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Memorandum of Understanding

ECT/FG/MMM/97.012

Contract Number : ESTEC/CONTRACT11549/95/NL/FG

TECHNICAL NOTE : 37.82

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

Version : 1

Issue : 0

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February 1998

Document Change Log

Version	Issue	Date	Observations
0	0	15/02/98	Draft
1	0	28/02/98	Original version

Table of Contents

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 in 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.

The carbon sources most likely to be found at the exit of Compartment I of MELISSA, and therefore the carbon sources that will be used in Compartment II by *Rhodospirillum rubrum* cells, have been identified as the following volatile fatty acids : acetic, propionic, butyric, isovaleric and isobutyric. The batch kinetics of cells growth and carbon source consumption has been investigated in an experimental set-up with controlled illumination, temperature, and buffered medium. This study has been performed at two levels of light intensity, 200 and 20 W/m^2, and using the five carbon sources mentioned above. Some experiments have been repeated to double check the accuracy of the result.

In a previous TN (Lenguaza *et al.* 1997, TN 37.81), the experimental set-up prepared to carry out these experiments was presented. It was also discussed the relationship between the spectra of light emission by the lamps, and the properties of light

absorption of *Rhodospirillum rubrum* cells. Finally, the results of cell growth at 200 W/m^2 incident light, using acetic, butyric, propionic, isovaleric and isobutyric acids as carbon sources where given.

In the present TN, the previous results are completed with those of cell growth kinetics at 20 W/m^2 incident light. Finally, in the discussion, a summary of all the results obtained with the 2 light levels and 5 carbon sources is given.

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 medium.

Culture medium 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 0.49 g/l K_2HPO_4 0.52 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.

Experiments were carried out in the experimental set up described in detail in a previous TN (Lenguaza *et al.* 1997, TN 37.81). Basically, the cultures were carried out in Roux flasks with controlled illumination from one side. Temperature (30 °C) was maintained by means of a water bath. Culture was maintained homogeneous using a magnetic stirrer.

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.

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 35 cm from culture vessel surface. On the second floor lamps were located at 30 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 in Lenguaza *et al.*, TN37.81)). Light intensity obtained, corresponded to an average value around 20 W/m^2 PAR 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 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 400-700nm, 0.291 for the range 350-750 nm, 0.425 for the range 350-950 nm. The result of the illumination measurements are given in table 1.

Biomass dry weight was calculated from the measured absorbance of a sample (A_{680}) and a calibration curve of dry weight versus absorbance at 680 nm (appendix 1).

The carbon sources and concentrations used in these tests were: acetic acid (2.5 g/l), propionic acid (2.06 g/l), butyric acid(1.84 g/l), isobutyric acid(1.84 g/l), and isovaleric acid (1.7 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, 0.67 g/l, 1.35 g/l, 1.35 g/l,1.23 g/l. The amounts of sodium carbonate for the cultures carried out on propionic, butyric and isobutyric acids were calculated taking into account the stoichiometries established under culture conditions with a C/N ratio equal to 5, by Poughon *et al.*, 1995.

Illumination conditions	$\mu mol \times m^2 \times s^{-1}$ +/- S.E. (PPFD)	W/m^2 (400-700nm) +/- S.E. (PAR)	W/m^2 (350-750nm) +/- S.E.	W/m^2 (350-950nm) +/- S.E.
first floor : 6 lamps, 9 V	109 +/- 2.2	22.2+/- 0.5	31.7+/- 0.6	46.3+/- 0.9
second floor : 6 lamps, 9 V	105.9 +/- 2.4	21.6+/- 0.5	30.8+/- 0.7	45.0+/- 1.0

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 109 +/- 2.2 mmol×m^2×s^{-1} (PPFD), (W/m^2: 22.2+/-0.58 (400-700), 31.7+/-0.6 (350-950)) The preparation of the initial medium was carried out in order to have 1 g/l of initial cell concentration and with respect to acetic acid 1 g of carbon / l. For this, 350 ml of a cell preculture with a concentration of 3.5 g/l were diluted to 1 l of total volume using 650 ml of concentrated medium (concentration factor 1 / 0.65).

Figure 2: Once diluted cell growth begins with a lag phase which gradually turns into the linear light limited phase at around 25 hours (figure 1). During this time there is a small increase in biomass of around 0.25 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, 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

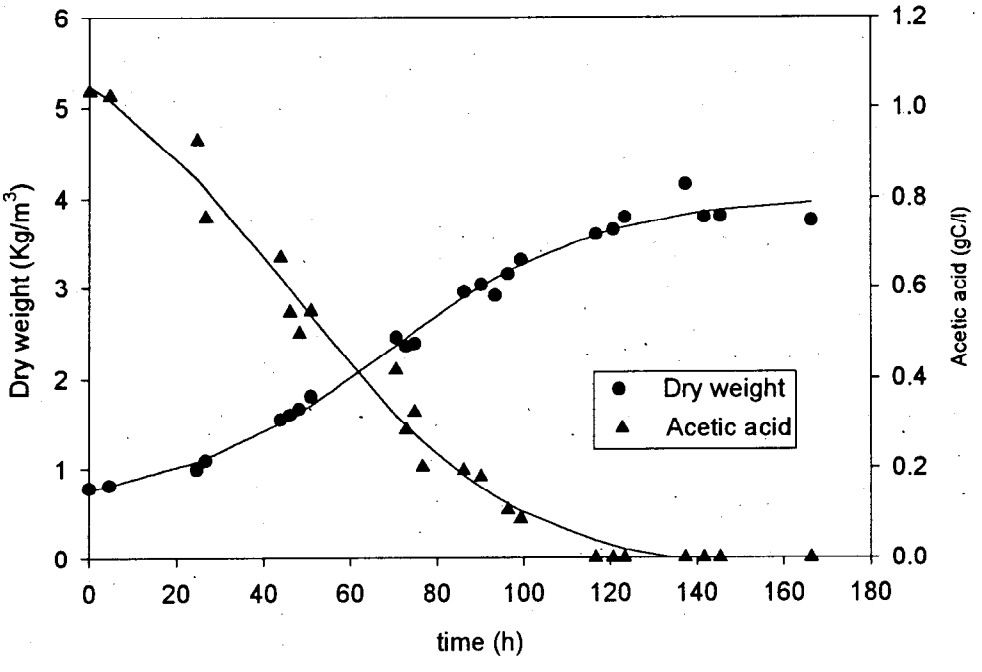

Figure 1: Experimental data obtained in acetic acid test A (floor 1).

compared with the change occurring when the culture is diluted directly from a high biomass concentration, as in the present case. After this point the cell growth begins a light limited linear phase. Total biomass dry weight at the end of the culture amounts 3.75 kg/m^3. The average yield is of 1.2 kg DW/kg acetic acid (1.4 kgDWC/kgC). The pH at the end of the culture was 7.32.

Table 2: Experimental data obtained in acetic acid test A . (floor 1)

Time(h)	DW (kg/m^3)	Acetic acid (C-kg/m^3)
0.00	0.78	1.04
4.67	0.81	1.03
24.67	0.99	0.93
26.67	1.09	0.76
43.84	1.55	0.67
46.00	1.60	0.55
48.17	1.67	0.50
50.84	1.81	0.55
70.50	2.46	0.42
72.84	2.36	0.29
74.84	2.39	0.33
76.67	ND	0.20
86.17	2.95	0.19
90.17	3.03	0.18
93.34	2.91	ND
96.34	3.15	0.11
99.34	3.31	0.09
116.67	3.60	0.00
120.67	3.65	0.00
123.34	3.79	0.00
137.34	4.15	0.00
141.67	3.79	0.00
145.34	3.80	0.00
166.34	3.75	0.00

III.2 TEST B : Propionic acid.

This test was done at 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 method to prepare the initial medium was the same as the previous one, that is, in order to have 1 g/l of initial cell concentration and with respect to propionic acid 1gC/l , 350 ml of a cell preculture (preculture was done in propionic acid) with a concentration of 3.5 g/l were diluted to 1 l of total volume using 650 ml of concentrated medium.

Once diluted the cell growth begins with a lag phase which gradually turns into the linear light limited phase at around 20 hours (figure 2). During this time there is a small increase in biomass of around 0.3 g/l of dry weight. After this point the cell growth begins a light limited linear phase. Total biomass dry weight at the end of the culture amounts 4.74 kg/m^3. The average yield is of 1.6 kg DW/kg propionic acid (1.6 kgDWC/kgC). The pH at the end of the culture was 7.32.

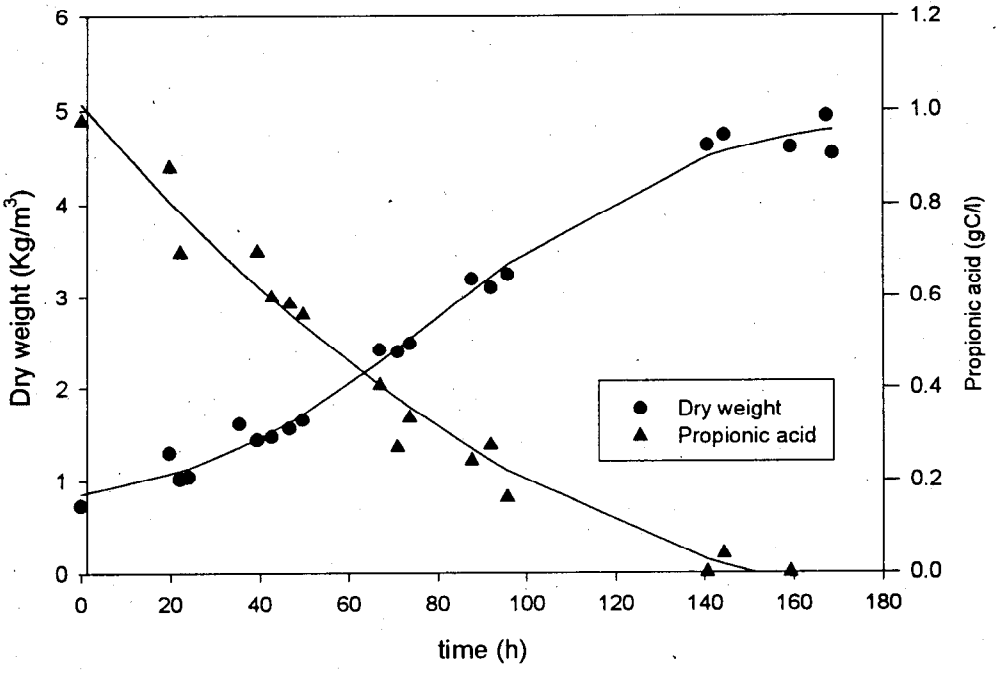

Figure 3: Experimental data obtained in propionic acid test B (floor 2).

Table 3: Experimental data obtained in propionic acid test B (floor 2).

Time(h)	DW (kg/m^3)	Propionic Acid (C-kg/m^3)
0.00	0.73	0.98
19.67	1.30	0.88
22.08	1.02	0.70
24.08	1.04	ND
35.33	1.61	ND
39.33	1.44	0.70
42.58	1.47	0.60
46.58	1.56	0.58
49.58	1.65	0.56
66.83	2.41	0.41
70.83	2.39	0.27
73.58	2.48	0.33
87.58	3.20	0.24
91.83	3.10	0.28
95.58	3.25	0.16
140.58	4.63	0.00
144.33	4.74	0.04
159.33	4.61	0.00
167.33	4.94	ND
168.67	4.54	ND

III.3 TEST C : Butyric acid.

This test was done at 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. This time, in order to have 1 g/l of initial cell concentration and with respect to butyric acid 1gC/l, 350 ml of the culture of the experiment test A, with a concentration of 3.8 gDW/l , in which the acetic acid is depleted, were diluted to 1 l of total volume using 650 ml of concentrated medium.

Once diluted the cell growth begins with a lag phase which gradually turns into the linear light limited phase at around 20 hours (figure 3). During this time there is a small increase in biomass, but is negligible. After this point the cell growth begins a light limited linear phase. Total biomass dry weight at the end of the culture amounts 4.97 kg/m^3 .The average yield is of 2.6 kg DW/kg butyric acid (2.3 kgDWC/kgC). The pH at the end of the culture was 7.48.

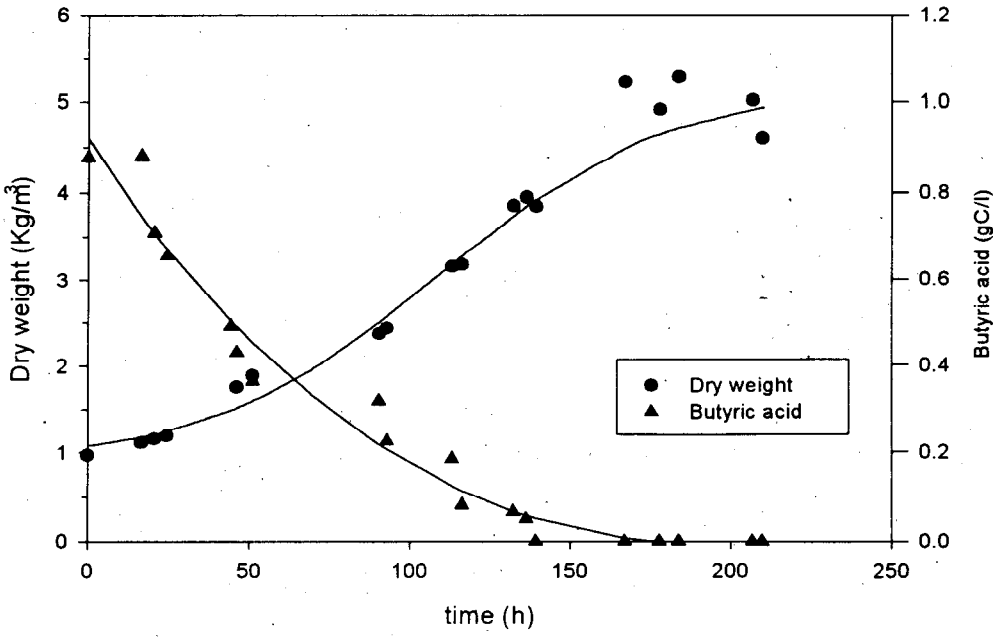

Figure 4: Experimental data obtained in butyric acid test C (floor 1).

Table 4: Experimental data obtained in butyric acid test C (floor 1).

Time(h)	DW (kg/m^3)	Butyric acid (C-kg/m^3)
0.00	0.97	0.88
16.50	1.12	0.88
20.50	1.16	0.71
24.50	1.20	0.66
44.25	ND	0.49
46.00	1.75	0.43
51.00	1.89	0.36
90.25	2.37	0.32
92.75	2.44	0.23
113.08	3.16	0.19
116.25	3.18	0.08
132.25	3.86	0.07
136.25	3.95	0.05
139.25	3.85	0.00
167.00	5.23	0.00
177.75	4.92	0.00
183.75	5.29	0.00
206.75	5.02	0.00
209.75	4.60	0.00

III.4 TEST D : Isovaleric acid.

This test was done at 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 using the same procedure as in the previous test C. Once diluted the cell growth begins with a lag phase which gradually turns into the linear light limited phase at around 25 hours (figure 4). During this time there is a small increase in biomass of around 0.3 g/l of dry weight. After this point the cell growth begins a light limited linear phase. Total biomass dry weight at the end of the culture amounts 4.8 kg/m^3. The average yield is of 2.4 kg DW/kg isovaleric acid (2. kgDWC/kgC). The pH at the end of the culture was 7.54.

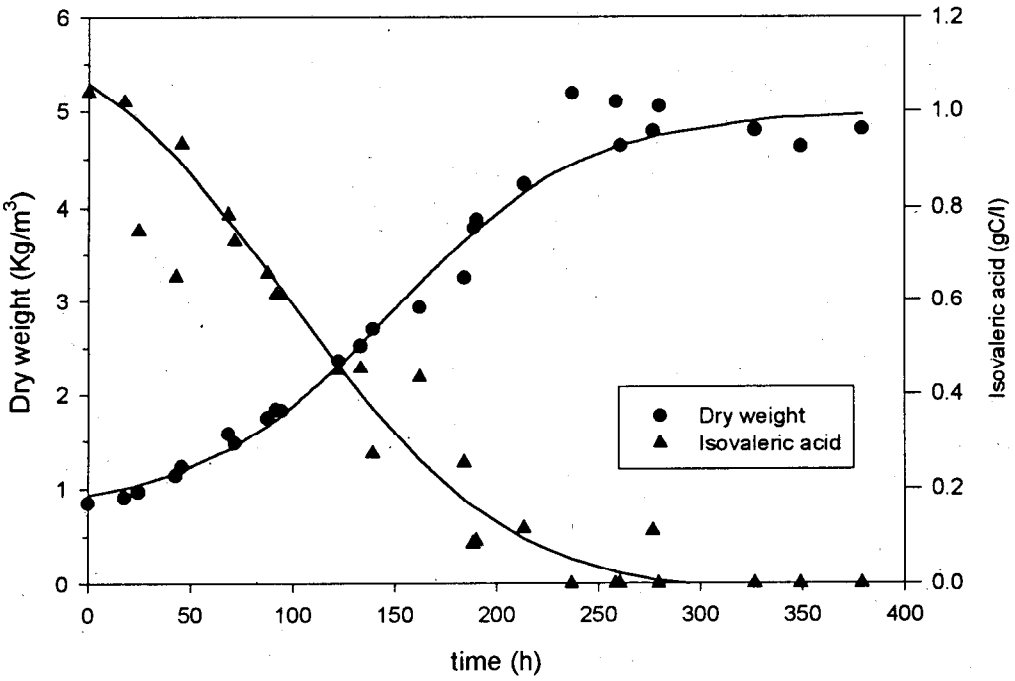

Figure 5: Experimental data obtained in isovaleric acid test D (floor 2).

Table 5: Experimental data obtained in isovaleric acid test D (floor 2).

Time(h)	DW (kg/m^3)	Isovaleric acid (C-kg/m^3)
0.00	0.85	1.04
17.67	0.91	1.02
24.50	0.97	0.75
42.75	1.14	0.65
45.50	1.23	0.93
68.33	1.58	0.79
71.50	1.48	0.73
87.50	1.75	0.66
91.50	1.84	0.62
94.50	1.83	0.61
122.25	2.36	0.45
133.00	2.52	0.46
139.00	2.70	0.27
162.00	2.93	0.44
184.00	3.25	0.25
188.50	3.79	0.09
190.00	3.88	0.09
213.50	4.24	0.12
237.00	5.19	0.00
258.50	5.10	0.00
260.75	4.64	0.00
279.75	5.05	0.00
276.75	4.79	0.11
326.75	4.80	0.00
349.25	4.63	0.00
379.25	4.81	0.00

III.5 TEST E1 : Isobutyric acid.

This test was done at 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. There is a lag phase which gradually turns into the linear light limited phase at around 20 hours (figure 5). During this time there is a small increase in biomass. After this point the cell growth begins a light limited linear phase. Total biomass dry weight at the end of the culture amounts 4.46 kg/m^3. The average yield is of 2.6 kg DW/kg isobutyric acid (2.3 kgDWC/kgC). The pH at the end of the culture was 7.38. Results obtained in this test can be found in table 6.

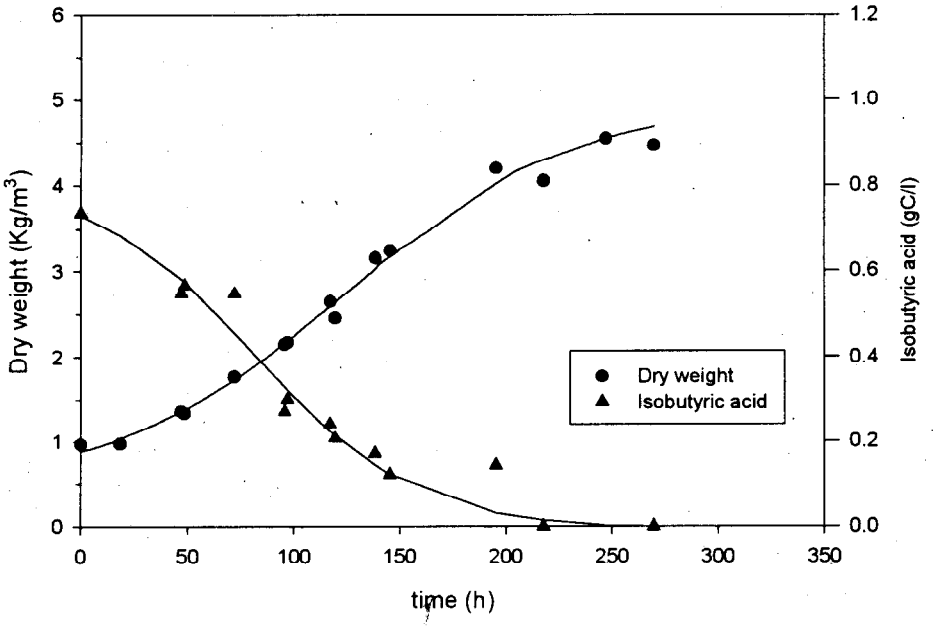

Figure 6: Experimental data obtained in isobutyric acid test E1 (floor 1).

Table 6: Experimental data obtained in isobutyric acid test E1 (floor 1).		
Time(h)	DW (kg/m^3)	Isobutyric Acid (C-
0.00	0.96	0.74
18.50	0.98	ND
47.00	1.37	0.55
48.50	1.34	0.57
72.00	1.77	0.55
95.50	2.15	0.27
97.00	2.17	0.30
117.00	2.65	0.24
119.25	2.46	0.21
138.25	3.15	0.17
145.25	3.24	0.12
195.25	4.20	0.14
217.75	4.05	0.00
247.00	4.54	ND
269.75	4.46	0.00

IV. DISCUSSION

The experimental results presented provide a set of data for the development of a kinetic growth model for *Rhodospirillum rubrum*, taking into account the effect of the carbon source and light limitation on the growth rate. As a first approach to evaluate the behaviour of these cells with different conditions, the maximum slopes of the light limitation phase of the growth curves obtained in the previous experiments have been calculated. The slopes were calculated giving the dry weight data in gC/l. In this case, as the C/N ratio in all the experiments with the different carbon sources has been maintained constant (C/N = 5), it was assumed that the biomass composition remains also fairly constant. In an acetic acid culture with a C/N ratio of 5 the carbon content of biomass is equal to 48.71%, as determined by Poughon L. *et al*, 1995. This value of carbon content was used in the calculations. The data of the calculated slopes from the different experiments are given in table 7.

Table 7: Maximum slope of DW vs time for tests at 20 W/m^2.

VFA	gDWC/lh
Acetic acid	0.0149 ± 0.0006
Propionic acid	0.0167 ± 0.0007
Butyric acid	0.0161 ± 0.0008
Isobutyric acid	0.0093 ± 0.0005
Isovaleric acid	0.0095 ± 0.0005

It can be observed that two groups of compounds can be identified. Acetic, propionic and butyric acids present higher growth rates than isobutyric and isovaleric acids.

Table 8 gives the results for the same kind of analysis performed with the growth curves obtained at 200 W/ m^2 , as reported previously (Lenguaza *et al.*, TN37.81). Again, the same two groups of compounds can be identified, where the isoacids present slower growth rates than those of the first group. Also, as a general trend, growth at 200 W/ m^2 of light intensity is generally faster than the ones at 20 W/ m^2, with the exception of isobutyric acid, where the growth at 200 W/ m^2 is very low. These results could be affected by the different innoculum, as have been performed in separate experimental series. In order to have a further indication of the reproducibility of the results it was decided to repeat the growth on isobutyric acid at 20 and 200 W/ m^2, simultaneously,

using the same initial medium and innoculum. The results of these tests, referred to as E2 and E3, are reported below.

Table 8: Maximum Slope of DW vs time for tests at 200 W/m^2 (gDWC/lh).

F1 and F2 indicate floor 1 and 2 of the set up.

VFA	200 W/m^2	
Acetic acid	0.0642 F1 ± 0.0071	0.067 F2 ± 0.0029
Propionic acid	0.0404 ± 0.0015	
Butyric acid	0.0451 ± 0.0012	
Isobutyric acid	0.0028 ± 0.0001	
Isovaleric acid	0.0146 ± 0.0004	

IV.1 TEST E2 : Isobutyric acid (20W/m^2 - R).

In this test, the illumination conditions were the same as in the previous isobutyric test, that is the light intensity corresponded to an average value around 20 W/m^2 PAR, and was done on the first floor of the set up. To have a medium with about 1 g/l of initial cell concentration and with respect to isobutyric acid 1gC/l, 350 ml of the culture of the experiment test E, with a concentration of 4.5 gDW/l , in which the isobutyric acid was depleted, were diluted to 1 l of total volume using 650 ml of concentrated medium.

Total biomass dry weight at the end of the culture amounts 4.75 kg/m^3. The average yield is of 2.0 kg DW/kg isobutyric (1.8kgCDW/kgC). The pH at the end of the culture was 7.35 . Results obtained in this test can be found in figure 7 and table 9

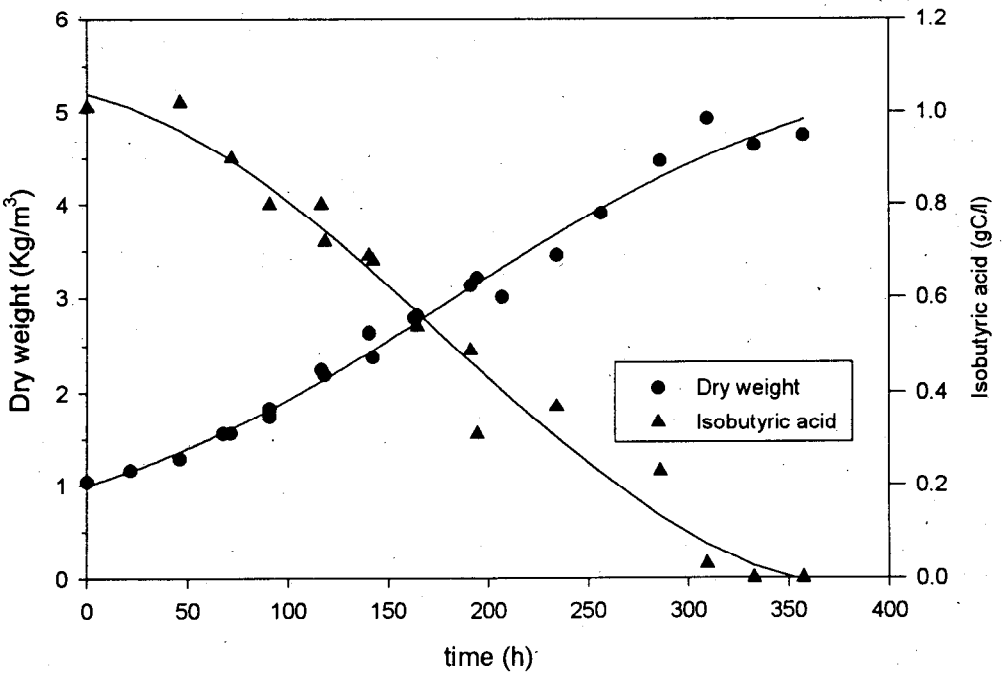

Figure 7: Experimental data obtained in isobutyric acid test E2 (floor 1).

Table 9: Experimental data obtained in isobutyric acid test E2 (floor 1).

Time(h)	DW (kg/m^3)	Isobutyric Acid (C-
0.00	1.04	1.01
21.50	1.16	ND
46.00	1.29	1.02
67.50	1.56	ND
71.50	1.56	0.90
90.50	1.83	0.80
90.83	1.75	ND
116.33	2.25	0.80
118.08	2.19	0.72
140.08	2.64	0.69
142.08	2.37	0.68
162.83	2.79	0.56
164.33	2.81	0.54
191.08	3.13	0.49
194.33	3.21	0.31
206.83	3.01	ND
234.03	3.45	0.37
256.43	3.90	ND
285.93	4.46	0.23
309.43	4.93	0.03
332.93	4.64	0.00
357.43	4.75	0.00

IV.2 TEST E3 : Isobutyric acid (200W/m^2 - R).

This test is equivalent to the test F in TN37.81, that is the light intensity corresponded to an average value around 200 W/m^2 PAR, and was done on the second floor of the set up, but the innoculum was the same as in the previous test.

Total biomass dry weight at the end of the culture amounts 2.48 kg/m^3. The average yield is of 0.9 kg DW/kg isobutyric (0.8 kgDWC/kgC). The pH at the end of the culture was 7.37. Results obtained in this test can be found in figure 8 and table 10.

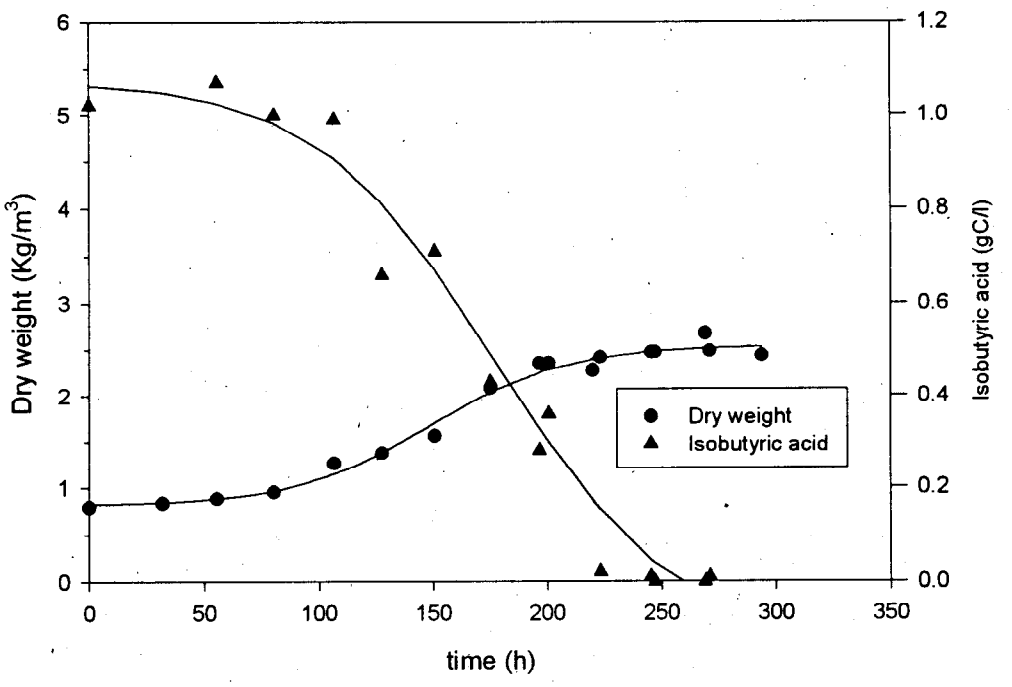

Figure 8: Experimental data obtained in isobutyric acid test E3 (floor 2).

Table 10: Experimental data obtained in isobutyric acid test E3 (floor 2).

Time(h)	DW (kg/m^3)	Isobutyric Acid (C-
0.00	0.79	1.02
31.75	0.83	ND
55.50	0.88	1.07
80.25	0.95	1.00
106.50	1.26	0.99
127.50	1.37	0.66
150.50	1.56	0.71
175.00	2.08	0.43
196.50	2.35	0.28
200.50	2.35	0.36
223.17	2.41	0.02
219.83	2.27	ND
245.33	2.47	0.01
247.08	2.47	0.00
269.08	2.67	0.00
271.08	2.48	0.01
293.83	2.43	ND

Table 11: Maximum Slope of DW vs time for tests at two light intensity levels (gDWC/lh). R represents repeated tests.

	200 W/m^2		20 W/m^2	
VFA		R		R
Acetic acid	0.0642 F1 ± 0.0071	0.067 F2 ± 0.0029	0.0149 ± 0.0006	
Propionic acid	0.0404 ± 0.0015		0.0167 ± 0.0007	
Butyric acid	0.0451 ± 0.0012		0.0161 ± 0.0008	
Isobutyric acid	0.0028 ± 0.0001	0.007 ± 0.0006	0.0093 ± 0.0005	0.0061 ± 0.0002
Isovaleric acid	0.0146 ± 0.0004		0.0095 ± 0.0005	

The slopes calculated from the two new tests are given together with the previous results in table 11. It can be seen that the new value obtained for the growth at 200 W/m^2 in isobutyric acid differs greatly from the first value. It is also observed that the slope variation found in repeating the experiment is higher than the one observed in other cases like in the acetic acid tests which was lower. The increased scattering can be the result of a high sensitivity to the combined effect of the low values of the slope and the variations between tests (culture media preparation, innoculum source, aeration during sampling etc.). In this conditions the weighted average of the 20 W/m^2 isobutyric tests can be calculated as being 0.0077 gC/lh. This value is equivalent to the one obtained in the repeated experiment at 200 W/m^2.

In any case, it seems clear that isobutyric and isovaleric acids present the lowest growth, with small changes between different illumination conditions. On the other hand, acetic, propionic and butyric acids present higher growth rates, with different values when illumination is different.

It was also calculated the global yields for all the tests. These yields were calculated taking the total biomass increase with respect to the total VFA consumed. A summary

of the results is reported in table 12. The analysis of this global yields from experimental data obtained in batch experiments is not straight forward. Indeed two different kinds of limitations exist in different phases of the growth curve: light limitation and VFA limitation. At the end of the batch probably both. In each phase the yield can be different. It is not possible to measure this variation in yield among different phases in this batch cultures. In consequence the calculated values presented in table 12 represent lumped values of the overall process and they give only general trends.

Table 12: Average yield.

F1 and F2 indicates floor 1 and 2. R represents repeated tests

	kgDW / kgVFA				kgDWC / kgVFAC			
VFA	200 W/m2	R	20 W/m2	R	200 W/m2	R	20 W/m2	R
Acetic	0,77 F1	0,8 F2	1.2		0,93 F1	0,97 F2	1.4	
Propionic	1.1		1.6		1.1		1.61	
Butyric	1.5		2.6		1.4		2.3	
Isobutyric	2.7	0.9	2.6	2.0	2.4	0.8	2.3	1.8
Isovaleric	2.1		2.4		1.7		2.0	

As a general trend the global yields are higher for the lower illumination conditions. Acetic acid presents the lower biomass yield from the carbon source. This fact can be the result of a lower incorporation of carbon into the biomass from a source other than the VFA supplied, namely carbon dioxide or any of its ionic forms. Indeed its reductance degree is the lowest among the VFA used(table 13) and it is also lower than the biomass reductance degree, assuming a biomass composition established for the growth on acetic acid with a C/N ratio of 5 by Poughon L. *et al*, 1995 , which formula is $C\ H_{1.5951}\ O_{0.3699}\ N_{0.2094}\ S_{0.0034}\ P_{0.0152}$. In consequence it can be expected that some carbon has been lost as a carbon dioxide because to convert the carbon source into a biomass of a higher reductance degree, some reduction equivalents must be added. From a global point of view, this fact is usually explained such as that some acetic acid is converted into carbon dioxide and the reduction equivalents collected used to convert the remaining acetic acid into biomass. Therefore this conditions would give carbon dioxide production.

Table 13: Reductance degree of biomass and carbon sources.	
Acetic acid	4.00
Biomass	4.29
Propionic acid	4.66
Butyric acid	5.00
Isobutyric acid	5.00
Isovaleric acid	5.2

Being the other VFAs of a reductance degree higher than the biomass, it is possible to incorporate more carbon into the biomass by using the excess of reducing power that is contained within them. From this point of view the yields from the other carbon sources have to be higher, which is indeed what has been observed.

However this effect can be modified by other factors, and therefore the amount of increase in yield . For example if lower illumination conditions make difficult the protein synthesis and promote a strong accumulation of PHB, the yield will also be modified by this factor. Biomass composition will be different (and probably its reductance degree) and the growth measured as dry weight increase will not only be the result of a real increase of the number of cells but the increase in weight suffered by individual cells as a result of PHB accumulation.

Therefore the increased yield observed in the low light intensity conditions might be the result of the combined effect effect of strong light limitation conditions (energy supply limitation) and carbon source on the storage polymers accumulation pattern. Different ratios of PHB/protein glycogen/protein might have been produced under the two different illumination conditions tested. If this is true, the yields have also been continuously changing during one growth curve, because as cells grow there is less energy (light) available for each cell, giving a variation in illumination conditions inside the same batch. And consequently biomass composition has changed also during the growth curve.

The variations that appear inside a batch culture along the time, in this case mainly the carbon source concentration and light availability, make difficult the study of this processes using only batch cultures. A more adequate alternative could be a continuous

culture in which light intensity, flow rate and carbon source, can be fixed, allowing for biomass analysis in fixed conditions. In this conditions a mass balance can be calculated for each steady state, and stoichiometric equations balanced. Carbon dioxide production or consumption can be in this way calculated. Nevertheless if it were possible to also measure the carbon dioxide production/consumption, it could help in validating the stoichiometric equations. In case both methods didn't match, prediction of the production of an a priori unexpected product, can be done.

V. CONCLUSIONS

This data represents the second part of the experiments done to adapt a mathematical model relating growth rate with light intensity.

In the tests reported in this technical note, a light intensity value of about 20 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, as were previously found at 200 W/m^2. It was noted that the light intensity has an effect on the growth rate and that there are two groups of volatile fatty acids that affect in a different way to the growth rate. That is, the bacteria does not grow on the isoacids as well as on the other ones tested. These data indicate that the carbon sources modify the effect of the light intensity on the growth rate. Therefore the modelling approach should take into account this fact. The global yield appear to be influenced by the light intensity in a inverse relationship, and a more deep study of the effect of the light intensity on the yield is desirable.

Therefore this preliminary results suggest that at least the combined effect of the carbon source and light intensity have to be investigated in a more defined system. Quimiostat cultures will allow to establish more stable and defined values for light intensity and carbon source concentrations allowing to determine the biomass composition for each different experimental set of conditions. In principle the tests can be done either in carbon limiting conditions or in light limiting conditions. If done in light limiting conditions, a refinement of the modelling of the light effect on the growth rate can be done. However if the effects of the carbon source and light intensity are linked, as it seems a priori, the tests should include both kind of conditions. Therefore light intensity can be varied from strong limiting conditions to a value were the effect of the carbon source concentration becomes the main limiting factor, even if it is never the only one. As an example for a determined flow rate, different light intensities can be tested and biomass analysis done at steady state. Measurements of carbon and nitrogen sources into the bioreactor or its output should be done. The test should be done for different dilution rates. This kind of test can be compared for different carbon sources. Flow rate conditions for the different carbon sources can be different because for example the isoacids will not sustain as high growth rates as the other ones. In fact if the growth measured for the isoacids is not a true growth, that is if it is not the result of an increase

of cell concentration but only an increase of cell weight, the continuous culture will be washed out. One test of this kind and its biomass analysis will give this information. In this last case, this kind of test will indicate if the second compartment can sustain an anomalous operation of the first compartment in which an increased production of long chain fatty acids could be produced.

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APPENDIX 1 : Biomass Dry Weight Determination From Optical Density

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 dried 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

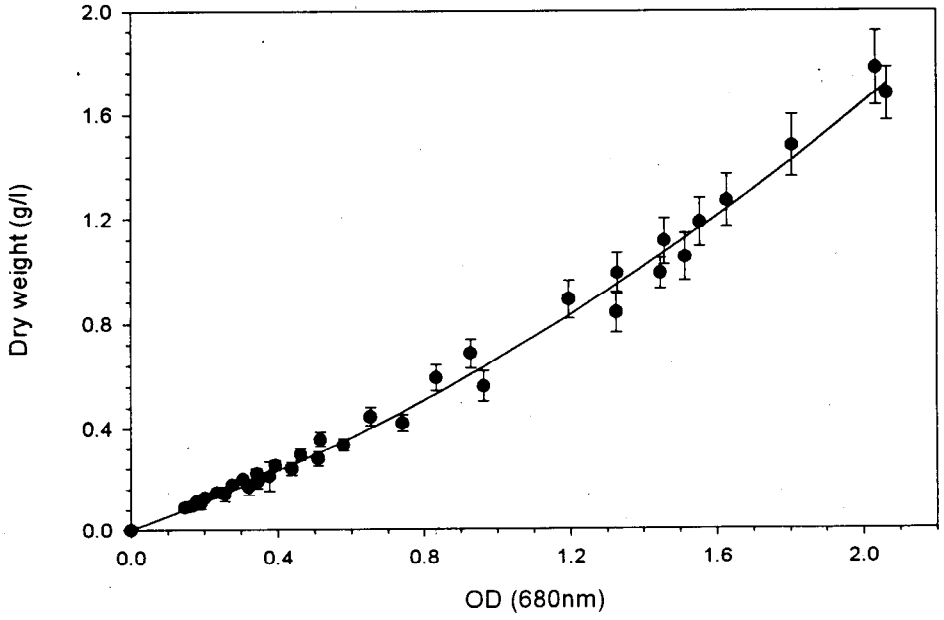

Figure 1: Dry weight vs. Optical Density used in this report.

APPENDIX 2 : Measurement Of Volatile Fatty Acids

Method : Gas Chromatography

Calibration : external standards from 0.25 gVFA/l up to 3 gVFA/l

Instrumentation:

- Gas chromatograph Hewlett Packard 5890
- Detector : FID
- Integration : Software Millenium 2.15.10

Chromatographic conditions

- Column : 25% NPAG + 2% H_3PO_4 WAW 100/200, 2.7 m x 1/8" , Supelco
- Carrier gas : Nitrogen.
- Pressure : 290 Kpa.
- Injection volume : 0.5 µl.
- Analysis time : 14 min.
- Oven temperature : 145 °C.
- Injector temperature : 250 °C.
- Detector temperature : 260 °C.
- Hydrogen pressure : 1.5 - 2 bar.
- Air pressure : 1.5 - 2 bar.

Samples were centrifuged 10 min at 12000 rpm , 4 °C, and filtered using 0.45 µm membranes (Millipore).