Universitat Autònoma de Barcelona
Dep. Enginyeria Química
08193 Bellaterra, Barcelona, Spain

MELISSA

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Control Laws of Photosynthetic Compartment.

Integration of Biomass Composition Prediction Model.

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ALBIOL, J.; VERNEREY A.; GODIA, F.

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Version	Issue	Date	Observations
Draft	0	15/1/98	Preliminary Version
1	0	20/6/98	First Issue

Table of Contents

I. INTRODUCTION

Development of the most appropriate control system for the IV compartment of the MELISSA loop requires a thorough knowledge of the behaviour of the compartment under different culture conditions. This knowledge is expressed in the form of a mathematical model that allows to predict the biomass behaviour under certain culture conditions. During the previous studies for this compartment, a model has been developed and validated under different conditions.

The knowledge obtained with the different tests performed for the photosynthetic compartment is progressively being incorporated into a more evolved mathematical model describing the *Spirulina* cells behaviour under different culture conditions. This model was implemented in the Photosim simulator (Cornet 1993a, 1993b and 1993c, TN 19.1, 19.2 and 19.3). As soon as more experimental data are being obtained the model is refined so as to be able to expand the range of operational conditions were it could be applied, consequently more advanced versions of the simulators are being issued.

In a subsequent step this model is going to be used by the control software developed by ADERSA and used for prediction of biomass evolution and composition. As a first step in the implementation of this model in the control algorithm, the model has been written in C code and incorporated in the GPS. The required arrangements have been done to allow for its use as an on line simulator (Pons 1997, TN 37.9), so that the GPS can display the biomass evolution based on a set of initial values of the variables, and defined conditions of flow and light intensity. This implementation of the software is the one that has been used in this technical note.

In order to test the software, different culture conditions and initial values have been chosen corresponding to a set of experimentally verified results. The conditions used and the results obtained are described in the following paragraphs.

During the preparation of this technical note, an updated version of Photosim has been issued. Not all the improvements included in Photosim V 2.0 have been yet included in this version of the GPS simulator. Therefore some discrepancies are observed between the two simulators, that are mentioned in the text were appropriate. The modifications included by this version of the GPS simulator with respect to Photosim V 1.0 are described in appendix 1.

II. SIMULATION

For all the tests, the initial conditions of biomass composition used for simulation were the ones obtained with Photosim as steady state solutions of the corresponding values of dilution rate, light intensity and input nitrate concentrations.

As mentioned previously the model used in Photosim has been verified in different culture conditions and it is being refined as a wider range of culture conditions are tested. The last tests done for compartment IV corresponded mainly to two different working conditions. One group was done at dilution rates higher than the ones tested previously (high dilution rate tests) and the other group was done under nitrogen limitation conditions (nitrogen limitation tests). The model predictions can be compared to those two groups of results in order to be able to determine the modifications required by the model to expand its range of applicability.

High dilution rate tests

These tests were done under light limiting conditions and dilution rates ranging from 0.025 h^{-1} to 0.035 h^{-1} , which were higher than the ones previously done. With them it can be tested the continuous culture behaviour of the biomass which, as biomass is maintained for a long term in a determined set of conditions, allows enough time for the adaptation of the culture to them, either by a long term metabolic adaptation response or by selection of the fittest individuals, all of which can result in a better performance.

In figure 1 it can be compared the total biomass values measured in two different continuous cultures. The model predicts a total biomass concentration quite near but below the levels of the experimentally measured values. In figure 2 the same comparison can be done but for a dilution rate of 0.035 h^{-1}. In this case, and although the real biomass concentration has decreased, the model predicts even a lower biomass level than the one measured. This behaviour has been corrected in the new version of the model. Indeed this can be seen in figures 6 and 7, were the new version of Photosim has been used. In this last case, the simulator follows the biomass evolution more closely.

As can be seen in figure 4 the increase in total biomass is mainly due to the increase in concentrations of active biomass and in exopolyssaccharide (EPS). The increased EPS content is higher than the increase in active biomass and results in an

increase in the mass fraction of EPS in total biomass, as can be seen in figure 3 and also calculated by the GPS. This is consistent with the results of the EPS measurements done in TN 25.110 for the dilution rate of 0.025 h^{-1}. However the increase in the mass fraction of EPS measured for higher growth rates (0.035 h^{-1}, TN 25.110) was smaller than the one obtained with the simulation data. The mass fraction obtained by the GPS allows the calculation of the different contents of hydrogen, oxygen, nitrogen, phosphorous and sulphur of the biomass (figure 5), this calculations can be used by the GPS for an elemental mass balance. The simulations indicate an increase in the content of oxygen and sulphur, per C mol of biomass, as a result of the increase in EPS content when light intensity is increased.

The model predicts a level of biomass concentration in which the average light intensity allows a growth rate equal to the dilution rate. As it predicts a lower biomass than the one experimentally found, either there is more light intensity available for the cells than the one calculated by the model, or the light requirement for the cells to maintain this growth rate is lower than calculated.

The new version of Photosim (2.0) results in a better prediction of the biomass evolution than the previous one. This improvements will be incorporated in the GPS in the next issue and the experimental results will be compared with the simulator. In the near future a new bioreactor with an increased volume and different ratio among illuminated and working volume will be tested and the model will be tested in even a wider range of culture conditions. It is therefore advisable to test the new issue of the GPS simulator with the results obtained in the new bioreactor.

One possible explanation for the disagreement among batch and continuous cultures, can be that the cells in continuous culture require less light intensity to maintain a given growth rate than it was calculated for batch cultures, due to cell selection or long term adaptation. One way to take this into account is to re-evaluate the kinetic model parameters, (such as Kj, μM ...etc.) to improve prediction accuracy. Future data obtained in continuous culture will allow to refine the mathematical models, based on the more defined controlled conditions of the continuous cultures.

Nitrate limitation tests.

In the second set of tests, nitrogen source concentration was decreased to 0.1 Kg/m^3 NO_3^-, which results in nitrogen limitation conditions on the growth rate. This fact is ascertained by the lower biomass concentrations found than the ones in the same conditions but with higher levels of nitrate, and also by the low levels of nitrate measured in the bioreactor (lower than 1 ppm).

In figures 8, 9 and 10 it can be observed the biomass evolution, under nitrate limitation conditions, for dilution rates of 0.018 h^{-1}, 0.012 h^{-1} and 0.016 h^{-1} respectively. During those tests light intensity was increased from 50 W/m^2 to 305 W/m^2. In all the experimental cases it is observed that the total biomass concentration is maintained stable in front of a change of light intensity. On one side this is consistent with the conditions of nitrogen limitation in which active biomass concentration is only expected to change slightly. Model prediction values are moving around the measured total biomass values. However the model indicates an increase in the total biomass when light intensity is increased. As it can be seen in figure 12 the active biomass concentration predicted by the GPS is maintained quasi constant, in front of the increase in light intensity. As can be seen in the same figure the increase in total biomass is mainly due to a predicted increase in the EPS content. This fact could not be verified experimentally. Indeed as shown in table 1, an increase in total carbohydrates was not measured during the nitrate limitation experimental tests, which indicates that under the conditions assayed there was not an increase in exopolysaccharide. In table 2 the elemental biomass composition measured in those tests could not confirm an increase in the oxygen and sulphur content and a decrease in phosphorous content, as a result of a strong increase in EPS in the biomass. This increase should be the result of an increase in the EPS content as seen in figure 13 where the elemental composition variation is represented as well.

In conclusion the data obtained until now indicate that under nitrate limitation conditions the EPS content of the biomass does not present an important increase (enough to be measured), when there is an increase in light intensity. If this data is confirmed, the model should be modified such as not to increase EPS production with an increase of light intensity, under nitrate limitation conditions.

T	DILUTION RATE (h^{-1})	LIGHT INTENSITY (W/m^2)	TOTAL BIOMASS (Kg/m^3) +/- SD	CALCULATED BIOMASS (Kg/m^3) +/-SD	TOTAL CARBOHYDR. (Kg/m^3) +/-SD	PROTEINS (Kg/m^3) +/-SD
A	0,018	305	0,47 +/-0,04	0,43	0,200 +/-0,01	0,15 +/-0,01
A	0,018	50.2	0,49 +/-0,03	0,5	0,22 +/-0,03	0,16 +/-0,01
C	0,016	305	0,50 +/-0,03	0,5	0,25 +/-0,04	0,19 +/-0,03
C	0,016	50.2	0,54 +/-0,03	0,53	0,24 +/-0,03	0,14 +/-0,03
B	0,012	305	0,44 +/-0,04	0,42	0,25 +/-0,02	0,15 +/-0,01
B	0,012	50.2	0,49 +/-0,03	0,48	0,27 +/-0,03	0,21 +/-0,03

Table 1 : Averaged macromolecular composition of the different samples for the different test conditions assayed in TN 25.120.

Table 2 : Averaged elemental composition of the different samples for the different test conditions essayed in TN-25.120.

TEST	A		C		B	
Dilution rate (h^{-1})	0,018		0,016		0,012	
Light intensity	305	50.2	305	50.2	305	50.2
%C	38.6 +/-2.2	36.4 +/-5.5	39.5 +/-4.9	38.4 +/-0.1	42.3 +/-1	42,3 +/-0,1
%H	5.6 +/-0.5	5.2 +/-0.9	5.74 +/-0.6	5.6 +/-0.01	6.13 +/-0.2	6.25 +/-0.01
%O	38.18 +/-1.74	39.44 +/-1.26	38.95 +/-0.42	40.8 +/-0.2	39,38 +/-0,29	37,52 +/-0.01
%N	5,2 +/-0.2	4.6 +/-0.96	4.6 +/-0.7	4.2 -	5,4 +/-0,3	4,7 +/-0,1
%P	1,0 +/-0.2	0,98 +/-0.2	0,45 +/-0.5	1,2 +/-0,05	0,75 +/-0,06	0,71 +/-0,2
%S	0,36 +/-0,05	0,38 +/-0,08	0,35 +/-0,05	0,38 -	0,31 +/-0,04	0,26 -
Ash	10.9 +/-3.6	12.96 +/-6.3	10.46 +/-7.0	9.355 -	5.73 +/-0.7	8.20 -

III. GRAPHICAL DATA REPRESENTATION

High dilution rate tests

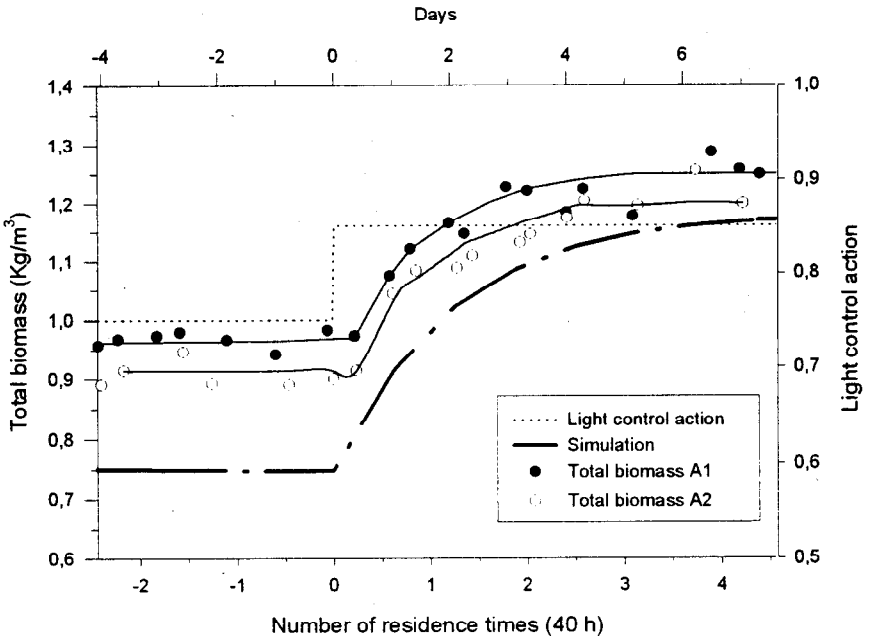

Figure 1: Comparison of the total biomass results measured obtained at a dilution rate of 0.025 h^{-1} during a change of illumination conditions from 133 W/m^2 to 305 W/m^2 at time 0, in light limiting conditions, with GPS simulation data.

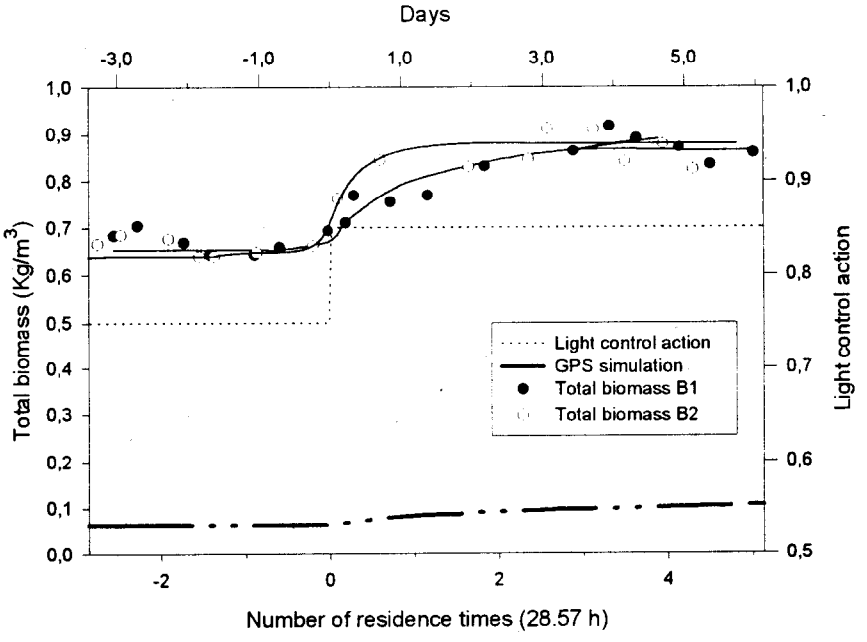

Figure 2: Comparison of the total biomass results measured obtained at a dilution rate of 0.035 h^{-1} during a change of illumination conditions from 133 W/m^2 to 305 W/m^2 at time 0, in light limiting conditions, with GPS simulation data.

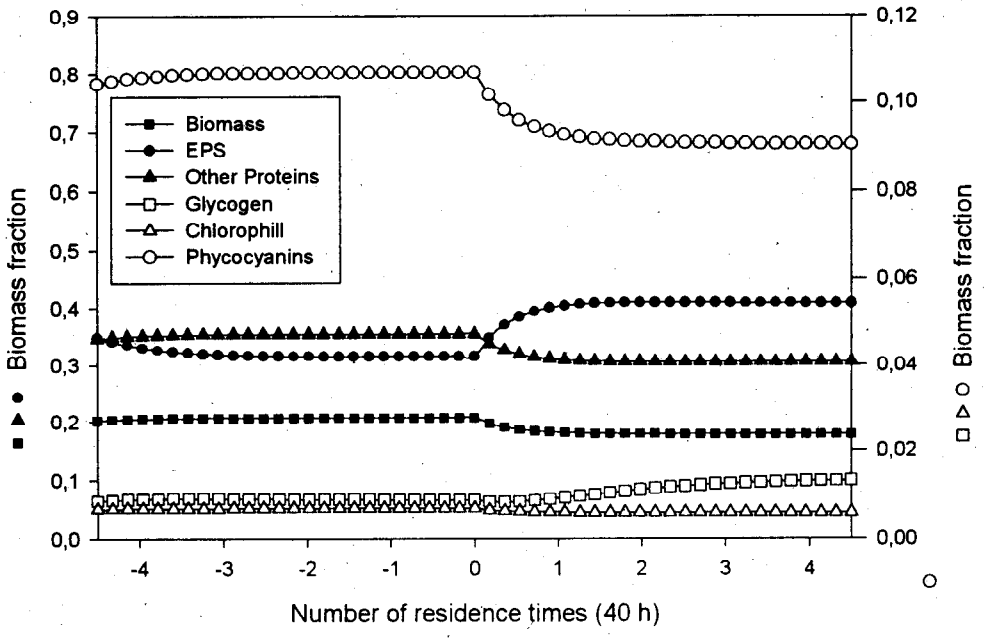

Figure 3: Values of the different biomass fractions calculated by the GPS simulation software corresponding to experimental data obtained at a dilution rate of 0.025 h^{-1} during a change of illumination conditions from 133 W/m^2 to 305 W/m^2 at time 0, in light limiting conditions, with GPS simulation data.

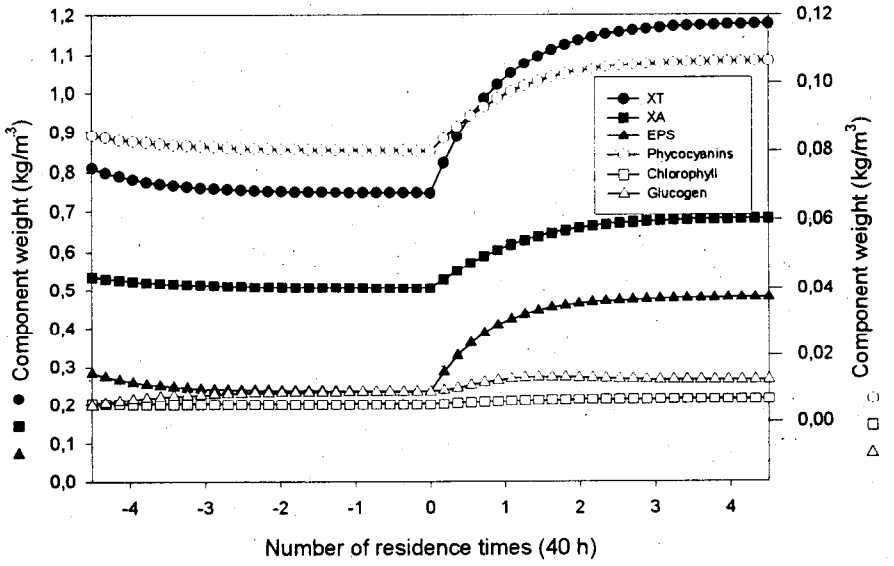

Figure 4: Values of the different component concentrations calculated by the GPS simulation software corresponding to experimental data obtained at a dilution rate of 0.025 h^{-1} during a change of illumination conditions from 133 W/m^2 to 305 W/m^2 at time 0, in light limiting conditions, with GPS simulation data.

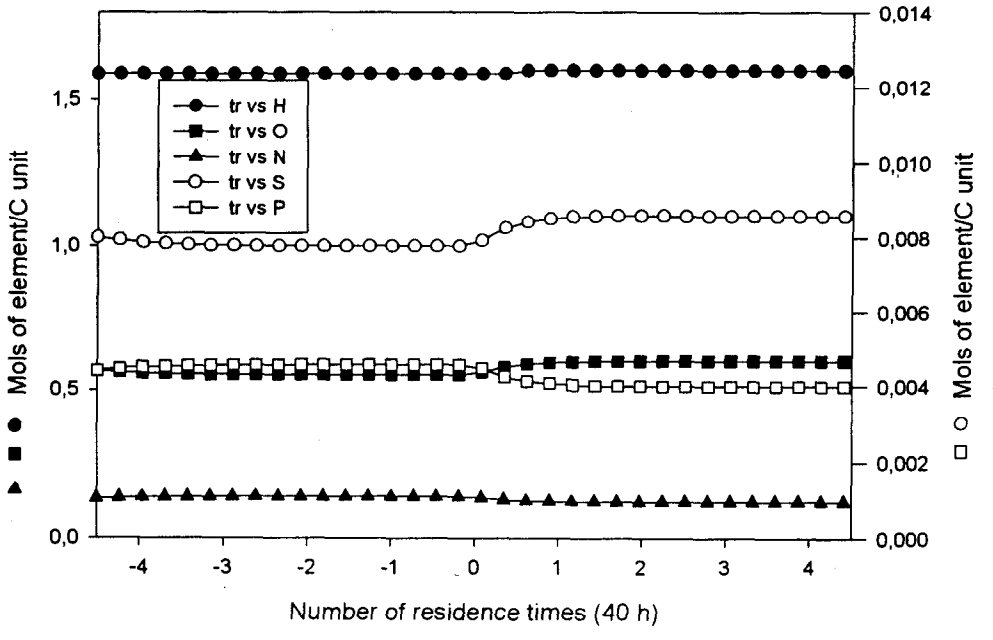

Figure 5: Values of the contents in different chemical elements, per mol of carbon, calculated by the GPS simulation software corresponding to experimental data obtained at a dilution rate of 0.025 h^{-1} during a change of illumination conditions from 133 W/m^2 to 305 W/m^2 at time 0, in light limiting conditions, with GPS simulation data.

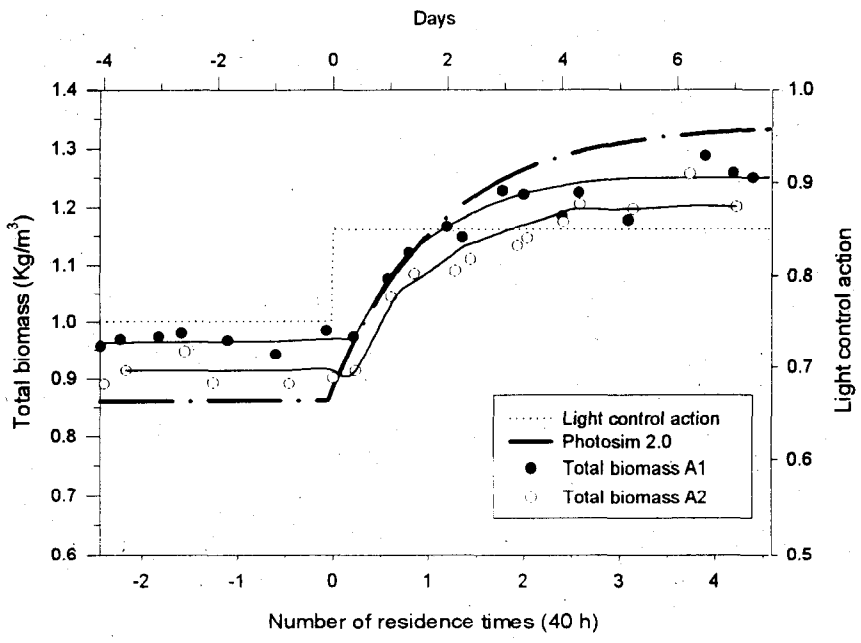

Figure 6: Comparison of the total biomass results measured obtained at a dilution rate of 0.025 h^{-1} during a change of illumination conditions from 133 W/m^2 to 305 W/m^2 at time 0, in light limiting conditions, with Photosim V2.0 simulation data.

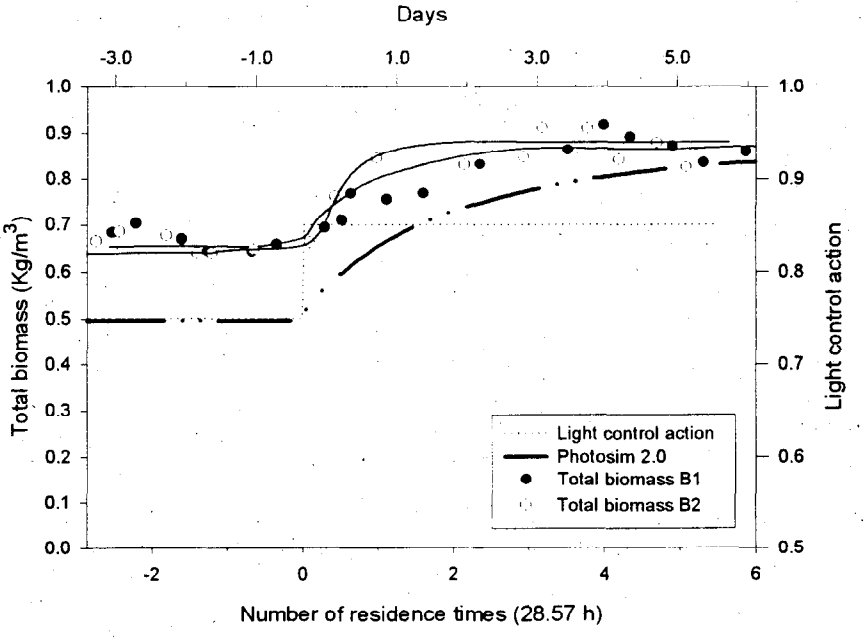

Figure 7: Comparison of the total biomass results measured obtained at a dilution rate of 0.035 h^{-1} during a change of illumination conditions from 133 W/m^2 to 305 W/m^2 at time 0, in light limiting conditions, with Photosim V2.0 simulation data.

Nitrate limitation tests.

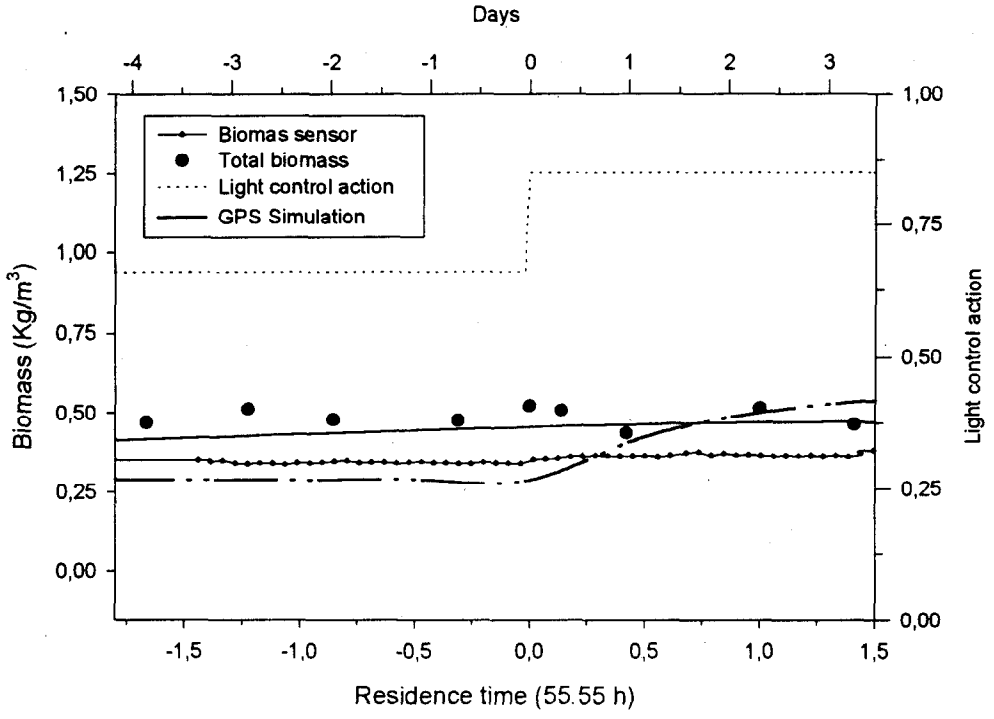

Figure 8: Comparison of the total biomass results measured, at a dilution rate of 0.018 h^{-1} during a change of illumination conditions from 50 W/m^2 to 305 W/m^2 at time 0, in nitrate limitation conditions, with GPS total biomass simulation data.

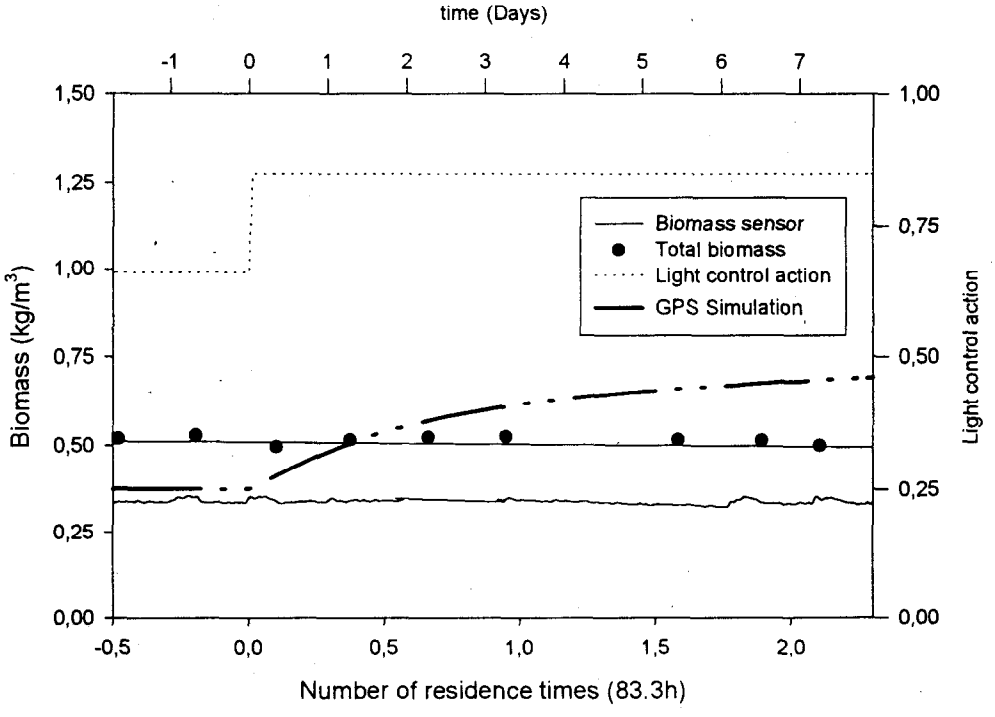

Figure 9: Comparison of the total biomass results measured, obtained at a dilution rate of 0.012 h^{-1} during a change of illumination conditions from 50 W/m^2 to 305 W/m^2 at time 0, in nitrate limitation conditions, with GPS total biomass simulation data.

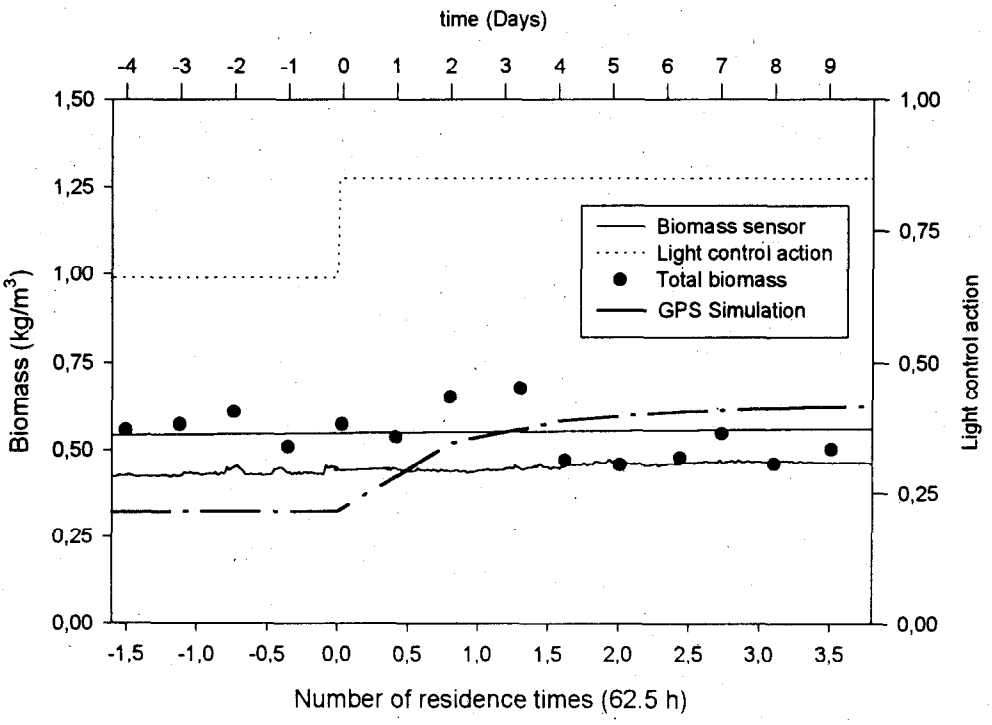

Figure 10: Comparison of the total biomass results measured, obtained at a dilution rate of 0.016 h^{-1} during a change of illumination conditions from 50 W/m^2 to 305 W/m^2 at time 0, in nitrate limitation conditions, with GPS total biomass simulation data.

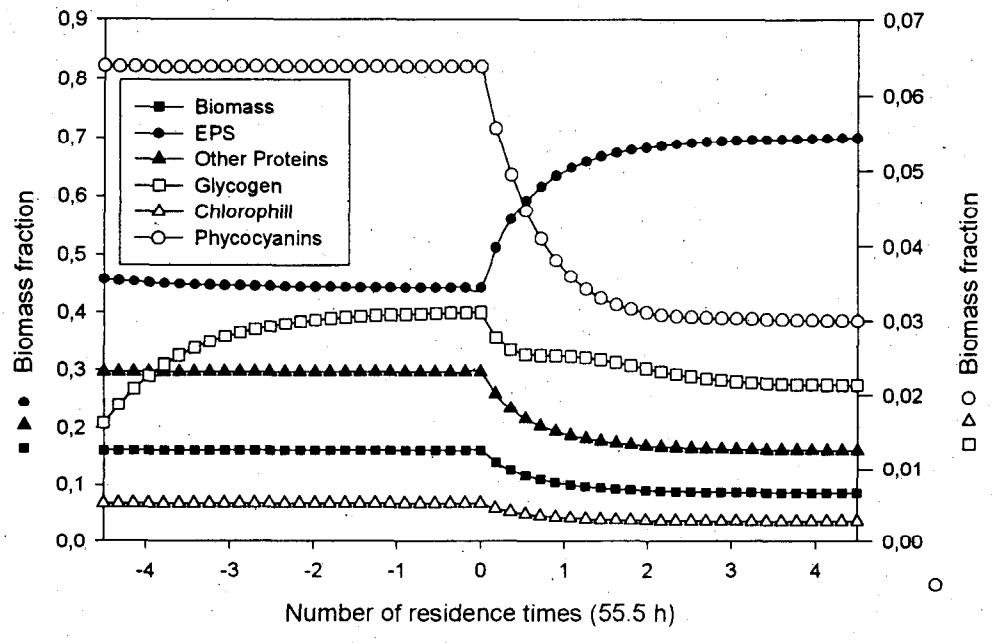

Figure 11: Values of the different biomass fractions calculated by the GPS simulation software corresponding to experimental data obtained at a dilution rate of 0.018 h^{-1} during a change of illumination conditions from 50 W/m^2 to 305 W/m^2 at time 0, in light limiting conditions, with GPS simulation data.

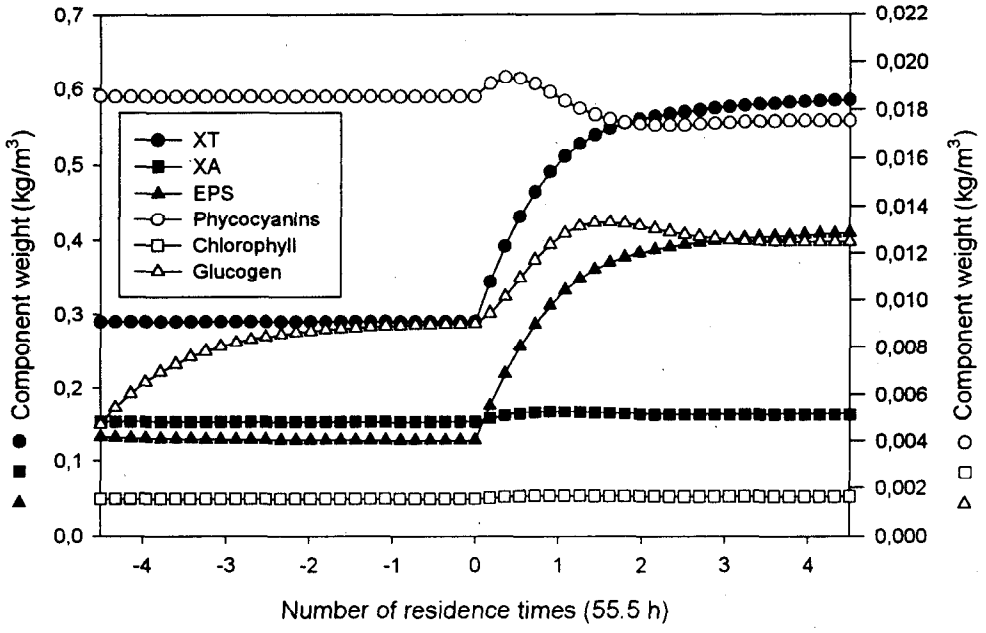

Figure 12: Values of the different component concentrations calculated by the GPS simulation software corresponding to experimental data obtained at a dilution rate of 0.018 h^{-1} during a change of illumination conditions from 50 W/m^2 to 305 W/m^2 at time 0, in light limiting conditions, with GPS simulation data.

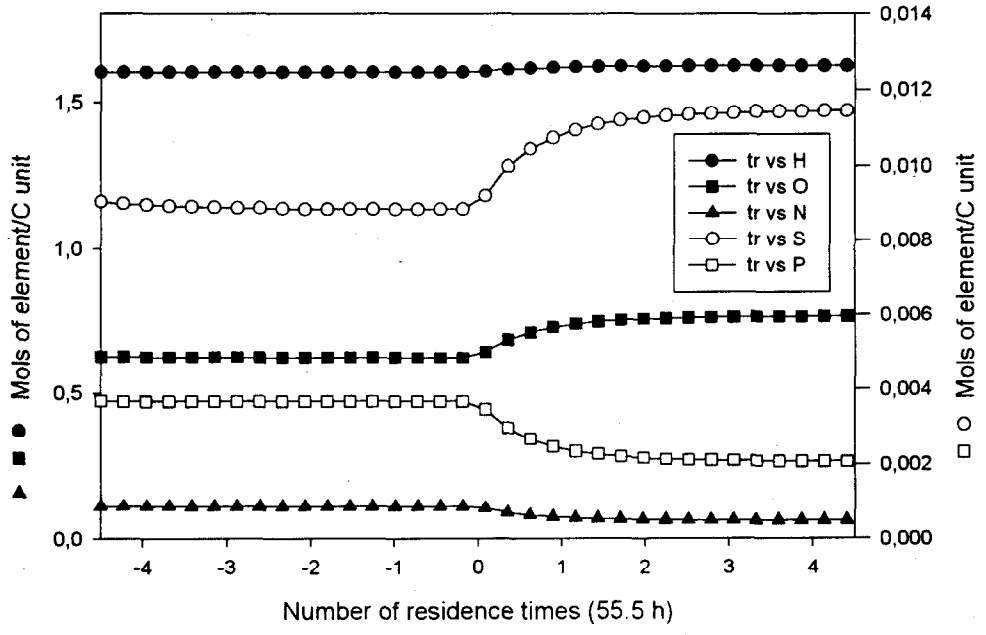

Figure 13: Values of the contents in different chemical elements, per mol of carbon, calculated by the GPS simulation software corresponding to experimental data obtained at a dilution rate of 0.018 h^{-1} during a change of illumination conditions from 50 W/m^2 to 305 W/m^2 at time 0, in light limiting conditions, with GPS simulation data.

IV. REFERENCES

CORNET J.F.; DUSSAP C.G.; GROSS J.B.; 1993[a]. Modelling of physical limitations in photobioreactors. Adaptation of the light energy transfer model to cylindrical geometry. ESA contract PRF 130-820. Technical Note 19.1.

CORNET J.F.; DUSSAP C.G.; GROSS J.B.; 1993[a]. Modelling of physical limitations in photobioreactors. Modelling of exopolysaccharide synthesis in cultures of *Spirulina platensis*. ESA contract PRF 130-820. Technical Note 19.1.

CORNET J.F.; DUSSAP C.G.; GROSS J.B.; 1993[a]. Modelling of physical limitations in photobioreactors. Applications to simulation and control of the *Spirulina* compartment of the MELISSA artificial ecosystem. ESA contract PRF 130-820. Technical Note 19.1.

FULGET N. 1994. MELISSA First approach of model based predictive control of Spirulina compartment. Contract ESA-ESTEC/ADERSA PRF n° 132443. Technical note 21.2

LECLERCQ J.,J. 1997. Software of computation of the biomass composition in the photoautotrophic compartment. Contract ESA-ESTEC/ADERSA PRF n° 151491. Technical note 28.4.

PONS P.; ALBIOL J.; GODIA F.;1996. General Purpose Station. Main Program Update II. ESTEC/CONTRACT 11549/95/NL/FG Technical Note. 37.9.

VERNEREY, A.; ALBIOL J.; GODIA F.; 1996. Nitrate limitation tests. ESTEC/CONTRACT 11549/95/NL/FG Technical Note 25.120.

VERNEREY, A.; ALBIOL J.; GODIA F.; 1996b. High dilution rate tests. ESTEC/CONTRACT 11549/95/NL/FG Technical Note 25.110.

VERNEREY, A.; ALBIOL J.; GODIA F.; 1997. Control Laws of the photosynthetic compartment. Biomass light studies. ESTEC/CONTRACT 11549/95/NL/FG Technical Note 37.110.

APPENDIX 1: PHOTOSIM AND GPS SIMULATORS UPGRADE

Previous to the tests presented in this technical note, either the Photosim simulator and the GPS model were updated with minor changes that have arisen since the Photosim V 1.0 was issued. Although a wider range of improvements will be included in the new Photosim version 2.0, only the following ones have already been included in the GPS simulator. Once Photosim V 2.0 will be issued a more complete upgrade of this simulator will be provided by ADERSA, and later included in the GPS.

In the first place, the previous Photosim version used two calculation procedures to obtain the amount of exopolyssaccharide generated (EPS) by the biomass, giving an average of both procedures as the end result. At this time it appears unnecessary to perform both procedures and one of them has been selected. Therefore the corresponding code modifications introduced with respect to the EPS are:

In Photosim: Previous code

```
      REPS1=WIV*MUEPS*Y(4)
C     CALCUL DE REPS2 PAR L'APPROCHE BIOCHIMIQUEMENT STRUCTUREE
C     (voir TN 19.2).
C_____________________________________________________________
      A=4*CI(1)*ALPHA*SINH(DELTA*RT)/(RT*(COSH(DELTA*RT)+ALPHA*SINH
     *(DELTA*RT)))
      PE=1.222E-5*A+1.267
      REPS2=(29.33*(PE*2.874-3.568)*RXA/23.096)/(3.33-PE*1.92)
C     CALCUL DE REPS PAR LA MOYENNE ARITHMETIQUE DE REPS1 ET REPS2
C_____________________________________________________________
      REPS=(REPS1+REPS2)/2
```

This code has been changed to:

```
      REPS=WIV*MUEPS*Y(4)
```

In consequence the corresponding code in the GPS has been modified to be equivalent and therefore:

Previous code:

```
REPS1 = 2. * mupMEPS * CPC * sEPS * wiv * kstep;
  A = 4*FR * alpha * sinh(delta) / RT / (cosh(delta) + alpha*sinh(delta));
  PE = 1.222e-5 * A + 1.267;
  REPS2 = 29.33 * (2.874*PE - 3.568) * *RXA / 23.096 / (3.33-1.92*PE);
  *REPS = (REPS1 + REPS2) / 2.;
```

New code:

```
*REPS = 2. * mupMEPS * CPC * sEPS * wiv * kstep;
```

During the first simulations using the GPS it appeared that under nitrate limitation conditions the addition of the different biomass fractions did not add to 1, that is the sum of the different parts of the biomass was not equal to the calculated total biomass. This resulted to be the consequence of a certain condition assumed in the software equations which was not fulfilled under nitrate limitation conditions. To overcome this problem, Photosim was also accordingly modified. The modifications done were:

Photosim:

Previous code

```
C    EXOPOLYSACCHARIDE
     F(9)=D*(CI(11)-Y(9))+REPS*(Y(6)/(PAR(10)+Y(6)))*(Y(7)/
     *(PAR(11)+Y(7)))+(RXA+REPS-RXV)*((PAR(10)/(PAR(10)+Y(6)))+
     *(PAR(11)/(PAR(11)+Y(7))))
```

Code Changed to:

```
C    EXOPOLYSACCHARIDE
     F(9)=D*(CI(11)-Y(9))+(F(1)-F(8))
```

Consequently the GPS software was modified to be equivalent and the modifications were:

Previous code:

```
/* calculation of the 9 mean volumic growth rates */
        ri[1] = RXA + REPS;                          /* rXT  */
        ri[2] = RXA * aa * bb;                       /* rXA  */
        ri[3] = zCH * ri[2];                         /* rCH  */
        ri[4] = zPC * RXA * (aa*bb - (dd+ee));       /* rPC  */
        ri[5] = zP * RXA * (aa*bb - qq*ee);          /* rP   */
        ri[6] = -YNXA * ri[2];                       /* rN   */
        ri[7] = -YSXA * ri[2] - YSEPS * REPS * aa * bb;   /* rS   */
        ri[8] = RXA * (aa*bb + cc*(dd+ee));          /* rXV  */
      ri[9] = REPS * aa * bb + (ri[1] - ri[8]) * (dd + ee);   / * rEPS */
```

New code:

```
/* calculation of the 9 mean volumic growth rates */

        ri[1] = RXA + REPS;                                  /* rXT  */

        ri[2] = RXA * aa * bb;                               /* rXA  */

        ri[3] = zCH * ri[2];                                 /* rCH  */

        ri[4] = zPC * RXA * (aa*bb - (dd+ee));               /* rPC  */

        ri[5] = zP * RXA * (aa*bb - qq*ee);                  /* rP   */

        ri[6] = -YNXA * ri[2];                               /* rN   */

        ri[7] = -YSXA * ri[2] - YSEPS * REPS * aa * bb;      /* rS   */

        ri[8] = RXA * (aa*bb + cc*(dd+ee));                  /* rXV  */

        ri[9] = ri[1] - ri[8];                               / * rEPS */
```

This modifications are included in the meantime until the new version of ADERSA for this simulator is issued as an adaptation to the Photosim V 2.0.