $K_J{}^{EPS}$ in equation (25).with the concept of working illuminated volume. The more accurate values (lower quadratic tolerance criterion between experimental and calculated volumetric rates) have been obtained for:

$$\mu_M{}^{EPS} = 0.30 \ h^{-1}$$
$$K_J{}^{EPS} = 750 \ W/m^2$$

Such an equation provides the rate of stoichiometric reaction (19) in the range 0-250 W/m^2 for the incident energy flux, but the structured-approach (equation 14) is not required. Thus, this approach is entirely independent from the metabolic pathway analysis and it is limited to one or two equations because of the increasing complexity with the extension of considered components. Conversely, equation (25) is always available if it exists a monodimensional equation giving the profiles of radiant available energy in different geometries (rectangular, cylindrical, spherical). Moreover, the monodimensional approximation provides better results for profiles of radiant energy than for volumetric rates of radiant energy absorbed, when the biomass concentration is low (CORNET *et al.*, 1993).

Examples:

The same two examples than in the preceding paragraph may be developed.

In the first example, the radial incident energy flux is taken at 195 W/m^2, and we have seen that the model for the volumetric rate in active biomass (equation 24 and TN 19.1) gave:

$$<r_{XA}> = 3.0 \ 10^{-2} \ kg.m^{-3}.h^{-1}$$

Then equation (25) provides the following value for the volumetric rate of exopolysaccharide synthesis:

$$<r_{EPS}> = 1.57 \ 10^{-2} \ kg.m^{-3}.h^{-1}$$

The percentage of exopolysaccharide in the total biomass produced is then 34% which is in good agreement with the values of Table 1 and with the model in paragraph 4 ($<r_{EPS}> = 1.63 \ 10^{-2} \ kg.m^{-3}.h^{-1}$).

In the second example, the incident flux was 21 W/m^2 and equation (24) leads to:

$$<r_{XA}> = 1.21 \ 10^{-2} \ kg.m^{-3}.h^{-1}$$

so equation (25) gives for the volumetric rate of exopolysaccharide synthesis:

$$<r_{EPS}> = 1.93 \ 10^{-3} \ kg.m^{-3}.h^{-1}$$

Then, the percentage of exopolysaccharide in the total biomass produced is 14% which is still in good agreement with the values in paragraphs 2 and 4 ($<r_{EPS}> = 1.73\ 10^{-3}$ kg.m^{-3}.h^{-1}).

6- Modifications required in the model presented in TN 19.1. New compartimentation of total biomass and new parameters.

The consideration of a free polysaccharide active biomass rather than a biomass with 20% of exopolysaccharide and the consideration of exopolysaccharide as an independent major component lead to a revision in the definition of biotic and abiotic state vectors defined in TN 19.1 (CORNET *et al.*, 1992b). The scheme of the new compartimentation is given in Table 3.

Table 3: Definitions of the biotic and abiotic phases in the model.

Biotic phase:

<table>
<tr><th colspan="4">Active Biomass (XA)</th><th rowspan="3">Glycogen
(G)</th><th rowspan="4">Exopoly-saccharide
(EPS)</th></tr>
<tr><th colspan="2">Proteins (P)</th><th rowspan="2">Chlorophylls
(CH)</th><th rowspan="2">Biomass
(B)</th></tr>
<tr><th>Phycocyanins
(PC)</th><th>Other Proteins
(OP)</th></tr>
<tr><th colspan="5">Vegetative Biomass (XV)</th></tr>
<tr><th colspan="6">Total Biomass (XT)</th></tr>
</table>

Abiotic phase:

Nitrates (N)	**Sulfates (S)**

Thus, the biotic state vector becomes: **X** = (C_{PC}, C_P, C_{CH}, C_{XA}, C_G, C_{EPS}) and the abiotic state vector remains **Y** = (C_N, C_S).

With the same assumptions that in TN 19.1 (CORNET *et al.*, 1992b), the mass biotic fraction of compound i is defined by $z_i = C_i/C_{XA}$ and remains constant whatever the energy flux. The vegetative biomass includes the additional intracellular glycogen appearing during mineral limitation (called total biomass in TN 19.1) and $C_{XV} = C_{XA} + C_G$. Moreover, the biosynthesis of the exopolysaccharide is taken into account in the total biomass and $C_{XT} = C_{XA} + C_G + C_{EPS}$.

Because the active biomass is free of exopolysaccharide, the mass biotic fractions (CORNET *et al.*, 1992b) are increased by 20% and become:

z_P = 0.684 kg P/kg XA (instead of 0.57)
z_{PC} = 0.162 kg PC/kg XA (instead of 0.135)
z_{CH} = 0.01 kg CH/kg XA (instead of 0.0085)

The conversion yields are also modified in regard with stoichiometric equations (8) and (14):

$Y_{N/XA} = 0.516$ kg NO_3^-/kg XA (instead of 0.42)
$Y_{S/XA} = 0.022$ kg SO_4^{2-}/kg XA (instead of 0.028)
$Y_{S/EPS} = 0.049$ kg SO_4^{2-}/kg EPS.

In the same way, the mass absorption and scattering coefficients are modified as follows:

$Ea = 872$ m^2/kg antenna (a = PC + CH)
$Es = 200$ m^2/kg of vegetative biomass XV

while the parameters α and δ remain unchanged:

$$\alpha = \left[Ea(C_{PC} + C_{CH}) / \left[Ea(C_{PC} + C_{CH}) + EsC_{XV}\right]\right]^{\frac{1}{2}}$$
$$\delta = \left[Ea(C_{PC} + C_{CH})\left[Ea(C_{PC} + C_{CH}) + EsC_{XV}\right]\right]^{1/2} R$$

with $z_G = C_G/C_{XA}$ or $(1 + z_G) = C_{XV}/C_{XA}$.

Furthermore, if the volumetric growth rate for active biomass $<r_{XA}>$ is given from the content in phycocyanins in the biomass, the maximum growth rate μ'_M becomes:

$$\mu'_M = 0.45\ h^{-1} \text{ (instead of } 0.54\ h^{-1})$$

the other kinetic parameters remaining unchanged:

$\mu_M = 0.073\ h^{-1}$ for active biomass itself
$K_J = 20\ W/m^2$
$K_N = 5.3\ 10^{-3}$ $kgNO_3^-/m^3$
$K_S = 2.5\ 10^{-4}$ kg SO_4^{2-}/m^3
$K_{PC} = 0.15\ kg^2/m^6$
$q = 0.55$

6.1- Kinetic equations under physical limitation by light only. Comparison between experimental and theoretical stoichiometries.

Under physical limitation by light only, the accumulation of intracellular glycogen is inexistant, and the vegetative biomass is equal to active biomass. The total biomass represents the active biomass and the exopolysaccharide. Additionally, no mineral limitation occurs on the volumetric rates, then the volumetric growth rate in active biomass is given by equation (24):

$$< r_{XA} > = \mu_M C_{XA} \frac{1}{V} \int_{V_2} \frac{4\pi J}{K_J + 4\pi J} dV \qquad (24)$$

with the working illuminated volume and the expression of $4\pi J$ along r axis in cylindrical coordinates are given in TN 19.1. With respect to phycocyanins and in cylindrical coordinates, equation (24) reduces to:

$$<r_{XA}> = <R_{XA}> = <\mu'> \gamma\, C_{PC} \quad (26)$$

$$<\mu'> \approx \frac{1}{\pi(R^2 - R_2^2)} \int_{R_2}^{R} 2\pi r \mu'_M \frac{4\pi J_r}{K_J + 4\pi J_r} dr \quad (27)$$

The other components of the biotic or abiotic phases are then given by:

$<r_{CH}> = z_{CH} <r_{XA}>$ (28)
$<r_{PC}> = z_{PC} <r_{XA}>$ (29)
$<r_P> = z_P <r_{XA}>$ (30)
$<r_N> = -Y_{N/XA} <r_{XA}>$ (31)
$<r_S> = -Y_{S/XA} <r_{XA}> - Y_{S/EPS} <r_{EPS}>$ (32)

Finally, the volumetric rate of exopolysaccharide synthesis is given either from the volumetric rate of radiant energy absorbed <A> by equations (6),(20),(23), or from profiles of radiant available energy by equation (25). Introducing the volumetric rate of exopolysaccharide under light limiting conditions only, we get $<R_{EPS}> = <r_{EPS}>$, and the volumetric rate of total biomass is given by:

$$<r_{XT}> = <R_{XA}> + <R_{EPS}> \quad (33)$$

These model equations enable to determine, for different incident fluxes, and from the stoichiometric equations given in paragraph 3 the global formulae provided by the model proposed in this TN. In Table 4, a comparison is made between the global formulae given by the model under different light limiting conditions and the experimentally observed values on continuous cultures for *S. platensis* (results of Table 1).

Table 4: Comparison between the experimental and theoretical global formulae of *S. platensis* obtained in continuous cultures and different light limiting conditions.

Mean radiant incident flux F_R (W/m^2)	**% of EPS in total biomass**	**Experimental global formulae obtained in continuous cultures of *Spirulina platensis***	**Global formulae given by the model presented in this Technical Note**	**Standard deviation (%)**
10	10	$CH_{1.579}O_{0.435}N_{0.162}S_{0.0064}P_{0.0057}$	$CH_{1.574}O_{0.459}N_{0.173}S_{0.0063}P_{0.0057}$	C/H=0.3 C/O=5 C/N=6 C/S=1.5 C/P=0
100	20	$CH_{1.590}O_{0.493}N_{0.140}S_{0.0073}P_{0.0047}$	$CH_{1.583}O_{0.514}N_{0.154}S_{0.0072}P_{0.0050}$	C/H=0.4 C/O=4 C/N=9 C/S=1.4 C/P=6
160	27	$CH_{1.600}O_{0.541}N_{0.120}S_{0.0078}P_{0.0043}$	$CH_{1.589}O_{0.552}N_{0.140}S_{0.0078}P_{0.0046}$	C/H=0.7 C/O=2 C/N=14 C/S=0 C/P=6.5
230	34	$CH_{1.607}O_{0.580}N_{0.105}S_{0.0084}P_{0.0039}$	$CH_{1.594}O_{0.590}N_{0.126}S_{0.0086}P_{0.0042}$	C/H=0.8 C/O=1.7 C/N=16.7 C/S=2.3 C/P=7.1

There is a good agreement between observed and theoretical values but more accurate results could be expected from a biochemically structured model providing an equation of volumetric rate for each component of the total biomass: proteins, fat, carbohydrates, nucleic acids, and exopolysaccharide.

6.2- Kinetic equations under light and mineral limitations.

The kinetic equations for the complete model under light and mineral limitations are almost the same as in TN 19.1, and will be recalled in paragraph 7. The aim of this paragraph is to describe the adaptation of equations in paragraphs 4 and 5, giving $<R_{EPS}>$ under light limitation. This imposes to consider the phycocyanin concentration as the reference component in biomass in order to remain valid and available under mineral limitation.

In the case of the approach presented in paragraph 4, equation (23) must be rewritten as a function of phycocyanin concentration using equations (26) and (27):

$$\boxed{<R_{EPS}> = 29.33\frac{\left(\frac{<J_P>}{<J_N>}2.874-3.568\right)\frac{<R_{XA}>}{23.096}}{\left(3.33-\frac{<J_P>}{<J_N>}1.92\right)}} \quad (34)$$

For the calculation of the volumetric rate in exopolysaccharide from the profiles in available energy $4\pi J$ presented in paragraph 5, equation (25) must be expressed also with respect to phycocyanins in the form (CORNET *et al.*, 1992b):

$$<R_{EPS}> = <\mu'^{EPS}> \gamma\, C_{PC} \quad (35)$$

$$\boxed{<\mu'^{EPS}> \approx \frac{1}{\pi(R^2-R_2^2)}\int_{R_2}^{R} 2\pi r \mu_M'^{EPS}\frac{4\pi J_r}{K_J^{EPS}+4\pi J_r}dr} \quad (36)$$

with $\mu'_M{}^{EPS} = 1.852\ h^{-1}$

When mineral limitation occurs, the protein synthesis is impaired and S. platensis synthesizes only carbohydrates such as glycogen and exopolysaccharide, but at the same rate as total biomass was produced under light limitation only. As intracellular glycogen synthesis is limited by the maximum size of the cells and stabilises rapidly during the decrease of phycocyanins (CORNET *et al.*, 1992b), the rate of exopolysaccharide synthesis is increased under these mineral limiting conditions. This fact has to be modelized by the equation giving $<r_{EPS}>$ in any condition and especially under mineral limitations. Thus, the volumetric rate of exopolysaccharide synthesis under light and mineral limitation appears as the sum of two term, each of them being preponderant under light or under mineral limitation:

$$\boxed{<r_{EPS}> = <R_{EPS}>\frac{C_N}{K_N+C_N}\frac{C_S}{K_S+C_S}+(<r_{XT}>-<r_{XV}>)\left[\frac{K_N}{K_N+C_N}+\frac{K_S}{K_S+C_S}\right]} \quad (37)$$

Then, the volumetric growth rate in total biomass is the sum of the rate in exopolysaccharide production and the rate in accumulation of intracellular glycogen, and it is the same as in light limiting condition:

$$<r_{XT}> = <R_{XA}> + <R_{EPS}> = <r_{EPS}> + <r_{XV}> \quad (38)$$

Moreover, the sulfates being consumed both for active biomass and for exopolysaccharide, the volumetric rate of sulfate consumption is given by:

$$\boxed{<r_S> = -Y_{S/XA}<r_{XA}> - Y_{S/EPS}<R_{EPS}>\frac{C_N}{K_N+C_N}\frac{C_S}{K_S+C_S}} \quad (39)$$

All the equations for the other components (XA, CH, PC, P, N) remain unchanged in regard to TN 19.1 and are listed with the equations of the model in paragraph 7.

7- Equations for the model.

$4\pi J_r$ calculation:

$$\frac{4\pi J_r}{F_R} = \frac{1}{Z}\frac{2\cosh\delta Z}{\cosh\delta + \alpha\sinh\delta}$$

$$\alpha = \left[Ea(C_{PC} + C_{CH})/\left[Ea(C_{PC} + C_{CH}) + EsC_{XV}\right]\right]^{\frac{1}{2}}$$

$$\delta = \left[Ea(C_{PC} + C_{CH})\left[Ea(C_{PC} + C_{CH}) + EsC_{XV}\right]\right]^{1/2} R$$

Calculation of the volumetric rate of radiant energy absorbed:

$$\frac{< A >}{2F_R/R} = \frac{2\alpha\sinh\delta}{\cosh\delta + \alpha\sinh\delta}$$

$$\lim_{C_X, R\to\infty}\left[\frac{< A >}{2F_R/R}\right] = \frac{2\alpha}{1+\alpha}$$

Working illuminated radius R_2 calculation:

$$\frac{1}{Z_2}\frac{2\cosh\delta Z_2}{\cosh\delta + \alpha\sinh\delta} - \frac{F_{min}}{F_R} = 0$$

$$Z_2 = \frac{R_2}{R}$$

$$F_{min} = 1\,W/m^2$$

Average of specific growth rates with respect to phycocyanins:

$$< R_{XA} > = < \mu' > \gamma C_{PC}$$

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

$$< \mu' > \approx \frac{1}{\pi(R^2 - R_2^2)}\int_{R_2}^{R} 2\pi r\mu_M' \frac{4\pi J_r}{K_J + 4\pi J_r} dr$$

or directly $< R_{XA} > \approx C_{PC}\dfrac{2\mu_M'}{R^2}\displaystyle\int_{R_2}^{R} r\frac{4\pi J_r}{K_J + 4\pi J_r} dr$

Obtention of the volumetric rate of exopolysaccharide synthesis:

- the non-structured approach:

$$<R_{EPS}>=<\mu'^{EPS}>\gamma C_{PC}$$

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

$$<\mu'^{EPS}>\approx\frac{1}{\pi(R^2-R_2^2)}\int_{R_2}^{R} 2\pi r\mu_M'^{EPS}\frac{4\pi J_r}{K_J^{EPS}+4\pi J_r}dr$$

or directly $<R_{EPS}>\approx C_{PC}\frac{2\mu_M'^{EPS}}{R^2}\int_{R_2}^{R} r\frac{4\pi J_r}{K_J^{EPS}+4\pi J_r}dr$,

-the biochemically structured approach:

$P/2e^- = <J_P>/<J_N> = 1.222\ 10^{-5} <A> + 1.267$

$$<R_{EPS}>=29.33\frac{\left(\frac{<J_P>}{<J_N>}2.874-3.568\right)\frac{<R_{XA}>}{23.096}}{\left(3.33-\frac{<J_P>}{<J_N>}1.92\right)}$$

Kinetic equations:

$$<r_{XT}>=<R_{XA}>+<R_{EPS}>=<r_{EPS}>+<r_{XV}>$$

$$<r_{XA}>=<R_{XA}>\frac{C_N}{K_N+C_N}\frac{C_S}{K_S+C_S}$$

$$<r_{CH}>=z_{CH}<r_{XA}>$$

$$<r_{PC}>=z_{PC}<R_{XA}>\left[\frac{C_N}{K_N+C_N}\frac{C_S}{K_S+C_S}-\left(\frac{K_N}{K_N+C_N}+\frac{K_S}{K_S+C_S}\right)\right]$$

$$< r_P >= z_P < R_{XA} > \left[\frac{C_N}{K_N + C_N} \frac{C_S}{K_S + C_S} - q \frac{K_S}{K_S + C_S} \right]$$

$$< r_N >= -Y_{N/XA} < r_{XA} >$$

$$< r_S >= -Y_{S/XA} < r_{XA} > -Y_{S/EPS} < R_{EPS} > \frac{C_N}{K_N + C_N} \frac{C_S}{K_S + C_S}$$

$$< r_{XV} >=< R_{XA} > \left[\frac{C_N}{K_N + C_N} \frac{C_S}{K_S + C_S} + \frac{C_{PC}}{K_{PC} + C_{PC}^2} \left(\frac{K_N}{K_N + C_N} + \frac{K_S}{K_S + C_S} \right) \right]$$

$$< r_{EPS} >=< R_{EPS} > \frac{C_N}{K_N + C_N} \frac{C_S}{K_S + C_S} + (< r_{XT} > - < r_{XV} >) \left[\frac{K_N}{K_N + C_N} + \frac{K_S}{K_S + C_S} \right]$$

Parameters:

$Ea = 872\ m^2/kg$
$Es = 200\ m^2/kg$
$z_{CH} = 0.01$
$z_{PC} = 0.162$
$z_P = 0.684$
$(1+z_G) = C_{XV}/C_{XA}$
$F_{min} = 1\ W/m^2$
$\mu_M = 0.073\ h^{-1}$
$\mu_M^{EPS} = 0.3 h^{-1}$
$\mu_M^{'} = 0.45 h^{-1}$
$\mu_M^{'EPS} = 1.852 h^{-1}$
$K_J = 20\ W/m^2$
$K_J^{EPS} = 750 W / m^2$
$K_N = 5.3\ 10^{-3}\ kg/m^3$
$K_S = 2.5\ 10^{-4}\ kg/m^3$
$K_{PC} = 0.15\ kg^2/m^6$
$q = 0.55$
$Y_{N/XA} = 0.516$
$Y_{S/XA} = 0.022$
$Y_{S/EPS} = 0.049$

Conclusion.

The purpose of this Technical Note was essentially to propose theoretical methods to obtain independently the volumetric rates of active biomass and of exopolysaccharide synthesis. The two approaches given in this Technical Note provide good results in regard to experimental data obtained on continuous cultures of *S. platensis* under different light limiting conditions. This is of prime importance in order to apprehend metabolic deviations occuring when microorganisms grow under physical or biological stresses, and especially under light limitation which is always observed in photobioreactors. Thus, the exopolysaccharide appears as a key component and the model developed in this Technical Note allows the following scopes to be considered:

- assessment of exopolysaccharide concentration produced in the reactor under light or mineral limitations in relation to technical continuous cultivation problem;
- establishment of variable total biomass stoichiometries for simulation of photosynthetic compartment;
- assessment of the produced biomass quality as source of single cell protein;
- development of control algorithms for the photosynthetic compartment.

Actually, the two approaches developed in this Technical Note provide almost the same results for the calculation of the independent rate of exopolysaccharide synthesis; nevertheless, the non-structured approach is limited to a few number of independent rate equations and presents the disadvantage to be of poor predictibility.

So, further investigations in the field of biochemically structured models might lead to considerate independent equations for each macromolecule involved in the biomass synthesis (proteins, fat, carbohydrates, nucleic acids, exopolysaccharide), providing then general and efficient tool for modeling, control, simulation and scaling up of photobioreactors.

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NOMENCLATURE

A	volumetric rate of radiant energy absorbed (W/m^3)
C_i	concentration of compound i (kg/m^3)
CMM_i	C-molar mass (kg/kmole)
Ea	global absorption mass coefficient (m^2/kg)
Es	global scattering mass coefficient (m^2/kg)
F	radiant energy flux (W/m^2)
F_0	incident radiant energy flux (W/m^2)
F_R	incident radiant energy flux (W/m^2)
J	mean light intensity (W/m^2)
J_i	molar volumetric rate of compound i (kmole.m^{-3}.h^{-1})
K_J	half saturation constant for available radiant energy in relation to active biomass synthesis (W/m^2)
K_J^{EPS}	half saturation constant for available radiant energy in relation to exopolysaccharide synthesis (W/m^2)
K_N	half saturation constant for nitrate concentration (kg/m^3)
K_S	half saturation constant for sulfate concentration (kg/m^3)
K_{PC}	half saturation constant for phycocyanin concentration (kg^2/m^6)
q	coefficient of proportionality (dimensionless)
r	radius (m)
R	reactor radius (m)
R_2	working illuminated radius (m)
r_i	mass volumetric rate of compound i (kg.m^{-3}.h^{-1})
R_i	mass volumetric rate of compound i in relation to phycocyanins (kg.m^{-3}.h^{-1})
V	volume (m^3)
V_2	working illuminated volume (m^3)
$Y_{i/j}$	mass conversion yield of compound i in compound j (dimensionless)
z_i	mass biotic fraction of compound i (dimensionless)

$Z = r/R$ dimensionless abscissa

$Z_2 = R_2/R$ dimensionless abscissa for working illuminated radius

$<>$ mean volumetric integral $= \frac{1}{V}\iiint_V dV$

Greek letters:

γ	working illuminated fraction (dimensionless)
μ'	growth rate in relation to phycocyanins (h^{-1})
μ_M	maximum growth rate for biomass (h^{-1})
μ'_M	maximum growth rate for biomass in relation to phycocyanins (h^{-1})
μ_M^{EPS}	maximum growth rate for exopolysaccharide (h^{-1})
$\mu_M^{'EPS}$	maximum growth rate for exopolysaccharide in relation to phycocyanins (h^{-1})

Subscripts:

a	antenna
CH	chlorophyll *a*
EPS	exopolysaccharide
G	glycogen
N	NADPH,H$^+$
N	nitrate
P	adénosine triphosphate ATP
P	protein
PC	phycocyanin
S	sulfate
XA	active biomass
XT	total biomass
XV	vegetative biomass

APPENDIX

Preliminary data of CCN5 about the reactor of ESTEC for given incident radiant energy fluxes in the range 20-200 W/m^2 and with *S. platensis* ($\gamma = 0.5$).

Active biomass concentration. C_{XA} (mg/L)	**Initial radiant energy flux. F_R (W/m^2)**	**Calculated volumetric rate of active biomass from model of TN 19.1. $<R_{XA}>$ (mg.L^{-1}.h^{-1})**	**Experimental volumetric rate of active biomass. $<R_{XA}>$ (mg.L^{-1}.h^{-1})**	**Standard deviation (%)**
770	193	21.0	18.9	10
700	195	18.0	18.9	5
473	23	7.6	8.5	10
470	68	12.7	12.5	1
460	45	10.7	10.2	5
460	21	7.2	6.5	11
238	26	6.0	6.6	9
155	23	4.2	5.0	15
90	31	3.0	2.5	15