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Scientific Tests for *R. rubrum.* Growth on different C sources. Part I.

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I. INTRODUCTION

The study of the growth of photosynthetic micro-organisms requires the evaluation of the light available for the growth of the microbial cell. This evaluation is also required for the implementation of control algorithms acting on the light energy supply, which allow to modify the bacterial behaviour by modification of the illumination conditions.

In the MELISSA project a light control system has been developed for the photoautotrophic compartment. The algorithm is based on a model describing light illumination conditions inside the bioreactor according to the light intensity supplied from the vessel surface and the biomass concentration and its characteristics. The model has been developed first for a simplified monodimensional version, and later extended to a more evolved one describing the illumination conditions in radial illuminated bioreactors. It has been validated in cultures of *Spirulina platensis* either in batch as in continuous cultivation conditions. It appears feasible to adapt this model for its use on the photoheterotrophic compartment. Such adaptation requires previously the evaluation of the absorption (Ea) and scattering (Es) coefficients, characteristic of the corresponding species. The experimental determination of this coefficients has already been done (Cornet 1996). To proceed further in the adaptation of the model describing the illumination conditions, it is necessary to collect bacterial growth data in different illumination conditions. In this case, the carbon source is also the electron source, therefore it is interesting to do some tests using different carbon sources.

It must be mentioned that in the previous models light intensity was measured in the wavelength interval of 350-750 nm which is appropriated for organisms having their reaction centres and accessory light collection pigments absorbing in this band. This is the case of the higher plants or the *Spirulina* cells. However other organisms have their reaction centres and light recovery pigment systems acting at other wavelengths. In the case of *R.rubrum* its reaction centres have a maximum of absorption around 880 nm. This implies that they can collect light in a much larger wavelength range. In this case the range of wavelengths chosen to be measured was from 350 to 950 nm.

With the purpose of knowing the light emission characteristic of the lamps used in this experiments, their light emission spectrum was measured in ESTEC XAL

laboratory (appendix 2). It is clear from those spectra that the maximum of emission of light intensity is located around 680-700 nm which coincides with the *Spirulina* absorption maximum. However *R.rubrum* does not have absorption maximums at this wavelength, but it has carotenoids absorbing at a lower wavelength (maximums around 520 nm) and chlorophills absorbing in the range 800-950 (peaks around 800 and 880nm). That is, they do not use light corresponding to the wavelengths used by the *Spirulina* cultures (wavelengths around the red light), but they use the light not used by *Spirulina* (wavelengths corresponding to the green light or the beginning of the infrared). Light around the 650-710 nm is not absorbed, being only scattered, and for this reason it is used for the biomass dry weight determinations.

It has also to be mentioned that the energy collected around 520 nm is, at the bacterial membrane level, ultimately sent to the reaction centres. As These reaction centres have an absorption maximum at 880 nm, the energy they can convey for physicochemical use is surely smaller than the energy of the photons they can collect. Consequently there is a loose of energy as heat at least of the same order than the difference of the energy content of the light photons of the light collected (p.e. 520 nm) to the one collected at the reaction centre (about 880nm). Therefore light collected at around 880 nm is more efficiently used than light collected at shorter wavelengths. On the other hand having pigments collecting at shorter wavelengths allows the bacteria to collect a higher number of photons at the same time and therefore to be more efficient in using the light available.

Consequently the illumination systems could be improved using lamps having an increased emission in the ranges around 450-580 nm and 800-900 nm. Light of other wavelengths is either less efficiently used or not used at all. As the lamps used currently have infrared filters, to decrease heat emission, it would be desirable to use lamps with infrared filters acting for wavelengths higher than 950 nm. Also desirable could be lamps that at the same time could have an important emission around at the 450 580 nm band.

Preliminary Roux flask data were reported in technical note 25.7 (Albiol 1996). The experiments were performed using flat culture vessels and monodimensional illumination conditions which allow a more simple and accurate way of light availability evaluation. In those tests the effect of illumination on cell growth was examined. In the

present TN, the effect of different carbon sources on the growth of the *R. rubrum* under monodimensional illumination conditions is reported.

II. MATERIAL AND METHODS.

The bacterial strain *Rhodospirillum rubrum* (ATCC 25903) was obtained from the American Type Culture Collection. It was revived and the subcultures were done using their recommended media.

Culture media was based on the basal salts mixture formulated by Segers & Verstraete as described by Suhaimi (Suhaimi et al 1987), using a volatile fatty acid as a carbon/electron source and biotin as the only vitamin. To maintain the culture pH and to decrease medium culture precipitation, that could affect the measurements, the following modifications were done. Phosphate concentration was decreased to the following levels: KH_2PO_4 0.2 g/l K_2HPO_4 0.3 g/l. Buffer capacity to maintain the pH culture was obtained using 3-Morpholino propane sulphonic acid (MOPS) 21 g/l. Phosphate was autoclaved separately. The pH was adjusted to 6.9.

Temperature (30 °C) was maintained by means of a water bath. Culture was maintained homogeneous using a magnetic stirrer.

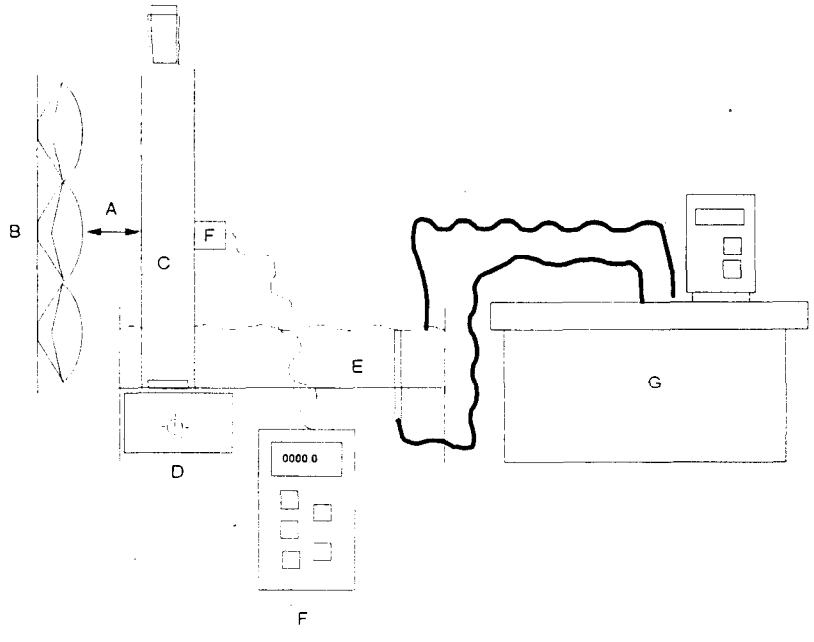

Figure 1: Experimental set up ; A : lamps-bioreactor distance. B : Lamps support. C : Bioreactor. D : magnetic stirrer. E : water bath. F : light sensor. G thermostatic bath.

The volume of the flat vessels was 1.13 litres. External dimensions of the bottom area of the vessel were 12x5.5 cm with and average 2 mm glass thickness. The top part of the vessel is round, however from the base area and the volume, a frontal area exposed to the light of 17.1x12cm (0.0205 m^2) can be calculated. A volume of 5 ml was extracted for each sample.

Figure 2 :View of the experimental set-up.

Experiments were carried out in the experimental set up shown in figures 1 and 2. Illumination was set up in monodimensional conditions inside a dark chamber with internal black surface. Two identical set-up were used during the experiments, corresponding to two floors on the dark chamber. On the first floor the lamps were located at 9 cm from culture vessel surface. On the second floor lamps were located at 7 cm from the vessel surface. Both set ups give the same average light intensity on bioreactor surface, the different distances are due to the different distribution of lamps on the light support. Lamps used were of the type Sylvania professional BAB 38° 12V 20W (new version, cool beam, UV filtered (Green box, code type 215)). Light intensity obtained, corresponded to an average value around 200 W/m^2 PAR as on to a flat glass surface located at the same position as the frontal part of the culture flasks. The averaged light intensity values measured is given in table 1.

Photosynthetically active radiation (PAR) was measured using a quantum sensor, of the same type as is used in the photoautotrophic compartment (Licor Li-190SA), attached to a LI-189 portable meter. The sensor gives the photosynthetic photon flux density (PPFD) in $\mu mols \cdot s^{-1} \cdot m^{-2}$. Conversion of quantum units to radiometric units (W/m^2) has been done by using a constant factor obtained by integration of the lamp spectral data (appendix 2). Factors used were: 0.204 for the range 300-700nm, 0.291 for the range 350-450 nm, 0.425 for the range 350-950 nm. The result of the application of those conversion factors is given in table 1.

Biomass dry weight was calculated from the measured absorbency of a sample (A_{700}) and its value interpolated on a calibration curve made using determined values of dry weight and optical density (appendix 1).

The carbon sources used in this tests were: acetic acid (2.5 g/l), butyric acid(1.84 g/l), propionic acid (2.06 g/l), isovaleric acid (1.7 g/l) and isobutyric acid(1.84 g/l). The concentrations used correspond to a 1 gC/l for all cases. Sodium carbonate concentrations for each case were respectively 0.25 g/l, 1.35 g/l, 0.67 g/l, 1.23 g/l,1.35 g/l.

Illumination conditions	$\mu mol \times m^2 \times s^{-1}$ +/- S.E. (PPFD)	W/m^2 (300-700) +/- S.E. (PAR)	W/m^2 (350-750) +/- S.E.	W/m^2 (350-950) +/- S.E.
first floor : 8 lamps, 12 V	1021 +/- 40	208 +/-8	297 +/- 11	434 +/-17
second floor : 8 lamps, 12 V	996 +/- 60	203 +/-12	289 +/- 17	423 +/-25

Table 1: Average light intensities for the different experiments. S.E. : Standard error ($SD/\sqrt{(n)}$).

III Experimental Results

III.1 TEST A: Acetic acid:

In this test acetic acid was used as a carbon and electron source. Light energy was supplied as explained in 'materials and methods', with an average light intensity of 996 +/- 60 mmol×m^2×s^{-1} (PAR), (W/m^2: 203+/-12 (400-700), 423+/-25 (350-950)) In order to obtain a high initial biomass concentration, about 400 ml of a preculture (preculture was done in acetic acid) of around 3.5 g/l were centrifuged and resuspended in 1 l of culture media. (the test was performed in floor 2).

Once diluted the culture begins with a lag phase wich gradually turns into the linear light limited phase at around 10-15 hours (figure 2). During this time there is a small increase in biomass of around 0.5 g/l of dry weight. This fact is due to the time required to adapt to the new cultivation conditions (average light intensity, carbon source concentration, ...). (If the culture is diluted, as soon as it enters the linear phase,

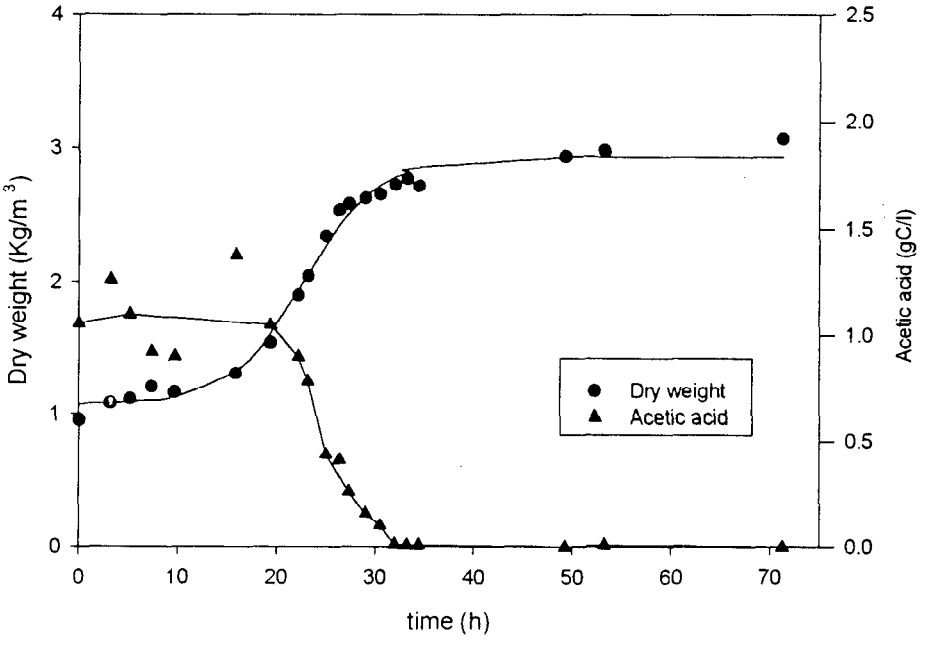

Figure 3: Experimental data obtained in Acetic acid test (floor 2).

so as to obtain the initial biomass concentration, the lag phase decreases and, eventually, disappears. This is due to the fact that the change in culture conditions is not severe compared with the change occurring when the culture is diluted directly from a high biomass concentration). After this point the culture begins a light limited linear phase. Total biomass dry weight at the end of the culture amounts of 3.07 kg/m^3, which gives a global yield of 0.8kg DW/kg acetic (2.1 gDW/gC).

Table 2: Experimental data obtained in acetic acid test . (floor 2)

Time(h)	DW (kg/m^3)	Acetic acid (C-kg/m^3)
0.0	0.95	0.38
3.2	1.08	0.43
5.2	1.11	0.45
7.3	1.20	0.48
9.7	1.16	0.46
15.8	1.31	0.52
19.3	1.54	0.62
22.2	1.90	0.76
23.2	2.04	0.82
25.0	2.35	0.94
26.3	2.54	1.02
27.3	2.58	1.03
29.0	2.63	1.05
30.5	2.66	1.06
32.0	2.73	1.09
33.3	2.78	1.11
34.4	2.72	1.09
49.3	2.94	1.18
53.3	2.98	1.19
71.3	3.07	1.23

III.2 TEST B : Acetic acid.

This test is equivalent to the previous one but it was done in the first floor of the set up using the same media and innoculum. Preculture was therefore done in acetic acid. Its purpose is to verify that there is no significant difference between using the first or the second floor of the experimental set-up, and to have a further indication of the reproducibility of the results. The culture presents a lag phase of about 5-10 hours, in contrast to the 15-hours of the previous case. The slightly different length of the lag phase can not be related, at this time, to any specific fact. Nevertheless, this difference is not considered significative for the comparison of this experiments, being more important to compare the specific growth rate obtained in both cases. Results obtained using both set ups appear to be equivalent, within the margin of experimental error, and therefore it is considered that experiments can be performed in either one with comparable results.

Total biomass dry weight at the end of the culture amounts of 3.07 kg/m^3 .This gives a global yield of 0.78 kg DW/kg acetic (1.96 gDW/gC).

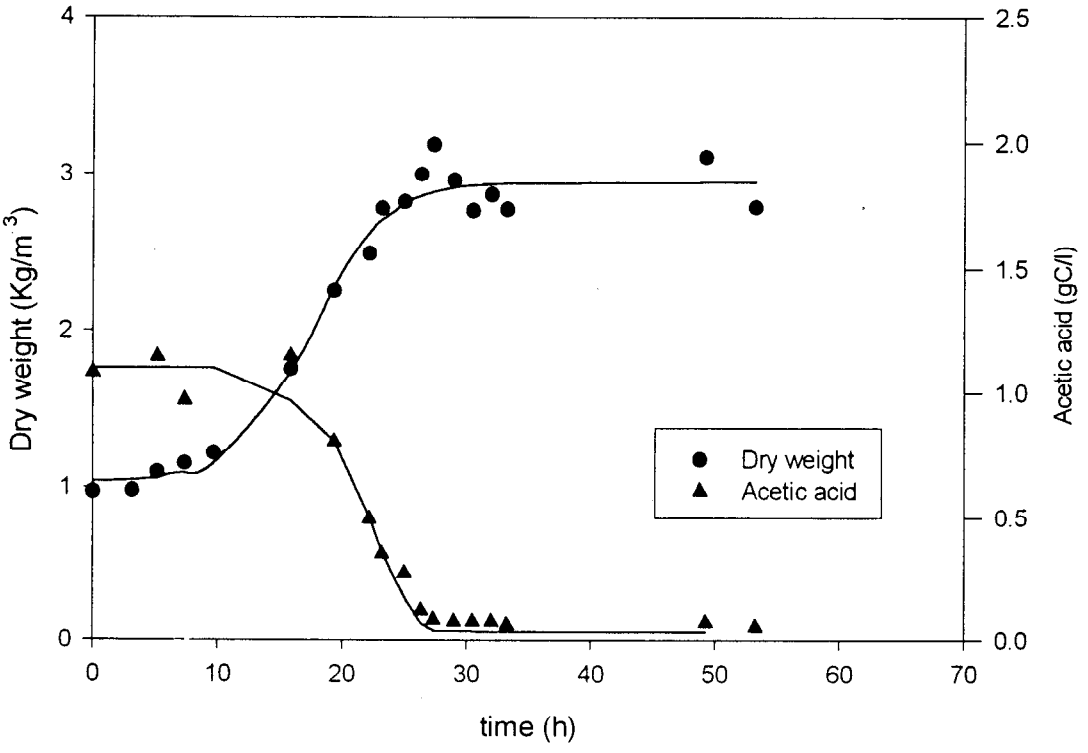

Figure 4: Experimental data obtained in acetic acid test B (floor 1).

Table 3: Experimental data obtained in acetic acid test (floor 1).		
Time(h)	DW(kg/m^3)	Acetic acid (C-kg/m^3)
0.0	0.97	1.08
3.2	0.98	-
5.2	1.09	1.14
7.3	1.15	0.97
9.7	1.22	-
15.8	1.75	1.15
19.3	2.26	0.80
22.2	2.50	0.5
23.2	2.78	0.35
25.0	2.82	0.27
26.3	3.00	0.12
27.3	3.18	0.08
29.0	2.96	0.075
30.5	2.77	0.075
32.0	2.87	0.074
33.3	2.78	0.061
49.3	3.11	0.072
53.3	2.79	0.054

III.1 TEST C : Propionic acid.

This test was done in the same illumination conditions as the previous one. However the carbon source used was propionic acid, which is also a possible compound to be obtained in the effluent from the first compartment. The culture presents no lag phase probably due to the fact that it comes from a previous propionic acid culture that had to be only slightly diluted. In that preculture the lag phase was finished but the biomass was diluted before concentration was too high. Total biomass dry weight at the end of the culture amounts of 3.1 kg/m^3 which gives a global yield of 1.77 kg DW/kg propionic (3.6 gDW/gC). The pH at the end of the culture was 7.2.

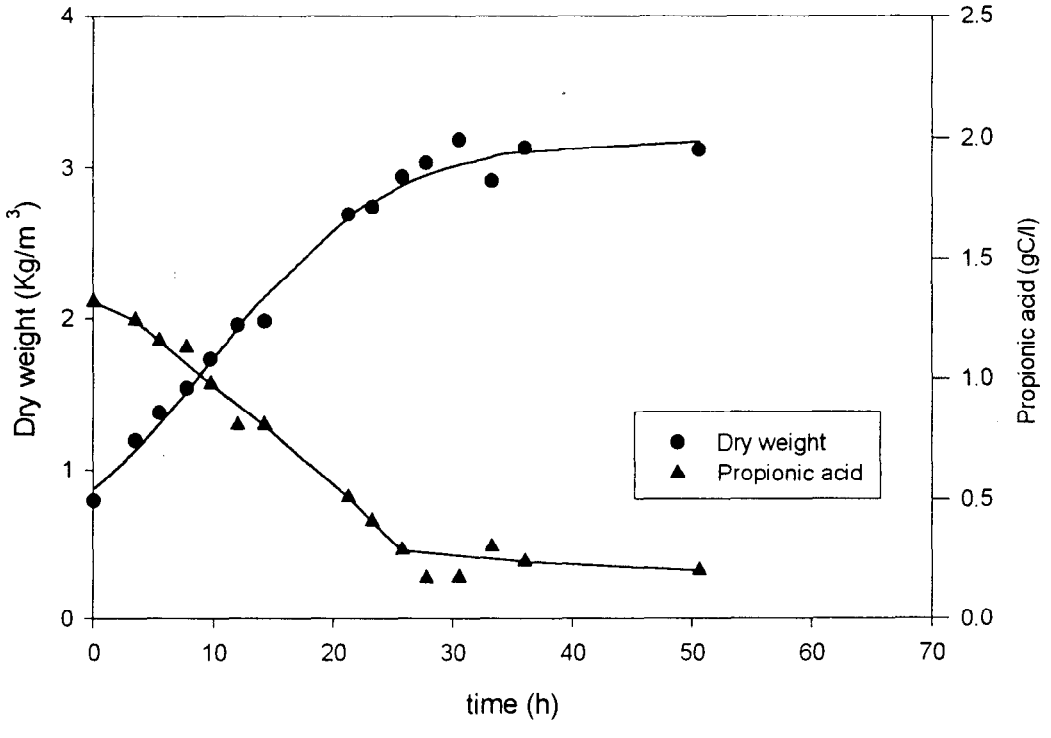

Figure 5: Experimental data obtained in propionic acid test C (floor 1).

Table 4: Experimental data obtained in propionic acid test (floor 1).		
Time(h)	DW (kg/m^3)	Propionic Acid (C-kg/m^3)
0.0	0.79	1.32
3.5	1.19	1.24
5.5	1.37	1.16
7.8	1.54	1.13
9.8	1.73	0.98
12.0	1.96	0.81
14.3	1.98	0.81
21.3	2.69	0.51
23.3	2.74	0.41
25.8	2.94	0.29
27.8	3.03	0.17
30.5	3.19	0.18
33.3	2.91	0.30
36.0	3.13	0.24
50.6	3.12	0.21

III.1 TEST D : Butyric acid.

This test was done in the same illumination conditions as the previous one. However the carbon source used was butyric acid, which is a possible compound to be obtained in the effluent from the first compartment. Innoculum was precultured in butyric acid. Total biomass dry weight at the end of the culture amounts of 3.6 kg/m^3 which gives a global yield of 1.54 kg DW/kg butyric (2.85 gDW/gC). The pH at the end of culture was 7.2.

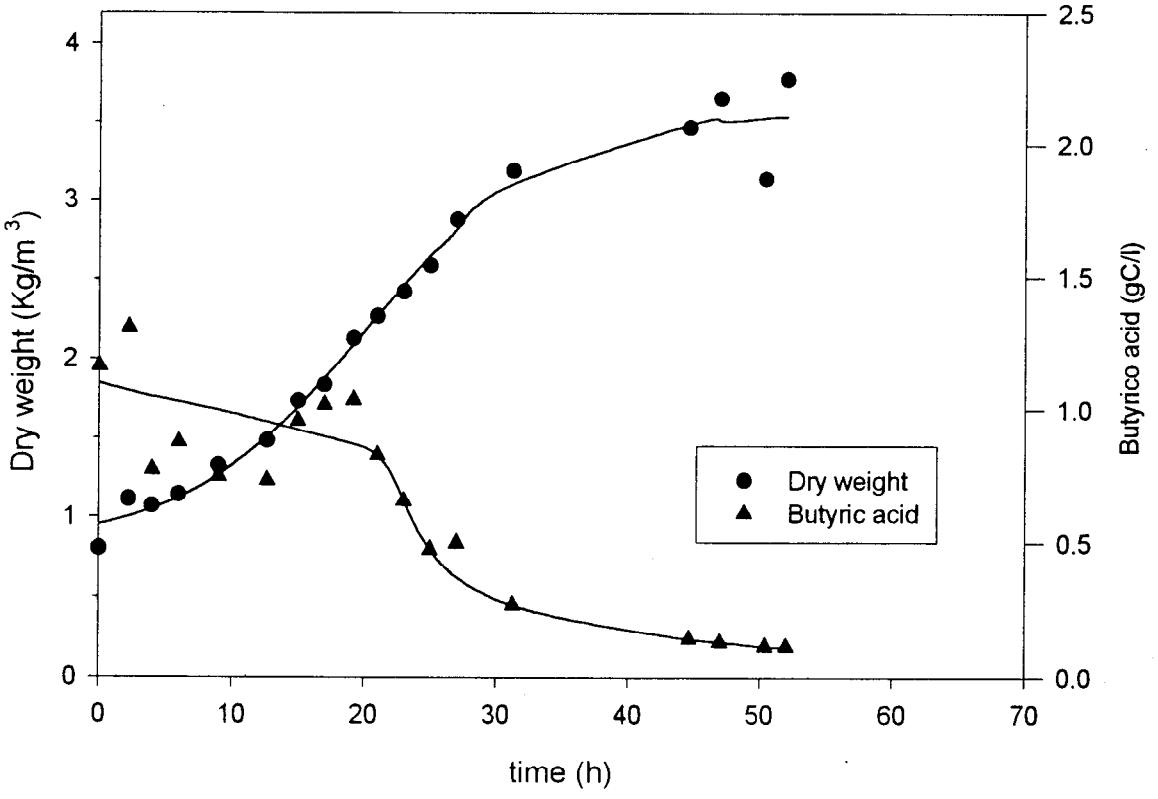

Figure 6 : Experimental data obtained in butyric acid test D (floor 1).

Table 5: Experimental data obtained in butyric acid test (floor 1).

Time(h)	DW (kg/m^3)	Butyric (C kg/m^3)
0.0	0.81	1.16
2.3	1.11	1.31
4.0	1.07	0.77
6.0	1.14	0.88
9.0	1.33	0.75
12.7	1.48	0.73
15.0	1.73	0.96
17.0	1.84	1.02
19.2	2.13	1.04
21.0	2.27	0.83
23.0	2.43	0.66
25.0	2.59	0.48
27.0	2.88	0.50
31.3	3.19	0.27
44.7	3.47	0.14
47.0	3.66	0.13
50.4	3.14	0.11
52.0	3.78	0.11

III.1 TEST E : Isovaleric acid.

This test was done in the same illumination conditions as the previous ones. However the carbon source used was Isovaleric acid, being it a possible compound to be obtained in the effluent from the first compartment. Preculture was done in isovaleric acid. Total biomass dry weight at the end of the culture amounts of 3.2 kg/m^3 which represents a global yield of 2.08 kg DW/kg isovaleric (3.54 gDW/gC). The pH at the end of culture was 7.2.

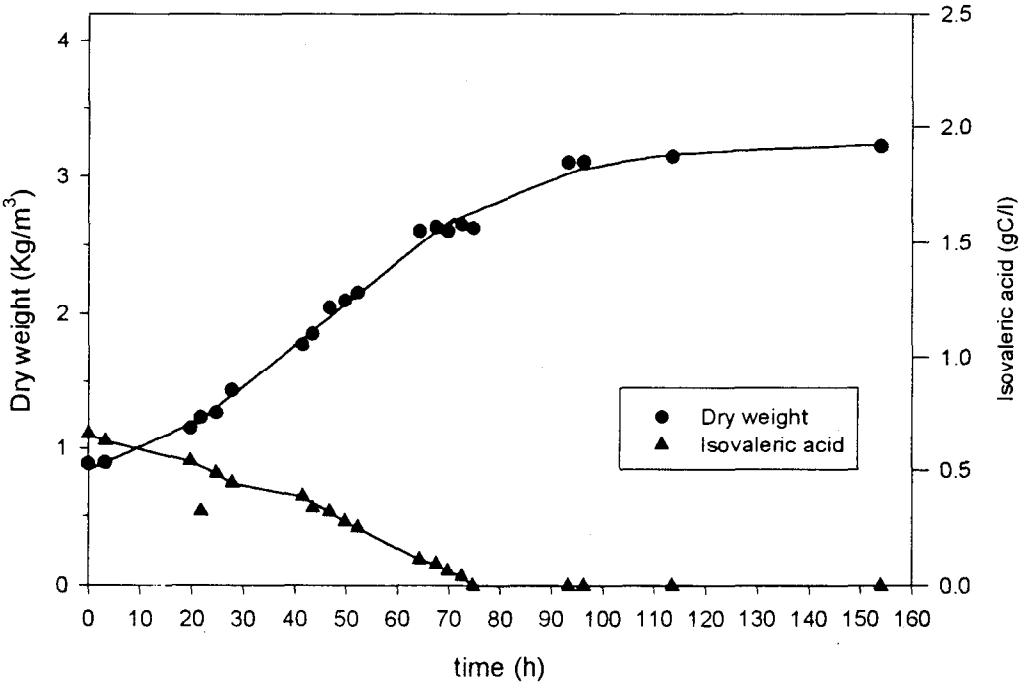

Figure 7: Experimental data obtained in isovaleric acid test E (floor 1).

Table 6: Experimental data obtained in isovaleric acid test (floor 1).		
Time(h)	DW (kg/m^3)	Isovaleric Acid (C-kg/m^3)
0.0	0.88	0.66
3.3	0.89	0.63
19.8	1.15	0.54
21.8	1.23	0.32
24.8	1.27	0.49
27.8	1.44	0.44
41.5	1.77	0.38
43.5	1.85	0.33
46.8	2.05	0.31
49.8	2.10	0.27
52.3	2.16	0.25
64.3	2.60	0.11
67.5	2.63	0.09
69.8	2.60	0.06
72.5	2.65	0.04
74.8	2.62	0.00
93.3	3.10	0.00
96.3	3.11	0.00
113.5	3.14	0.00
154.0	3.22	0.00

III.1 TEST f : Isobutyric acid..

This test was done in the same illumination conditions as all the previous tests. However the carbon source used was Isobutyric acid, another possible component of the incoming flow of this compartment. Preculture was done in isobutyric acid. Total biomass dry weight at the end of the culture amounts of 3.15 kg/m^3 which represents a global yield of 2.7 kg DW/kg isobutyric (5. gDW/gC). Results obtained in this test can are found in figure 8 and table 7.

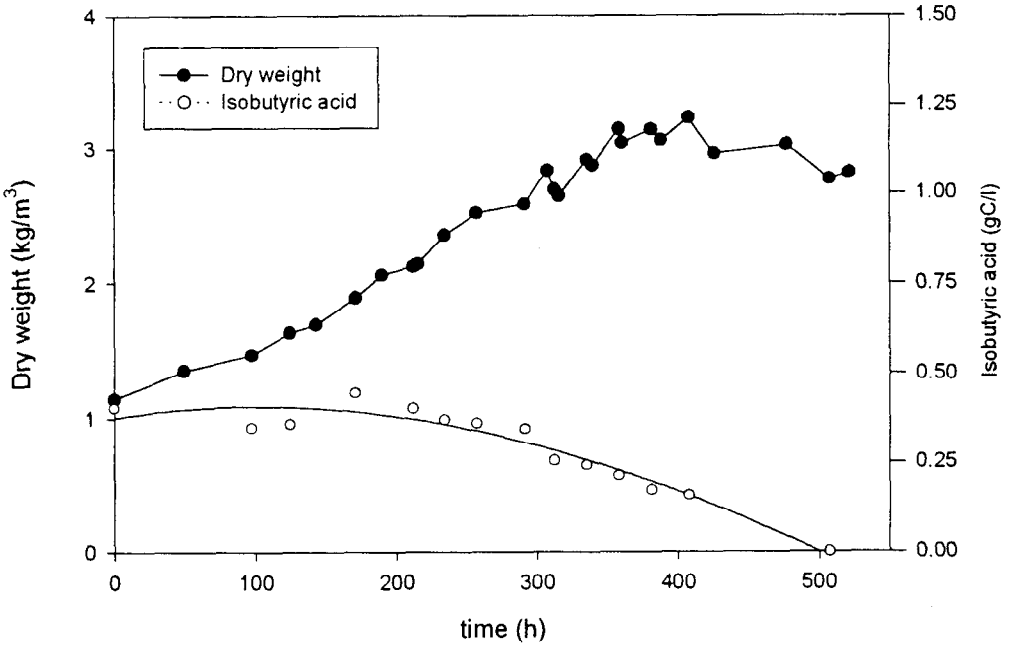

Figure 8: Experimental data obtained in isobutyric acid test F (floor 1).

Table 7: Experimental data obtained in isobutyric acid test (floor 1).		
Time(h)	DW (kg/m^3)	Isobutyric Acid ($C\text{-}kg/m^3$)
0.0	1.14	0.44
49.0	1.35	-
97.0	1.47	0.35
124.2	1.63	0.359
142.7	1.69	-
170.7	1.89	0.45
189.7	2.06	-
212.0	2.12	0.40
215.2	2.14	-
234.0	2.35	0.37
257.0	2.51	0.36
291.2	2.58	0.34
307.2	2.83	-
312.5	2.69	0.26
315.5	2.64	-
335.2	2.92	0.24
339.5	2.87	-
358.2	3.15	0.21
360.5	3.04	-
381.2	3.14	0.17
388.3	3.06	-
407.7	3.23	0.16
426.	2.96	-
477	3.03	-
507	2.77	0
521	2.82	-

CONCLUSION

This data represents the first part of the experiments done to adapt a mathematical model relating growth rate with light intensity. This model is similar to the one used with *Spirulina* cultures by Cornet (Cornet 1992 and 1996).

In the tests reported in this technical note, a light intensity value of around 200 W/m^2 PAR was chosen. With this light intensity different carbon sources, at concentrations of 1 g/C/l were used. The results show, a linear phase which presumably corresponds to the light limitation phase. Some of the cultures show a biomass final concentration of 3.1-3.2 g/l of biomass at which point they cease to grow. In some of those cases there appears to be still some carbon source to consume. It might be suggested that light intensity is not sufficient to allow further consumption of the substrate, and therefore that they are under strong light limitation. This are adequate conditions for testing the model behaviour under light limitation.

In the next batch of tests light intensity will be reduced 10 times and experiments repeated. This will allow to test the mathematical model for the biomass behaviour under light limitation conditions. After that step, some continuous cultures will be done under light limiting conditions, which will allow the fine tuning of the above mentioned mode.

It will be interesting to see what is the influence of the different carbon sources, on the growth rate, due to its possible use as electron source by the bacteria, besides being used as a carbon source.

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APPENDIX 1 : BIOMASS DW CALIBRATION

Biomass calibration curve for the spectrophotometric determinations. Data represents average values from tree different determinations. Dry weight was measured using biomass from different culture stages filtered using Sartorius 0.2 µm filters (Cat n° : SM11107-043N) and dryed at 100 °C until constant weight.

$$BM = aDO^3 + bDO^2 + cDO$$

Where :

BM= biomass concentration (g/l)

DO= optical density

a=0.0113 +/-0.0035

b=0.1247 +/-0.096

c=0.5262 +/-0.061

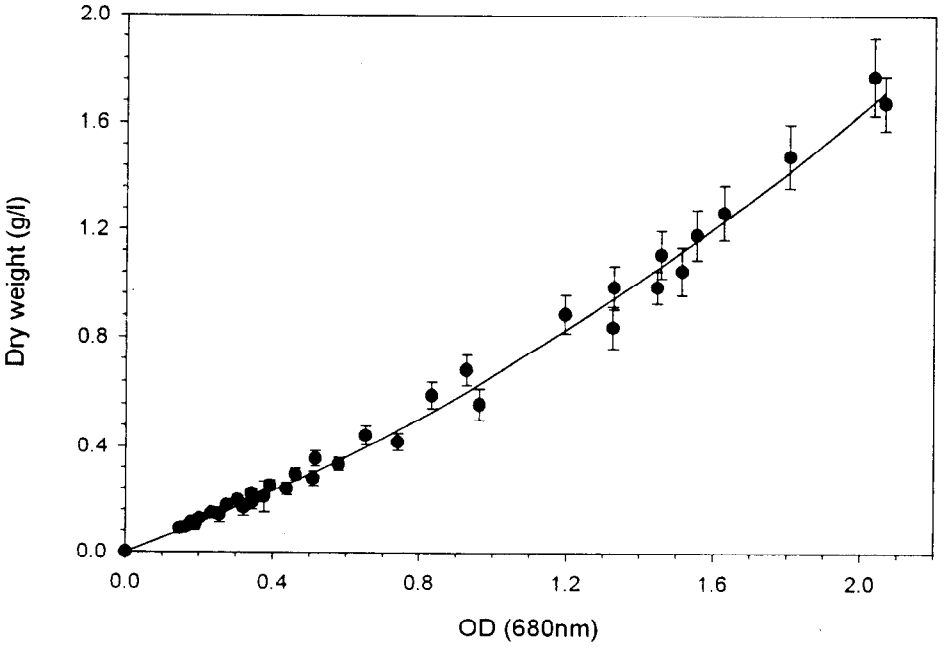

Figure 9: Dry weight vs. Optical Density used in this report.

APPENDIX 2 : MEASUREMENT OF LAMP SPECTRAL IRRADIANCE.

In order to be able to know the amount of radiation emitted by the lamps in the part of the spectrum used by *R.rubrum*, the spectral irradiance of the currently used lamps was measured. Measurements were done at XAL section of the ESTEC centre of ESA by D. Doyle.

The instrument used was a 1m Monochromator, Jobin-Yvon THR1000 with PMT (350-450 nm) and Si (450-1100 nm)detectors. 40W Spectral irradiance standard lamp and calibrated integrating sphere. The following parameters were used in the instrument :

Spectral resolution : 2.4 nm (450-1100nm) & 0.8 nm (350-450 nm).

Measurement interval : 10 nm.

Spectral range : 350 to 1100 nm.

Wavelenght accuracy : +/- 0.05 nm.

Irradiance accuracy : +/- 5% (500-850nm) & +/-10% (350-500 ;850-1100)

Measurement location : On axis at 55 mm from the centre of the frontal lamp window.

The spectral illuminance for the three different lamp models used can be seen in the following figures, for the 12, 10, 9 and 6 Volts applied to the lamps. The lamps used in this TN were the those with 38° UV filtered clear window (type: 215). The lamp spectra for the other type of lamps used previously, are shown for comparison purposes. In TN 25.7 the lamps used were 38° clear window (type: 104).

Lamps (type: 323) 38 ° frosted window were similar to the 36° ones used on the bioreactor experiments in Albiol 1994 (appendix 9 top curve) while a type similar to the (104) 38° clear window, was used in test tube light experiments as is shown in the spectral data given in the mentioned report (appendix 9, lower curve). It should be mentioned that in that report preliminary spectral data, done also at XAL-ESTEC, is given, were the relative intensities of both curves is correct, however the total power stated was inaccurate. Nevertheless the relative intensities can be used to compare the spectral distribution of the lamps.

The conclusions of the lamp spectral analysis were :

-Lamps 104 and 215 show a similar irradiation levels around the visible part of the spectrum but show a clear difference around 910 nm were lamp 104 exhibits bigger subsidiary peak than model 215.

-Lamp 323 with its frosted window shows a significant reduction in total output compared with the other two lamps, showing also a peak around 910 nm.

-Lamp 215 shows the best IR filtering.

-The total UV emission (<400 nm) is not significant for all the lamps.

-Lamp 104 has the highest total irradiance.

-All the lamps show maximum peak spectral irradiance in the range 680-720 nm.

-The ratio of IR (>800 nm) to the visible (<800 nm) increases with decreasing operating voltage.

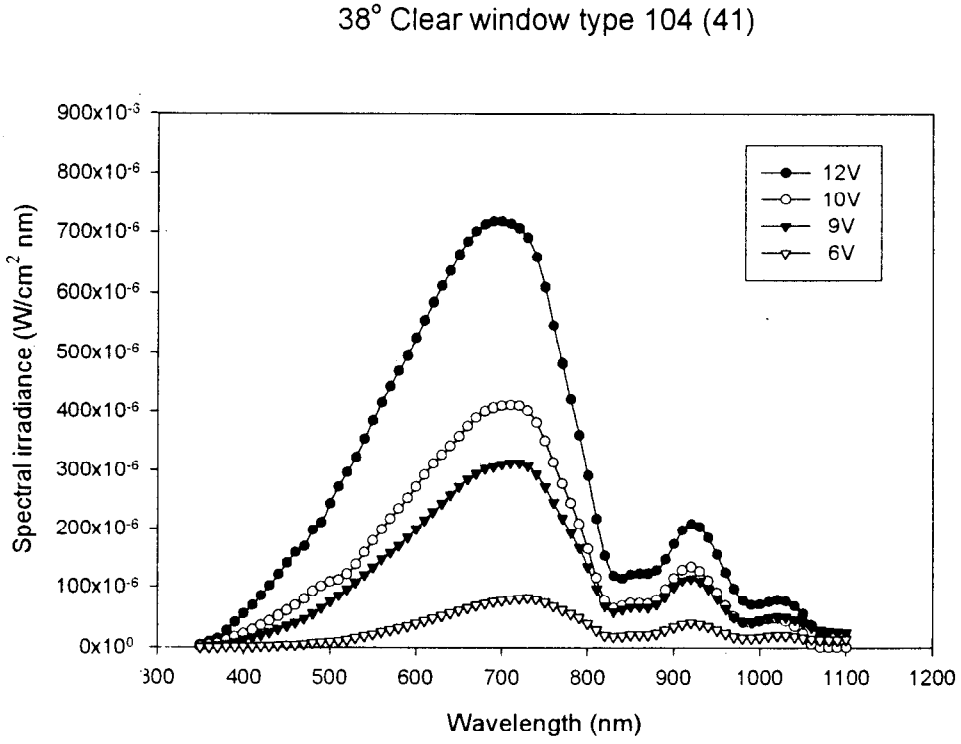

Figure 10: Emision spectra of lamps of type model 104.

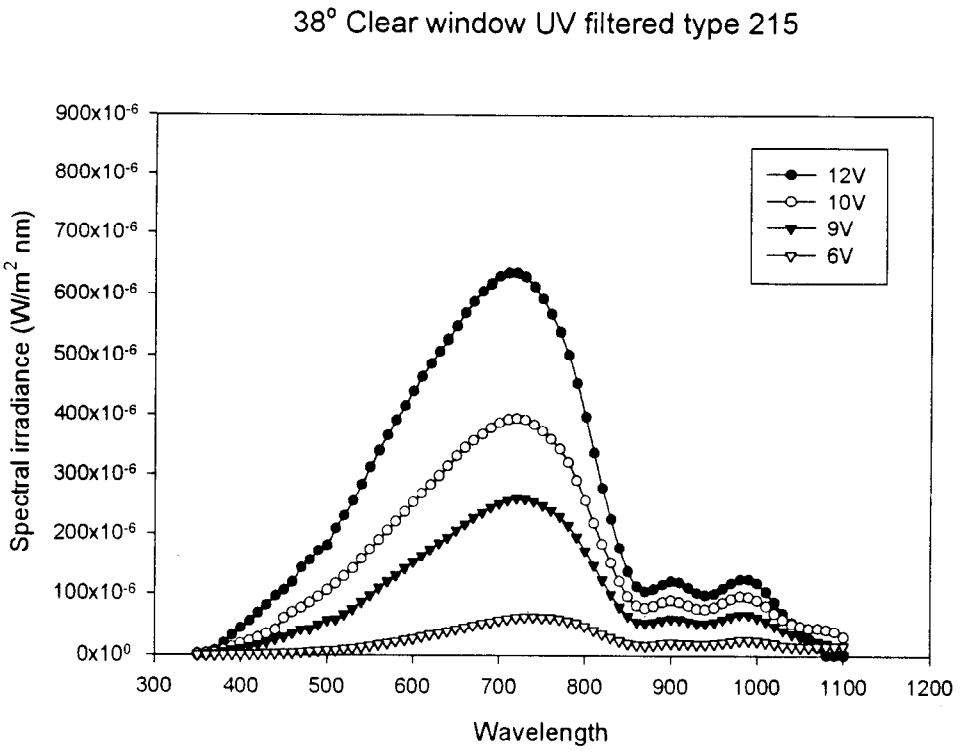

Figure 11: Emision spectra of lamps of type model 215

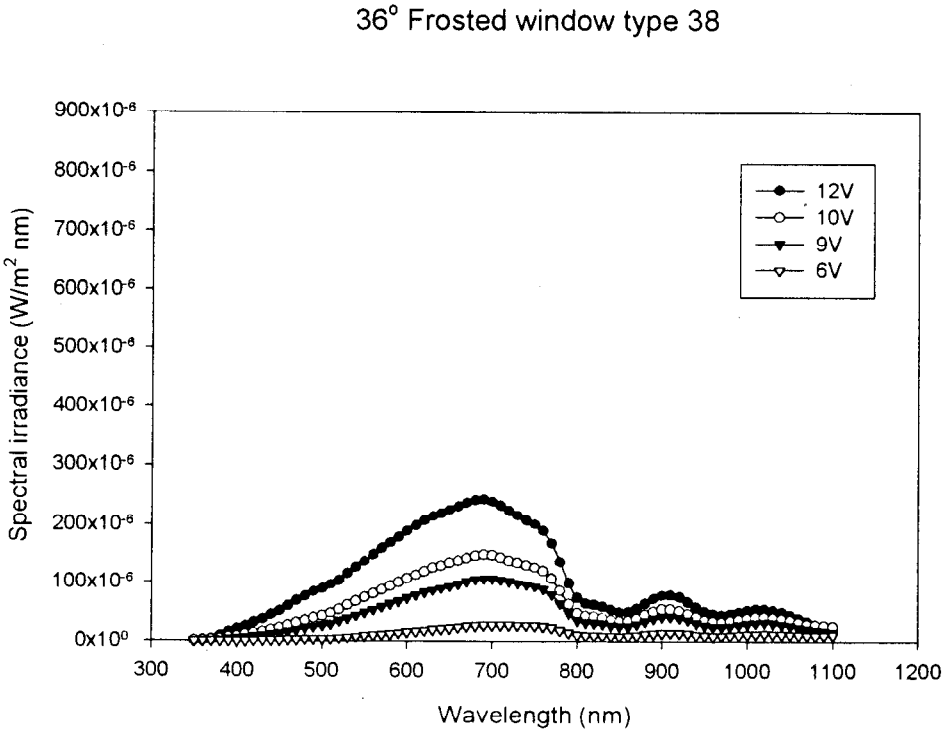

Figure 12: Emision spectra of lamps of type model 323

APPENDIX 3: DETERMINATION OF THE INCIDENT RADIANT LIGHT FLUX USING REINECKE'S SALT METHOD

Accurate determination of the incident light flux is a key issue for any study relating light intensity and biomass growth. Different determination methods are available ranging from the use of light sensors to photochemical methods. In TN 25.7 a set up was used with lamps located at 7 cm of the surface vessel and light intensity was changed by varying the number of lamps used. Average light intensity was measured on the surface of the rear part of the vessel filled with culture medium (Blank). To allow a better determination of the light incident flux available, an alternative photochemical method of light flux measurement has been used for comparison purposes. The method is based on the revised Reinecke's salt technique of Cornet (Cornet 1997) and light intensities tested were the same used in TN 25.7.

The average light energy flux F_0 irradiating the front surface of the reactor can be determined from a chemical actinometer as proposed by Aiba (1982). The chemical actinometer principle consists on obtaining F_0 from the rate of a given photochemical reaction from which the quantum yield is well-known. Reinecke's salt irradiated in the visible range releases thiocyanate ions SCN^-, which can be easily measured spectrophotometrically to determine the rate of the reaction.

During the photolysis of Reinecke's salt, the main reaction is as follows :

$$Cr(NH_3)_2(NCS)_4^- \xrightarrow{h\nu} NCS^- + Cr(NH_3)_2(NCS)_3(H_2O)$$

or :

$$A \Rightarrow B + C$$

where the local volumetric reaction rate r_A is proportional to the local volumetric rate of radiant light energy absorbed A :

$$r_A = \phi A$$

being ϕ the energetic yield of conversion (moles/J) calculated from the quantum yield of the reaction. The rate of light absorbed A may be obtained from the knowledge of the local mean light intensity J from.

$$A = 4\pi E_a C_a J$$

Integrating the local rates over the total volume it is possible to obtain the mean spatial macroscopic measurable quantities $<r_A>$ and <A>. In this case, assuming that the energetic yield is constant in the reactor, then:

$$\langle r_A \rangle = \phi \langle A \rangle$$

When A is integrated over the total reaction volume and divide by the total volume to give <A> it is obtained :

$$\frac{\langle A \rangle}{\langle A_0 \rangle} = 1 - \exp(-\beta C_A)$$

In the case of a rectangular photobioreactor illuminated from one side β=EaL and $\langle A_0 \rangle = F_0/L$. Where L is the light path. (Cornet *et al.*, 1997).

From an elemental mass balance for species A it can be deduced that :

$$-\langle r_A \rangle = \frac{dC_A}{dt}, \text{and therefore } \frac{dC_A}{dt} == -\phi \langle A_0 \rangle (1 - \exp(-\beta C_A))$$

The integration of the equation allows to calculate the time course of the reaction. The integrated form can be simplified for case were C_{A0} and C_A are high to a linear relation.

$$C_A = C_{A0} - \phi \langle A_0 t \rangle$$

From a mass balance from the stoichiometric equation it can be seen that $C_B=C_{A0}-C_A$. Therefore from the above simplified equation :

$$C_B = \phi \langle A_0 t \rangle$$

or considering the molar volumetric rate of B production, r_B, and if C_A and C_{A0} are high, gives

$$r_B = \phi \langle A_0 \rangle$$

If the Reinecke's salt concentration C_A is sufficiently high (10^{-2} mol/l) to absorb all the incident radiation in the vessel, the molar volumetric rate of thiocyanate released r_B is constant, and can be determined from the slope of the C_B versus time experiment.

In this case, the radiant light incident flux is directly approximated from the molar volumetric rate of thiocyanate released, r_B , from equation :

$$F_0 = \frac{r_B L}{\rho \phi}$$

where :

F_0 radiant light incident flux

r_B molar volumetric rate of thiocyanate

L light path

ρ correction factor for incident flux

ϕ = (1.33 ± 0.07) x 10-^{6} mole B J-^{1}

1.- Correction factor for incident flux

Reinecke's salt absorbs light mainly between 350 and 650 nm, whereas the considered visible spectrum (photosynthetically active radiation, PAR, for microorganisms) is in the range 350 - 750 nm. Thus it is necessary to define a correction factor, ρ, to calculate true incident radiant fluxes in the considered range of wavelength. This factor is defined as the ratio of the incident radiant light flux emitted in the range 350 and 650 nm to that emitted in the range 350 and 750 nm.

The value of ρ is 0.611, calculated for halogen filtered lamps Claude BAB 38 - 20 W by Cornet *et al.*, 1997, which emission spectrum is very similar to the type used in this report.

2.- Determination of the molar extinction coefficient

The molar extinction coefficient, ε, has to be determined to calculate the number of moles of thiocyanate (SCN-) released by the photochemical reaction.

Reactants

Reinecke's salt

The commercially available Reinecke's salt as the ammonium salt must be converted into the potassium salt since ammonia enhances the photochemical reaction. A 0.1 mol/l KOH solution was prepared and warmed at 40°C. Then a quantity of ammonium Reinecke's salt was dissolved to obtain the desired final concentration, 10^{-2}

mole/l . The flask was keept away from light. The solution was continuously stirred with a magnetic rod during the dissolution. It is important to keep the temperature of the KOH solution between 40 and 45°C because Reinecke's salt is damaged at higher temperatures (>50°C). The solution was then transferred into a dark bottle, cooled in ice and shaken manually to strip ammonia. Ammonia stripping is not increased by working in a vacuum and the Reinecke's salt is damaged by sustained sonication.

Procedure

Consists in preparing thiocyanate standard solutions between 0 and 4 x 10^{-4} mole/l. To a volume of thiocyanate solution, is added one volume of Reinecke's salt, three volumes of a 0.1 mol/l $Fe(NO_3)_3 . 3H_2O$ in a 0.5 mol/l $HClO_4$ solution. This mixture is well mixed and the optical density immediately measured in a double beam spectrophotometer at 450 nm. The Reinecke's salt has to be protected from light. The blank solution used as reference was the solution without thiocyanate. The value of the molar extinction coefficient is the slope obtained by plotting the optical density versus the thiocyanate concentration in the sample.

The OD values measured for each thiocyanate concentration are listed in the following table :

ε (b)		ε (c)	
Thiocyanate (mole/l)	OD 450nm	Thiocyanate (mol/l)	OD 450nm
0	0	0	0
8,0E-05	0,25	8,0E-05	0,26
1,6E-04	0,5	1,6E-04	0,49
2,4E-04	0,74	2,4E-04	0,74
3,2E-04	0,99	3,2E-04	0,97
4,0E-04	1,26	4,0E-04	1,23

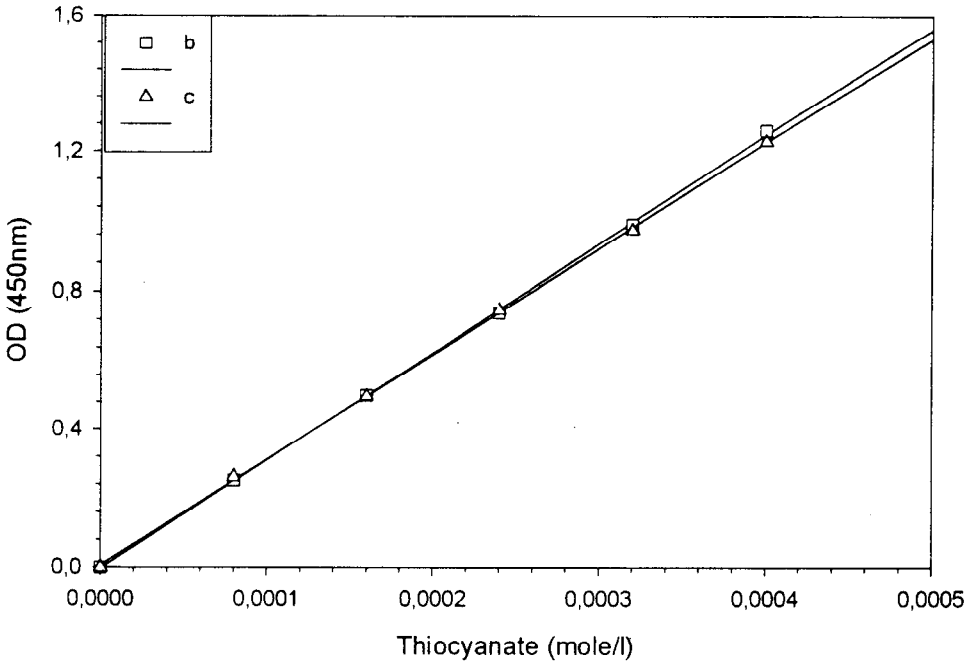

Figure 13: Measured OD v.s. thiocyanate concentration.

The following ε values correspond to Reinecke's salt solutions prepared at different days. As it can be observed , there is no notable difference between them.

a : $\varepsilon = 3200.0$ l mol^{-1} cm^{-1}

b : $\varepsilon = 3116.5$ l mol^{-1} cm^{-1}

c : $\varepsilon = 3072.7$ l mol^{-1} cm^{-1}

3.- Determination of volumetric reaction rate

The determination of the volumetric reaction rate of thiocyanate released is performed in a rectangular vessel (Roux flask) with 1.1 l total volume, bottom area of 12x5.6 cm with a 3 mm glass thickness, irradiated on one side with lamps type Sylvania professional BAB 38° 12V 20W. The Reinecke's solution volume is 1l , then from the base area and this volume, the frontal area exposing to the light is 17.5x11.4 cm (0.0200

m^2). The light path L is 0.055 m. The solution was maintained homogeneous using a magnetic stirrer. Experiments are carried out in the experimental set up shown in figure 1, on the first floor of the dark chamber described oi 'Materials and Methods'. The lamps are located at 7 cm from the vessel surface. Lamp voltage is fixed at 12 V.

Procedure

In the photolysis of Reinecke's salt, changing the number of lamps irradiating the solution vessel changes the molar volumetric rate of thiocyanate production. Just before being used, the Reinecke's salt is prepared and its molar extinction coefficient measured. The solution is gently and continuously stirred. The temperature is regulated at 30 °C. The test was repeated using different number of lamps (1, 2, 4, and 6).

Samples of 1 ml are taken at regular intervals. Each sample is treated with 3 ml of a 0.1 mol/l $Fe(NO_3)_3 \cdot 3H_2O$ in a 0.5 mol/l $HClO_4$ solution and 20 volumes of distilled water to determine spectrophotometrically at 450 nm the concentration of released thiocyanate C_B . A sample of the irradiated solution at the beginning of the reaction is taken as a blank.

The volumetric rate is defined by :

$$r_B = \frac{m}{\varepsilon} \times 24$$

where:

r_B	molar volumetric rate of thiocyanate, mol $m^{-3}s^{-1}$
m	slope from thiocyanate concentration vs time for each number of lamps
24	dilution factor

For each number of lamps, the evolution of OD values are :

	time (h)	OD 450nm
1 lamp (a)	0.00	0.000
	51.25	0.256
	56.25	0.295
	61.25	0.313
	66.25	0.346
	73.25	0.364
2 lamps (a)	0.00	0.000
	5.00	0.040
	10.00	0.085
	15.00	0.107
	20.00	0.149
	25.00	0.167
	30.00	0.218
4 lamps (b)	0.00	0.000
	5.00	0.087
	10.00	0.146
	16.00	0.227
	21.00	0.286
	26.00	0.363
	31.00	0.414
	36.00	0.456
	41.00	0.473
6 lamps (c)	0.00	0.000
	30.00	0.602
	37.00	0.684
	46.00	0.805
	52.00	0.88
	61.00	0.935
	66.33	0.974

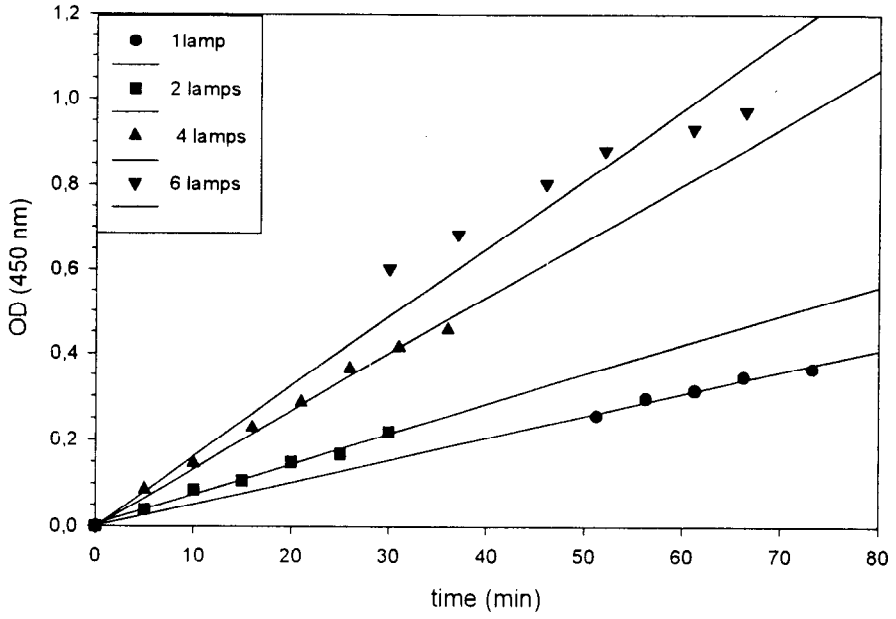

Figure 14: Variation in absorbance v.s. time.

Then, r_B results :

Number of Lamps	r_B (mol m^{-3} s^{-1})		
	a	b	c
1	0.000638		
2	0.000900		
4		0.001643	
6			0.002213

F_0 values are listed and are confronted with F_o values from TN25.7 with the same number of lamps.

Number of Lamps	F_0 (W m^{-2})		
	Fo Reinecke	Fo *	Fo flat glass surface
1	43	27	28
2	61	49	51
4	111	101	102
6	156	145	151

*TN25.7 : Measured with the sensor in the rear part of the bottle. Bottle was filled with culture medium.

As it is shown, the results of Reinecke'salt method are in close agreement with the F_o flat glass surface.