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- TECHNICAL NOTE 25.7-

PHOTOHETEROTROPHIC COMPARTMENT STUDIES

Complementary tests for Ea and Es determination.

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INTRODUCTION

The study of the growth of photosynthetic micro-organisms requires the evaluation of the availability of light used by them. This evaluation is also required for the implementation of control algorithms acting on the light energy supply.

In the MELISSA project a light control system has been developed for the photoautotrophic compartment. The algorithms make use of models describing light illumination conditions inside the bioreactor according to the input light supplied to the vessel surface. It appears feasible to adapt this models for its use on the photoheterotrophic compartment. The adaptation requires the evaluation of the absorption (Ea) and scattering (Es) coefficients characteristic of the corresponding species. Experimental determination of this coefficients is being done by another group within the MELISSA activities. However for the further adaptation of the model to the actual working conditions, some complementary tests must be done. The purpose of this technical note is to report on those complementary tests according to the SOW (RFQ/3-8453/95/NL/FG). The experiments were performed using flat culture vessels and monodimensional illumination to be able to use simplified models.

MATERIALS AND METHODS

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

Culture media was based on the basal salts mixture of SEGERS & VERSTRAETE as described by Suhaimi (Suhaimi et al 1987), using acetic acid as a carbon source and biotin as the only vitamin. To maintain the pH of the culture media 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 (MES) 21 g/l. Phosphate was autoclaved separately. The pH was adjusted to 6.9. At the end of the culture the pH was found to be 7.4.

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

Flat vessels have a volume of 1.09 litres. External dimensions of the bottom area of the vessel were 12x5.6 cm with a 3mm 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 19.1x11.4cm (0.0218 m^2) can be calculated. A volume of 5 ml was extracted for each sample.

Experiments were carried out in the experimental set up shown in figure 1. Illumination was set up in monodimensional conditions inside a dark chamber with black surface. Lamps used were of the same type as the ones used in the test tube experiments done by Albiol (Albiol 1994) (Sylvania professional 25 BAB 38° 12V 20W). Different light intensities were obtained either by using a different number of lamps at 12V or one lamp at different voltages. Lights were positioned at 7 cm of the reactor surface. Photosinthetically 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 has been done

by using a constant factor (1/4.9). . To measure the PPFD leaving the bioreactor, the sensor was placed in direct contact with the rear surface of the vessel. Positioning of the sensor showed to be critical for the light measurement, since the values measured varied strongly while moving the sensor away from the centre of one lamp. Therefore to obtain a more accurate description of the input light flux distribution initial values of the PPFD (F_0) were measured using a culture bottle filled with medium and measuring the flux at different points on the rear surface of the vessel as described in appendix A. On the other hand it was decided to fix the sensor centred with one lamp to measure the flow leaving the vessel, at different culture times, either in the culture conditions or in a parallel ones with the illuminating lamp also centred but at a different distance. That is, for each sampling time two different measurements were done. In type A the lamp was in front of the light sensor but the lamp was in direct contact with the vessel. That is the distance from the bottle was 0 cm. In this case the voltage of the lamp was decreased so as to have the same measurement with the blank as the one measured, also with the blank, but in the culture conditions. That is F0 is the same. In type B the measurement was done in the culture conditions, that is the light sensor was in front of one lamp and the lamp distance was distance A (7 cm). This has been done to better describe de experimental set up (Appendix B).

Biomass dry weight was calculated from the measured absorbency of a sample (A_{700}) and its value interpolated on a calibration curve taken from previous determinations (ALBIOL 1994).

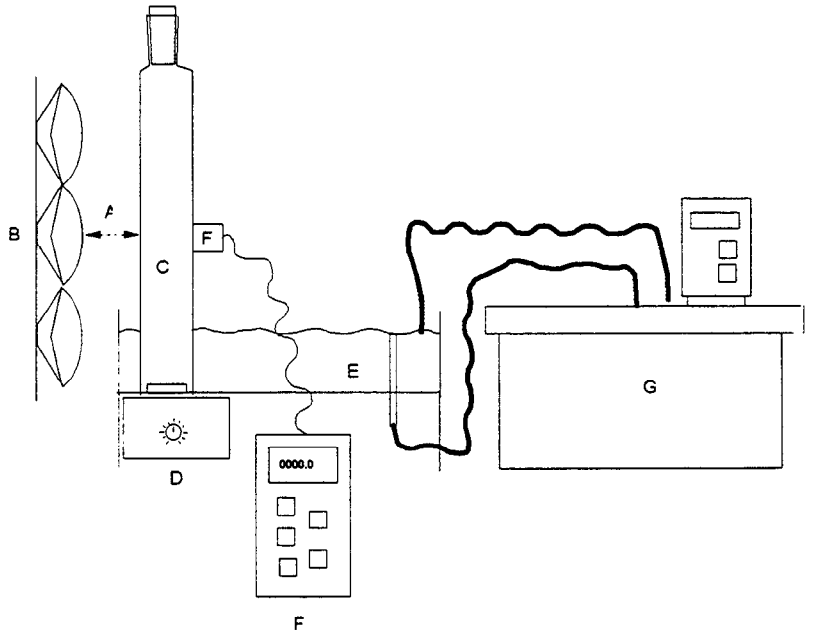

Figure 1: Experimental set up ; A : lamps-bioreactor distance (7 cm). B : Lamps support. C : Bioreactor. D : magnetic stirrer. E : water bath. F : light sensor. G thermostatic bath.

RESULTS AND DISCUSSION

TEST A

For this test six lamps were used, and the voltage applied was 12V. In this case the average F_0 value measured was of 717 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (145 W/m^2). The value measured with the blank medium at the fixed point of measurement (F_{I0}) was 1303 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (263 W/m^2).

The time course of the experiment is shown in figure 2 and data in table 1. Biomass growth takes place until around 2 g/l of biomass concentration. According to the acetic acid yields obtained in previous experiments (Albiol 1994), this biomass level corresponds to the exhaustion of the carbon/electron source. Allowing for some variation due to the fact that the culture media is diluted with the inoculum volume.

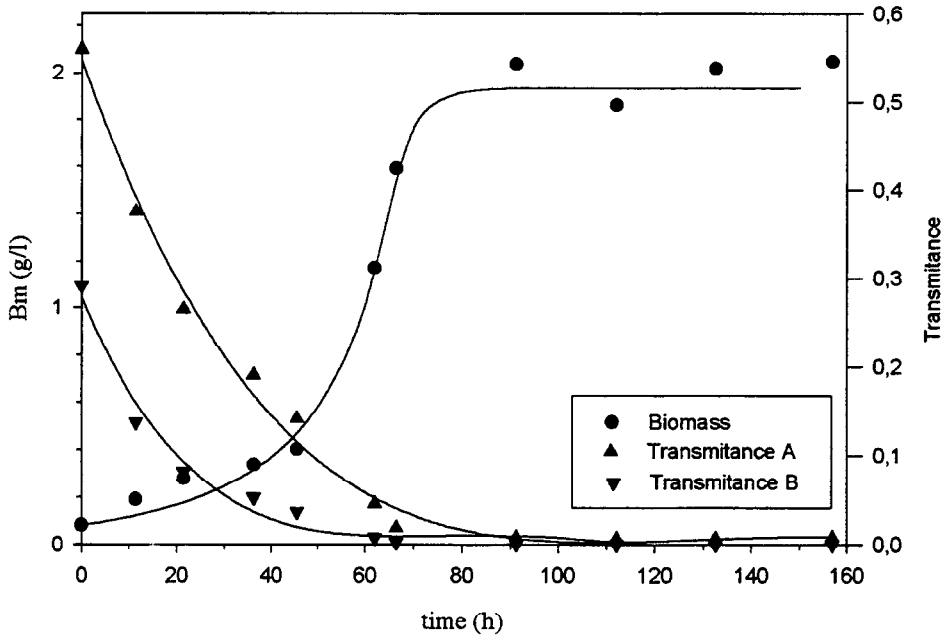

Figure 2: Plot of the results obtained in test A

After several hours the culture appears to enter a linear phase of growth, during the second part of the culture (60-80h). This part corresponds to the lower light intensities measured leaving the bioreactor. In this case it can be assumed that in this part of the time course, growth rate would be light limited. This can be accounted for by assuming the establishment of an gradient of light intensity between the frontal and the rear part of the vessel. However, due to agitation, cells are moving from one part of the illuminated field to another, and therefore it can be considered that they are exposed to an averaged light intensity.

The existence of a threshold of light intensity under which the energy supplied is not enough for growth but only for maintenance would allow to theoretically consider the bioreactor divided in two volumes. A dark one were cells do not have light to grow and an illuminated one were growth takes place. This illuminated part corresponds to a working illuminated volume as

described by Cornet (Cornet 1992). The relative volumes of those areas would be continuously changing as biomass concentration increases, and therefore average growth rate would be continuously decreasing. Cells would be maintained in movement from one area to the other by means of the stirring.

Table 1 Row data and illumination conditions of test A.

Illumination conditions: 6 lamps 12 Volts.
F_0: 717 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (145 $W \cdot m^{-2}$) ; F_{I0} : 1303 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (263.$W \cdot m^{-2}$)

Time (h)	Biomass (g/l)	Fl (A) ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	FI (A) ($W \cdot m^{-2}$)	Transmit. A	Fl (B) ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	FI (B) ($W \cdot m^{-2}$)	Transmit. B
0.	0.08	380	76.8	0.292	730	147.6	0.560
11.5	0.19	180	36.3	0.138	490	99.1	0.376
21.5	0.28	107	21.7	0.082	345	69.8	0.265
36.2	0.34	69	13.9	0.053	249	50.3	0.191
45.5	0.40	49	9.9	0.037	185	37.4	0.142
62.0	1.17	10	1.98	0.008	60	12.1	0.046
66.5	1.59	4	0.89	0.003	25	5.1	0.019
91.2	2.04	2	0.32	0.001	11	2.2	0.008
112.1	1.86	1	0.27	0.001	9	1.8	0.007
132.5	2.02	1	0.28	0.001	9	1.8	0.007
157.0	2.05	2	0.31	0.001	10	2.0	0.008

TEST B

For this test, illumination was decreased by removing two lamps from the set up. The averaged initial light flux (F_0), measured with the blank medium, was of 499 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (101 W/m^2), as described in appendix A . Light flux at the point of measurement (F_{I0}) with the blank was of 1223 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (247 W/m^2). The decrease corresponds only to the light influence of the removed lamps on the measurement of the sensor. This results from the fact that this light measurement is taken in front of one lamp. Time course of the experiments can be seen in figure 3 and table 2.

As in the previous test, according to the acetic acid yields obtained in previous experiments (Albiol 1994), the maximum biomass level corresponds to the exhaustion of the carbon/electron source. Growth of the cells takes place until there is barely no light leaving the vessel. As the average of light intensity (or the working illuminated volume) decreases with the increase of biomass concentration, growth rate decreases. In this case, for the same biomass concentrations the growth rate appears to be lower than in the previous case, due to the fact that the average light intensity entering the bottle has decreased.

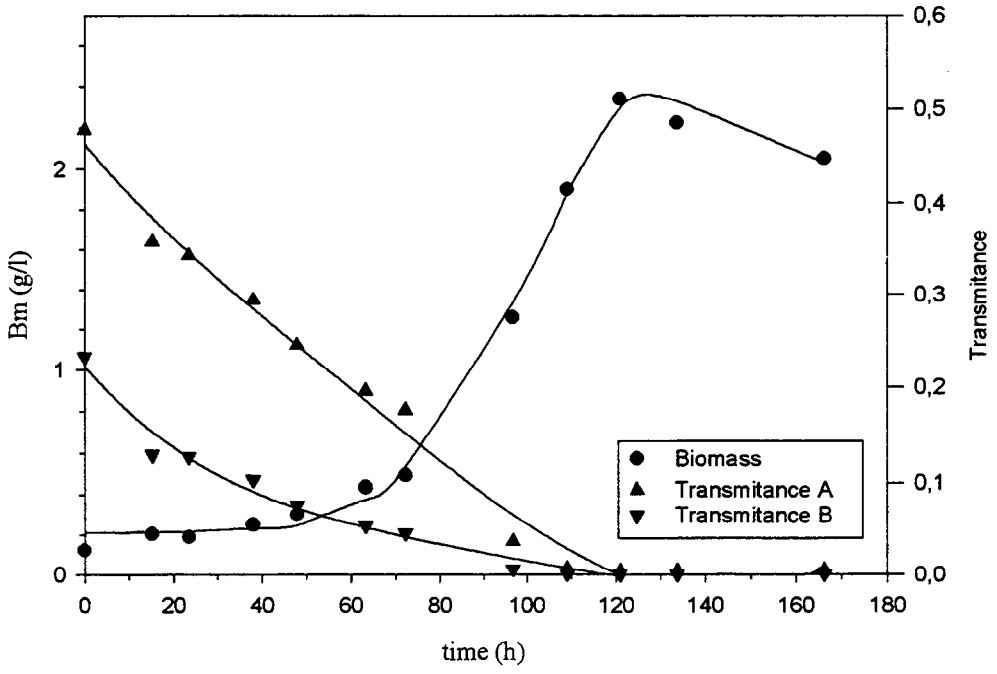

Figure 3: Plot of the results obtained in test B

Table 2 Row data and illumination conditions of test B.

Illumination conditions: 4 lamps 12 Volts. F_0: 499 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (101 $W \cdot m^{-2}$) ; F_{I0} : 1223 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (247 $W \cdot m^{-2}$)							
Time (h)	**Biomass (g/l)**	**Fl (A) ($\mu mol \cdot m^{-2} \cdot s^{-1}$)**	**FI (A) ($W \cdot m^{-2}$)**	**Transmit. A**	**Fl (B) ($\mu mol \cdot m^{-2} \cdot s^{-1}$)**	**FI (B) ($W \cdot m^{-2}$)**	**Transmit. B**
0.0	0.12	284	57.5	0.232	585	118.3	0.478
15.2	0.21	159	32.2	0.130	438	88.6	0.358
23.5	0.19	156	31.6	0.128	422	84.9	0.343
37.9	0.25	127	25.7	0.104	361	72.8	0.294
47.8	0.31	93	18.9	0.076	303	60.7	0.245
63.2	0.44	66	13.4	0.054	240	48.5	0.196
72.2	0.5	56	11.35	0.046	215	43.5	0.176
96.7	1.26	8	1.59	0.006	45	9.1	0.037
109.0	1.9	1.3	0.26	0.001	8	1.6	0.007
120.9	2.3	0.7	0.14	0.001	5	1.0	0.004
133.7	2.2	0.8	0.16	0.001	5	1.0	0.004
166.2	2.0	0.85	0.17	0.001	6	1.2	0.005

TEST C

In this test only two lamps were left in the set up. The average light intensity measured with the blank (F_0) was found to be 239 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (48 W/m^2). The light intensity measured by the sensor with the blank in front of one lamp (F_{I0}) was of 1188 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (240 W/m^2). The results obtained during this test are depicted in figure 4 and recorded in table 3. As in the previous cases the growth presents a linear phase during the second part of the culture which corresponds to the effect of light limitation.

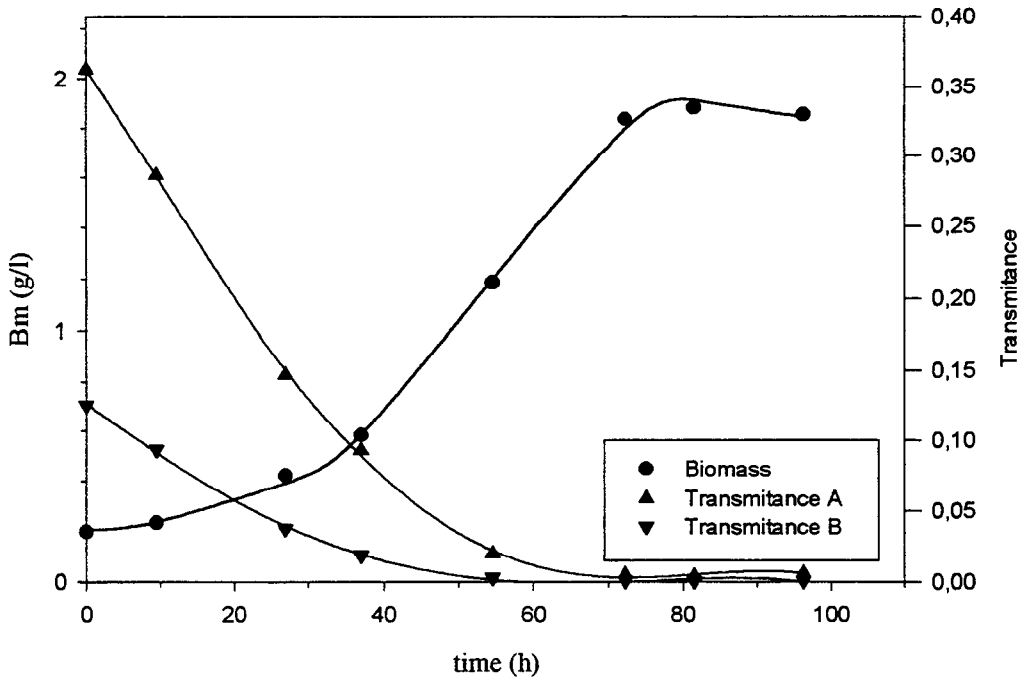

Figure 4: Plot of the results obtained in test C

Table 3 Row data and illumination conditions of test C.

Illumination conditions: 2 lamps 12 Volts. F_0: 239 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (48 $W \cdot m^{-2}$) ; F_{10}: 1188 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (240 $W \cdot m^{-2}$)							
Time (h)	**Biomass (g/l)**	**Fl (A) ($\mu mol \cdot m^{-2} \cdot s^{-1}$)**	**FI (A) ($W \cdot m^{-2}$)**	**Transmit. A**	**Fl (B) ($\mu mol \cdot m^{-2} \cdot s^{-1}$)**	**FI (B) ($W \cdot m^{-2}$)**	**Transmit. (B)**
0.	0.2	149.44	30.21	0.126	430	86.9	0.362
9.5	0.24	111.60	22.56	0.094	340	68.7	0.286
27.0	0.42	45.10	9.12	0.038	175	35.4	0.147
37.0	0.58	22.36	4.52	0.019	110	22.2	0.093
54.5	1.19	3.9	0.79	0.003	24	4.9	0.020
72.25	1.84	0.88	0.18	0.001	6	1.2	0.005
81.5	1.88	0.76	0.15	0.001	5	1.0	0.004
96.25	1.87	0.84	0.17	0.001	7	1.4	0.006

TEST D

In this test only one lamp, positioned focusing the middle of the bottle, was used. Averaged light flux (F_0), determined as explained in appendix A, was found to be 134 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (27 W/m^2). Light flux, measured with the sensor in front of the lamp (F_{l0}), was of 1159 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (234 W/m^2). The decrease being, as in the previous cases, due to the influence of the other lamps on the measurement of the sensor. The results obtained in this test are depicted in figure 5 and recorded in table 4. Growth takes place as before until light is depleted. The growth rate has further decreased from the previous cases due to the lower averaged light intensity.

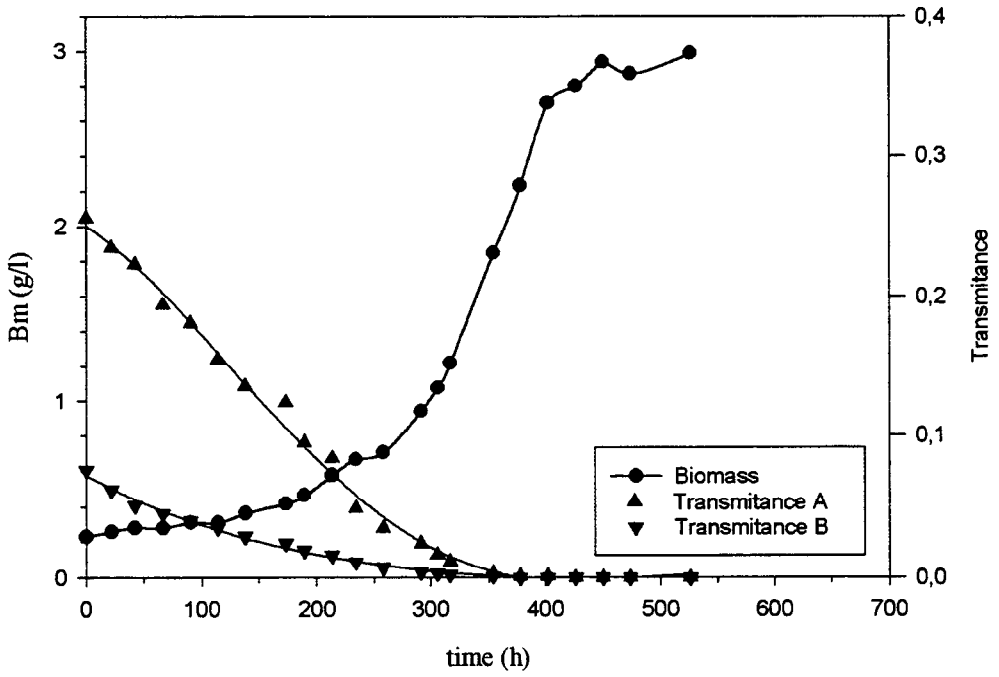

Figure 5 Plot of the results obtained in test D

Table 4 Row data and illumination conditions of test D.

ILLUMINATION CONDITIONS: 1 LAMP 12 VOLTS. F_0: 134 mmol×m^{-2}×s^{-1} (27 W·m^{-2}) ; F_{L0}:917 mmol×m^{-2}×s^{-1}; (185 W·m^{-2})							
Time (h)	Biomass (g/l)	Fl (A) (mmol×m^{-2}×s^{-1})	Fl (A) (W×m^{-2})	Transmit, A	Fl(B) (mmol×m^{-2}×s^{-1})	Fl (B) (W×m^{-2})	Transmit. B
0.00	0.23	850	171.9	0.076	225	45.5	0.245
22.00	0.26	700	141.5	0.062	200	40.4	0.218
42.33	0.28	570	115.2	0.051	190	38.4	0.207
66.33	0.28	505	102.1	0.045	170	34.4	0.185
90.33	0.31	445	90.0	0.040	153	30.9	0.167
114.33	0.31	399	80.7	0.035	135	27.3	0.147
138.33	0.36	328	66.3	0.029	120	24.3	0.131
173.33	0.42	272	55.0	0.024	108	21.8	0.118
189.50	0.46	218	44.1	0.019	94	19.0	0.103
213.50	0.58	185	37.4	0.016	74	15.1	0.081
234.33	0.67	126	25.5	0.011	45	9.1	0.049
258.33	0.70	80	16.2	0.007	30	6.1	0.033
291.87	0.94	47	9.5	0.004	21	4.3	0.023
306.25	1.07	31	6.2	0.003	14	3.0	0.016
317.00	1.22	22	4.4	0.002	10	2.0	0.011
354.25	1.85	6.0	1.2	0.001	3	0.6	0.003
378.25	2.23	2.4	0.5	0.000	1.2	0.2	0.001
402.25	2.70	1.2	0.2	0.000	0.6	0.1	0.001
426.67	2.80	0.8	0.2	0.000	0.4	0.1	0.000
450.33	2.94	0.6	0.1	0.000	0.3	0.1	0.000
474.25	2.87	0.5	0.1	0.000	0.28	0.1	0.000
526.00	2.99	0.4	0.1	0.000	0.23	0.0	0.000

TEST E

This is the last test that was done. In this case the light intensity was decreased by means of decreasing the voltage of the lamp. The averaged light intensity (F_0) was of 77 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (15 W/m^2). The light intensity measured by the sensor in the front of the lamp (F_{10}) was of 584 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (118 W/m^2). The results obtained can be seen in figure 6 and table 5.

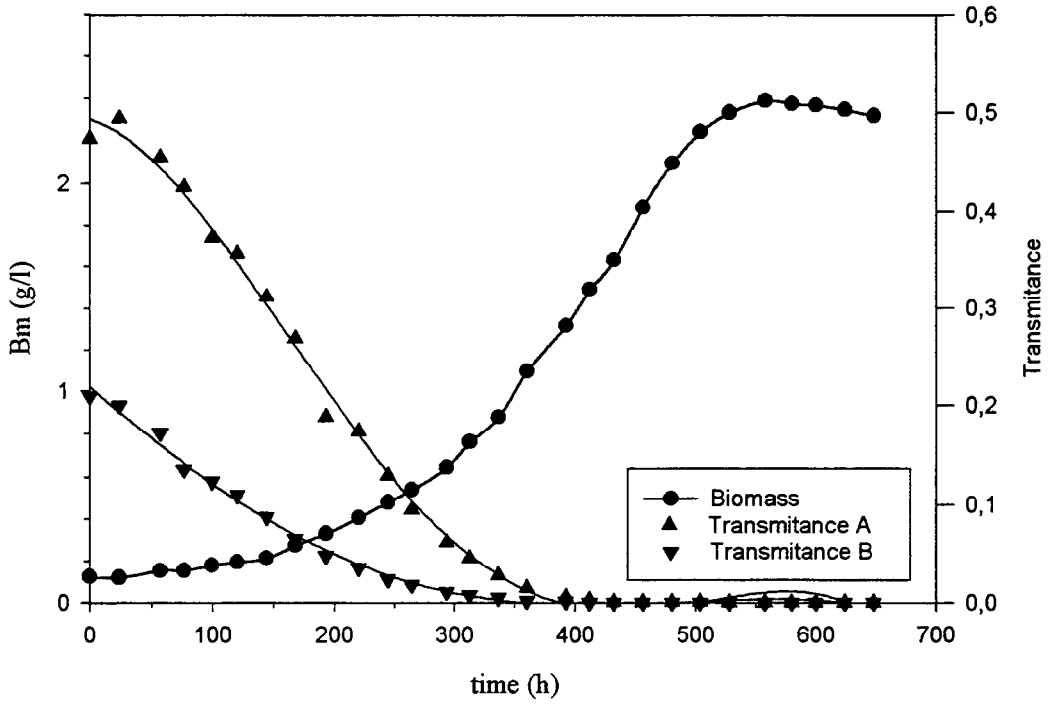

Figure 6: Plot of the results obtained in test E.

Illumination conditions: 1 lamp 10 Volts. F_0: 77 mmol×m^{-2}×s^{-1} (15 W·m^{-2}). F_{10}: 506 mmol×m^{-2}×s^{-1}; 102 W×m^{-2}							
Time (h)	**Biomass (g/l)**	**Fl (mmol×m^{-2}×s^{-1})**	**FI (W×m^{-2})**	**Transmit.**	**Fl (mmol×m^{-2}×s^{-1})**	**FI (W×m^{-2})**	**Transmit.**
0.00	0.13	2315	468.1	0.209	240	48.5	0.474
24.12	0.12	2220	448.8	0.200	250	50.5	0.494
56.87	0.15	1910	386.2	0.172	230	46.5	0.455
76.28	0.15	1505	304.3	0.136	215	43.5	0.425
99.12	0.18	1370	277.0	0.124	188	38.0	0.372
120.12	0.19	1215	245.7	0.110	180	36.4	0.356
144.12	0.21	970	196.1	0.088	158	31.9	0.312
167.95	0.28	729	147.4	0.066	136	27.5	0.269
192.95	0.33	530	107.2	0.048	95	19.2	0.188
220.12	0.41	395	79.9	0.036	88	17.8	0.174
244.62	0.48	271	54.8	0.024	66	13.3	0.130
264.37	0.54	195	39.4	0.018	48	9.7	0.095
293.37	0.64	117	23.7	0.011	31	6.4	0.062
312.28	0.76	85	17.1	0.008	23	4.8	0.046
336.28	0.88	53.	10.7	0.005	14	2.8	0.028
360.28	1.10	27	5.5	0.002	7.6	1.5	0.015
392.45	1.32	9.6	1.9	0.001	2.8	0.6	0.006
412.12	1.49	4.59	0.9	0.000	1.4	0.3	0.003
432.12	1.63	2.34	0.5	0.000	0.7	0.1	0.001
455.87	1.89	1.56	0.3	0.000	0.5	0.1	0.001
480.20	2.10	1.31	0.3	0.000	0.38	0.1	0.001
503.73	2.24	0.83	0.2	0.000	0.29	0.1	0.001
527.87	2.33	0.8	0.2	0.000	0.25	0.1	0.000
557.62	2.39	0.6	0.1	0.000	0.21	0.04	0.000
579.62	2.37	0.57	0.1	0.000	0.18	0.04	0.000
599.95	2.37	0.5	0.1	0.000	0.15	0.03	0.000
623.95	2.35	0.44	0.1	0.000	0.13	0.03	0.000
647.95	2.32	0.41	0.1	0.000	0.12	0.02	0.000

Table 5 Row data and illumination conditions of test E.

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

APPENDIX A

Due to the fact that the light flux was found to be non homogeneous, it was decided to measure the light flux at different points of the vessel (1 cm apart, and in the rear surface of the blank vessel) in order to provide a better description of the illumination conditions. The light flux was measured in all those positions for the different illumination conditions. A bottle with culture media without bacteria was used as a blank. Measurements were taken at the rear surface of the bottle. The results obtained are presented in the following tables and depicted in the graphs that follow. Once the light flux was mapped, the values measured in all the points, for a single light intensity, were averaged so as to obtain an approximation of the mean light flux available to the culture. The calculated values are given in table 7.

Illumination conditions	**$\mu mol \cdot m^{-2} \cdot s^{-1}$**	**W/m^2**
6 lamps, 12 V	717	145
4 lamps, 12 V	499	101
2 lamps, 12V	239	49
1 lamp, 12 V	134	27
1 lamp, 10 V	77	15

Table 6: Average light intensities for the different experiments.

Illumination in test A

In test A illumination was obtained by using six lamps at 12 volts. Results obtained in the measuring of the light flux at different positions can be seen in table 8 and figure 7.

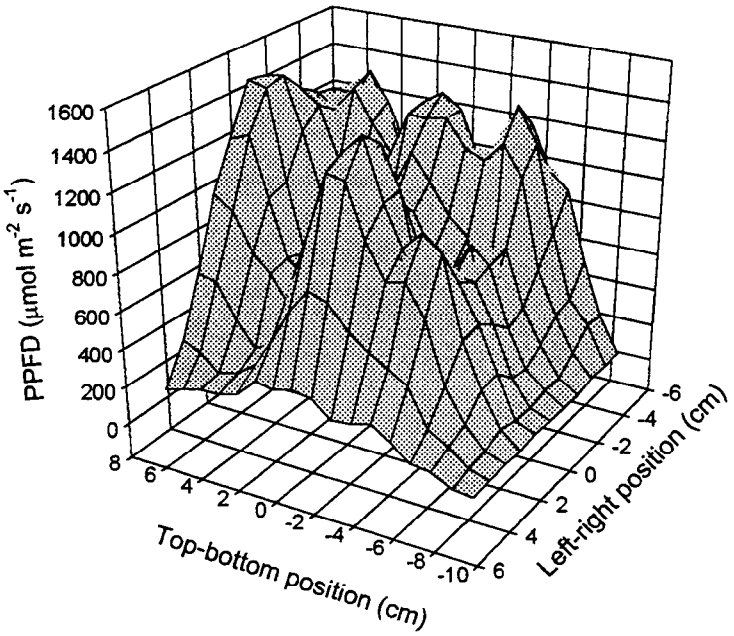

Figure 7: Light flux measured for test A

Vertical position	Horizontal position										
	-5	-4	-3	-2	-1	0	1	2	3	4	5
7	-	880	950	1126	1460	1480	1230	980	630	330	-
6	1200	1260	1300	1370	1470	1440	1260	930	600	290	150
5	1300	1250	1230	1280	1200	990	950	720	430	200	175
4	1160	1385	1340	1020	890	640	670	640	300	230	180
3	980	1260	1200	830	638	575	580	490	360	290	220
2	720	890	1060	870	719	735	770	775	650	460	300
1	980	1300	1267	930	860	860	1070	1170	960	630	300
0	1205	1360	1260	1130	935	1030	1170	1350	1295	770	325
-1	950	1284	1290	1000	900	1000	1360	1450	1270	727	310
-2	840	1040	1160	850	710	843	1135	1133	1000	603	230
-3	810	1090	1100	770	615	560	760	920	830	520	225
-4	1230	1350	1200	247	803	690	840	1040	845	470	250
-5	1000	1170	1065	740	720	693	750	950	670	400	200
-6	900	730	600	450	406	480	555	525	360	220	130
-7	530	390	360	265	190	220	310	305	190	160	100
-8	260	280	170	113	100	88	100	117	90	70	60
-9	60	54	50	49	52	52	52	50	50	45	45

Table 7: Light flux values obtained for illumination conditions in test A

Illumination in test B

In test B illumination was obtained by using four lamps at 12 volts. Results obtained in the measuring of the light flux at different positions can be seen in table 9 and figure 8.

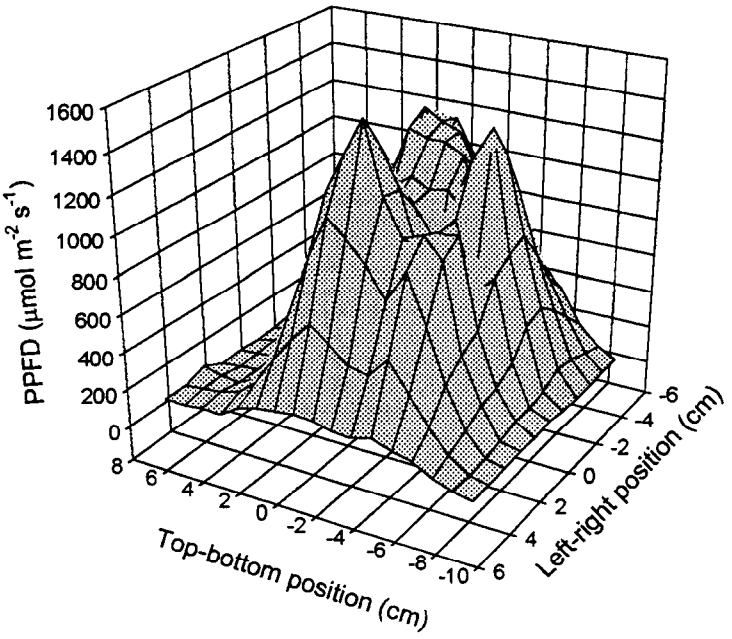

Figure 8: Light flux measured for test B

Vertical position	Horizontal position										
	-5	-4	-3	-2	-1	0	1	2	3	4	5
7	-	109	134	101	90	86	82	88	128	100	-
6	110	150	101	93	96	98	92	86	103	110	95
5	133	122	104	105	104	103	106	104	102	120	98
4	160	160	150	129	128	150	165	162	144	140	100
3	350	430	330	310	300	390	430	420	290	230	150
2	700	820	925	690	640	670	770	725	580	370	185
1	1000	1260	1190	950	890	900	1075	1130	860	500	200
0	1165	1205	1170	1030	960	1085	1118	1326	1090	615	210
-1	1000	1160	1170	980	970	1125	1350	1520	1010	530	200
-2	850	985	1116	1000	490	1000	1150	1170	890	450	175
-3	510	688	795	880	950	850	830	990	740	415	165
-4	500	630	850	1190	1240	1000	980	1032	910	540	200
-5	490	640	990	1237	1410	640	990	920	660	370	170
-6	380	490	680	840	800	740	530	470	390	240	140
-7	200	300	400	490	440	320	250	240	166	135	90
-8	99	130	150	163	118	95	110	80	70	62	55
-9	40	35	38	38	43	48	44	46	42	35	35

Table 8: Light flux values obtained for illumination conditins in test B.

Illumination in test C

In test C illumination was obtained by using two lamps at 12 volts. Results obtained in the measuring of the light flux at different positions can be seen in table 10 and figure 9.

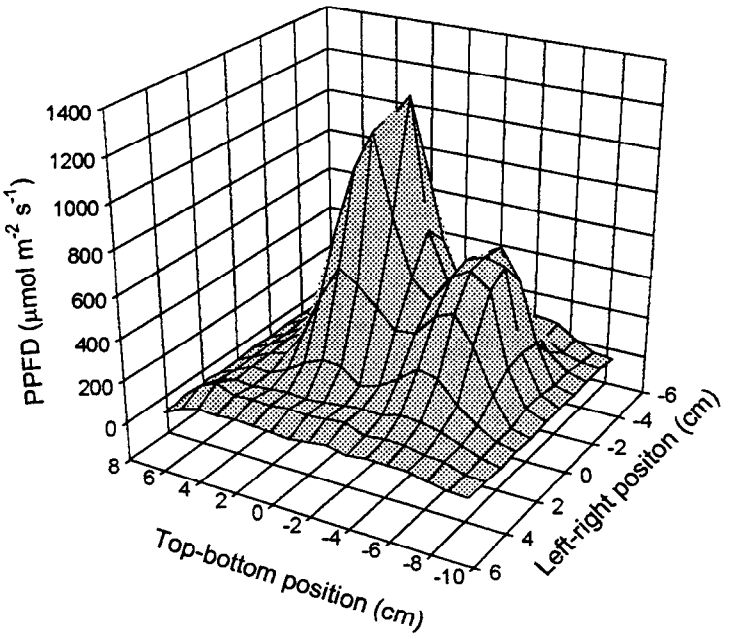

Figure 9: Light flux measured for test C

Vertical position	Horizontal position										
	-5	-4	-3	-2	-1	0	1	2	3	4	5
7	10	11	11	13	12	12	12	11	9	9	8
6	102	60	49	53	53	58	51	50	57	97	50
5	150	99	92	105	106	98	90	82	87	125	74
4	115	95	126	187	200	161	106	77	76	96	63
3	125	170	300	460	500	380	236	117	81	94	64
2	137	310	540	770	912	700	432	171	91	93	64
1	170	390	700	1029	1050	995	592	214	90	95	67
0	130	385	709	1144	1185	1170	535	199	99	91	55
-1	136	290	576	982	1300	800	443	133	105	112	70
-2	140	204	378	620	721	560	370	165	108	109	72
-3	120	167	281	424	500	470	366	218	104	78	55
-4	138	211	370	586	620	600	470	277	134	96	65
-5	145	193	375	647	655	650	510	264	133	94	62
-6	133	127	266	510	624	580	375	170	99	73	55
-7	73	68	125	240	250	230	176	99	66	61	45
-8	33	35	45	54	61	60	40	37	35	35	27
-9	25	26	25	25	21	25	24	24	23	23	18

Table 9: Light flux values obtained for illumination conditions in test C.

Illumination in test D

In test D illumination was obtained by using one lamp at 12 volts. Results obtained in the measuring of the light flux at different positions can be seen in table 11 and figure 10.

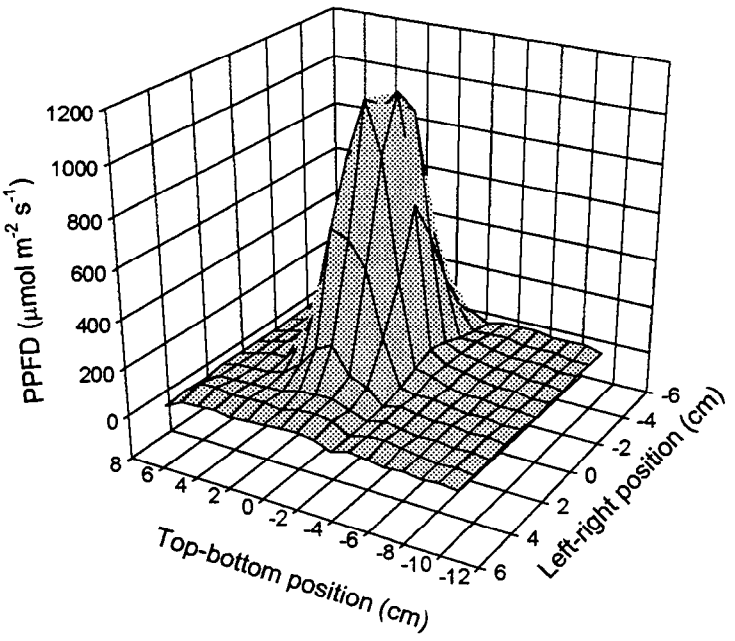

Figure 10: Light flux measured for test D

Vertical position	Horizontal position										
	-5	-4	-3	-2	-1	0	1	2	3	4	5
7	25	30	20	23	20	26	21	22	30	50	30
6	65	47	45	46	47	43	38	36	39	61	36
5	53	47	52	83	85	78	50	34	34	48	32
4	60	98	200	315	340	240	130	57	42	48	33
3	72	180	395	560	616	530	320	106	52	48	32
2	76	290	560	900	950	890	633	185	60	48	30
1	106	330	660	1050	1050	1120	615	225	73	56	35
0	80	280	580	1010	1115	916	500	105	67	65	39
-1	50	130	300	520	690	425	230	86	45	57	29
-2	22	30	54	80	110	68	49	22	16	15	11
-3	30	38	45	58	61	53	39	36	30	33	25
-4	38	32	32	33	34	33	32	32	29	32	22
-5	29	24	26	27	26	25	22	23	17	20	15
-6	23	20	16	17	17	17	16	16	16	17	13
-7	12	11	14	12	12	12	12	12	13	13	10
-8	10	10	9	9	9	9	9	9	9	9	9
-9	9	8	7	6	6	6	6	6	5.7	4.5	5

Table 10: Light flux values obtained for illummination conditions in test D.

Illumination in test E

In test E illumination was obtained by using one lamp at 10 volts. Results obtained in the measuring of the light flux at different positions can be seen in table 12 and figure 11.

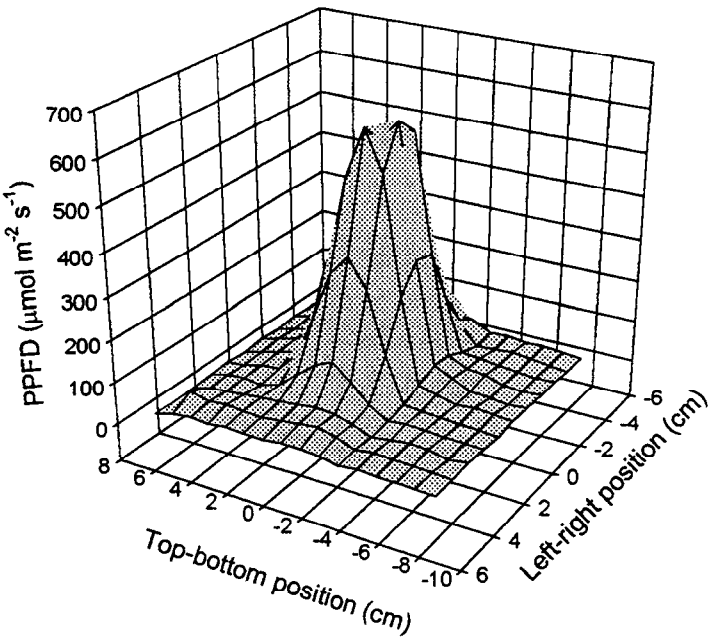

Figure 11: Light flux measured for test E

Vertical position	Horizontal position										
	-5	-4	-3	-2	-1	0	1	2	3	4	5
7	3.7	3.7	3.5	3.7	2.7	2.6	2.6	2.8	2.9	3	3
6	20	18	15	14.5	14.5	14.5	10	11	12	36	12
5	30	28	24	24	25	22	20	19	20	26	20
4	30	22	30	40	48	40	25	18	18	30	17
3	30	38	90	170	180	130	65	30	22	26	18
2	37	98	215	327	355	296	180	62	28	26	17
1	50	160	310	495	515	485	290	99	32	25	16
0	60	180	368	555	550	600	334	118	36	30	20
-1	52	155	310	560	605	505	271	86	35	34	20
-2	40	65	158	285	300	240	130	40	21	25	15
-3	13	16	27	45	55	35	20	11	9	7.5	6
-4	19	19	23	30	30	26	19	16	11	17	13
-5	18	17	16	18	15	16	16	15	13	15	10
-6	18	16	12	13	11	13	13	13	12	12	9
-7	14	9.5	9	9	8.5	10	9	9	8	10	7
-8	7	7	7	7	7	7	7	7	7	8	6.5

Table 11: Light flux values obtained for illumination conditions in test E.

APPENDIX B

As mentioned in the introduction, it has been observed that the transmitance of the cultures calculated for different distances between the lamp and the surface of the culture vessel heavily changes for lamp distances smaller than about 20 cm. As the measurement of the light intensity inside our bioreactors or at the rear part of the vessels can be used for the Fr estimation, it has been considered that this fact may be of importance. To illustrate this point a test was done in which the transmitance was measured in a culture vessel for different distances of the lamp from the culture media. It can be seen in figure 12 that as the distance of the lamp to the culture vessel increases, so it does the transmitance, up to a value around 20 cm. At this point the value of the transmitance has a very small increase.

On the other hand in can be expected that while changing the distance of the lamp to the vessel surface, the Fr also changes. To check if this fact had an impact on the measured transmitance, another test was done (figure 13). The lamp distance was fixed at either at 5 and at 0 cm of the surface vessel and the light intensity was changed by changing the voltage supplied to the lamp and therefore changing the Fr. It can be seen that, while the transmitance when changing the distance has changed, there is a very small variation in the transmitance when the change is done on the lamp voltage. The effect of the distance on the transmitance might be the result of of the combined effect of different elements of the experimental set up (type of lamps, refraction index of the air-glass-whater interfaces...) wich may influence the Fr calculated in different systems.from the light sensor values measured.

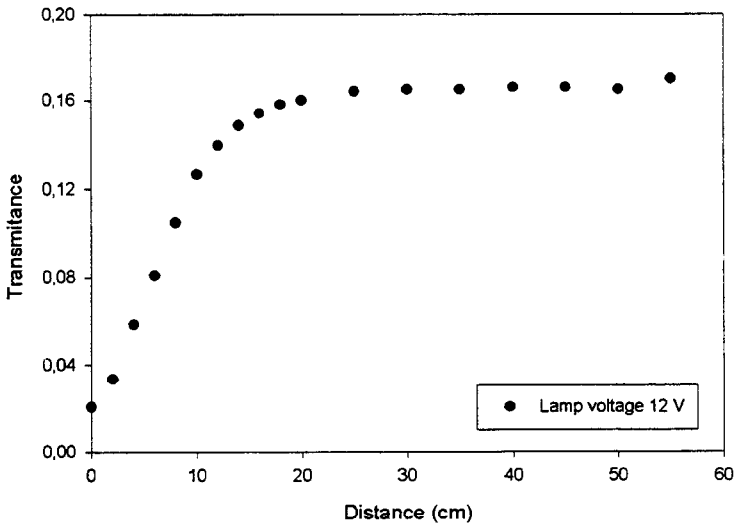

Figure 12: Effect of lamp distance on transmitance.

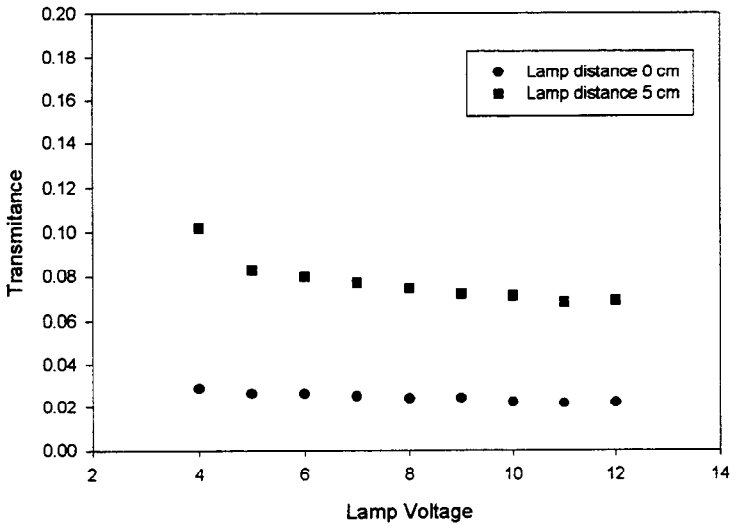

Figure 13: Effect of light intensity on transmitance.