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Test bench evaluation of IR enriched lamps

H_2 consumption test proposal

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TEST BENCH EVALUATION OF IR LAMPS

I. INTRODUCTION

In the growth of photosynthetic microorganisms the nature of the light source, especially its spectra distribution, plays an important role. In order to determine its effect on the growth of the *Rhodospirillum rubrum* cells several tests have been carried out using two different kinds of light source. On one side halogen lamps were used as the reference point because they have been used in all the previous tests. Incandescent lamps are the subject of this investigation due to the fact that they have a light emission spectra with maximum values closer to the absorption peaks of the bacterial photosynthetic pigments.

With the purpose of knowing the light emission characteristic of the lamps used in this experiments, their light emission spectra was measured at ESTEC XAL laboratory (appendix 3). It is clear from those spectra that the maximum of emission of light intensity in the halogen lamps is located around 680-700 nm, which coincides with the *Spirulina* absorption maximum. In the case of incandescent lamps the light emission maximum is located around 960 nm. Opposite to this, *Rhodospirillum rubrum* does not have absorption maximums at the 680-700 nm (like *Spirulina*), but it has carotenoids absorbing at a lower wavelength (maximum around 520 nm) and chlorophylls absorbing in the range 800-950 nm (peaks around 800 and 880 nm). That means they do not use light corresponding to 680-700 nm, being only scattered, and for this reason it is used for the absorbance-biomass dry weight correlations .

It has also to be mentioned that the energy collected around 520 nm, at the bacterial membrane level, is ultimately driven to the reaction centres. These reaction centres have an absorption maximum at 880 nm. Consequently the energy they can convey for physicochemical use is surely smaller than the energy of the photons they can collect. As a result, some of the energy collected at 520 nm is lost as heat in an amount at least of the same order than the difference of the energy content of the light photons of the light collected (e.g. 520 nm) to the one collected at the reaction centre (880 nm). Thus, light collected at around 880 nm is more efficiently used that light collected at shorter wavelengths. On the other hand, having pigments collecting light at

longer and also at shorter wavelengths, allows the bacteria to collect a higher amount of energy under the same illumination conditions, therefore being more efficient in using the light available in different habitats.

So, it would be very interesting to carry out some tests in order to study which spectra distribution allows to the cell to attain a higher specific growth rate.

In the present TN, the effect of the use of lamps with different kind of light emission spectra, on the growth of *R. rubrum* under monodimensional illumination conditions, is reported.

The tests have been carried out in the experimental set-up used previously (Lenguaza et al. 1997, TN 37.81 and TN 37.82). It has been modified to allow the use of the incancescent lamps and the heat removal has been improved.

The light emission spectrum was measured for three different kinds of lamps (appendix 3), the halogen one and two of the incandescent type (15 W and 30 W). However the tests have been carried out using only one kind of incandescent lamp, the one which had the maximum of emission further than the halogen peak (15 W lamps).

II. MATERIAL AND METHODS

The bacterial strain *Rhodospirillum rubrum* (ATCC 25903) was obtained from the American Type Culture Collection. The strain was received freeze-dried and was revived using R8AH medium (ATCC medium 550). This media was also used for routinely subculture of the stock strain.

The culture media used during the tests was based on the basalt salt mixture formulated by Segers & Verstraete as is described by Suhaimi (Suhaimi *et. al.* 1987). Volatile fatty acids were used 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. In order to improve the buffer capacity of the culture media and allow to maintain the pH value around 6.9, 3-Morpholino propane sulphonic acid (MOPS) was added to the media. The culture media composition is described in Appendix 1.

Temperature was regulated by means of a water bath and a refrigeration coil around 30 °C. A fan also contributed to maintain the temperature constant and allowed to avoid small temperature gradients on the glass surface. Cultures were maintained homogeneous using a magnetic stirrer.

The volume of the flat vessels was 1.4 litres. External dimensions of the bottom area of the vessel were 120 x 55 mm with and average 2 mm glass thickness. The top part of the vessel is round, however from the base area and the volume, the normal area exposed in the light direction can be calculated as 171 x 120 mm (0.0205 m^2). An approximate volume of 4 mL was extracted for each sample.

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-ups were used during the experiments, corresponding to two different floors on the dark chamber. On the first floor the lamps used were of incandescent type (Philips Practitone, 220 V, 15 W) and were located at 130 mm from culture vessel surface. On the second floor lamps were of the halogen type (Sylvania professional BAB 38° 12V 20W, improved version, cool beam, UV filtered, green box, code type 215) and they were located at 280 mm from the roux flask surface. Light intensity obtained, corresponded to an average value around 38

$\mu mols \cdot s^{-1} \cdot m^{-2}$ as measured on to a flat glass surface located at the same position as the frontal part of the culture vessels (F_0). The average light intensity values measured and its distribution are given in Appendix 2.

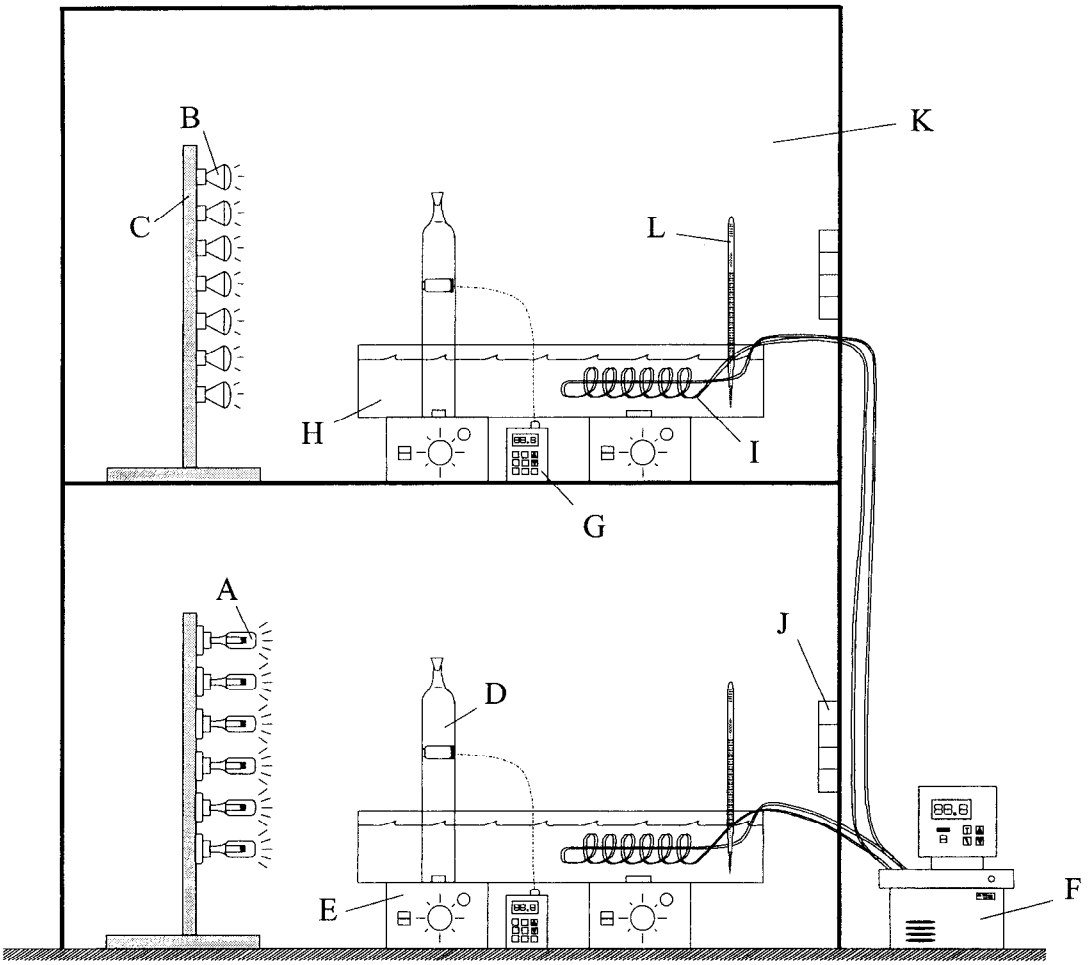

Figure 1: Experimental set-up. A: incandescent lamps; B: halogen lamps; C: lamps support; D: bioreactor (roux flask); E: magnetic stirrer; F: thermostatic bath; G: light sensor; H: water bath; I: coil; J: fan; K: dark chamber; L: thermometer

Photosynthetically active radiation (PAR) was measured using a quantum sensor (Licor Li-190SA) attached to a LI-189 portable meter. The sensor gives the photosyntethic 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 3 and 4) for both types of lamps. The factors used were 0.264 for the halogen lamps and 1.032 for the incandescent lamps,

both for the 350-950 nm range. The result of the application of those conversion factors is given in table 1.

Test	Illumination conditions	PPFD ± S.E. $\mu mols \cdot s^{-1} \cdot m^{-2}$	W/m^2 ± S.E. (350-950)
1, 2, 3, 4, and 5	Halogen lamps 8 lamps, 6.5 V	38.04 ± 0.70	13.98 ± 0.25
	Incandescent lamps 9 lamps, 220 V	38.00 ± 0.51	39.21 ± 0.53
6	Halogen lamps 8 lamps, 6.5 V	38.04 ± 0.70	13.98 ± 0.25
	Incandescent lamps 9 lamps, 220 V	13.48 ± 0.33	13.91 ± 0.34

Table 1: Average light intensities for the different set-ups. S.E.: Standard error = $\left(SD/\sqrt{n}\right)$. SD: Standard deviation

Biomass dry weight was calculated from the measured absorbance of a sample at 700 nm and its value interpolated on a calibration curve made using previously determined values of dry weight and optical density (Appendix 4).

The carbon sources used in this test were acetic acid and propionic acid. The carbon source profiles were followed during the tests by liquid chromatography analysis providing supplemental data for analysis and performance evaluation.

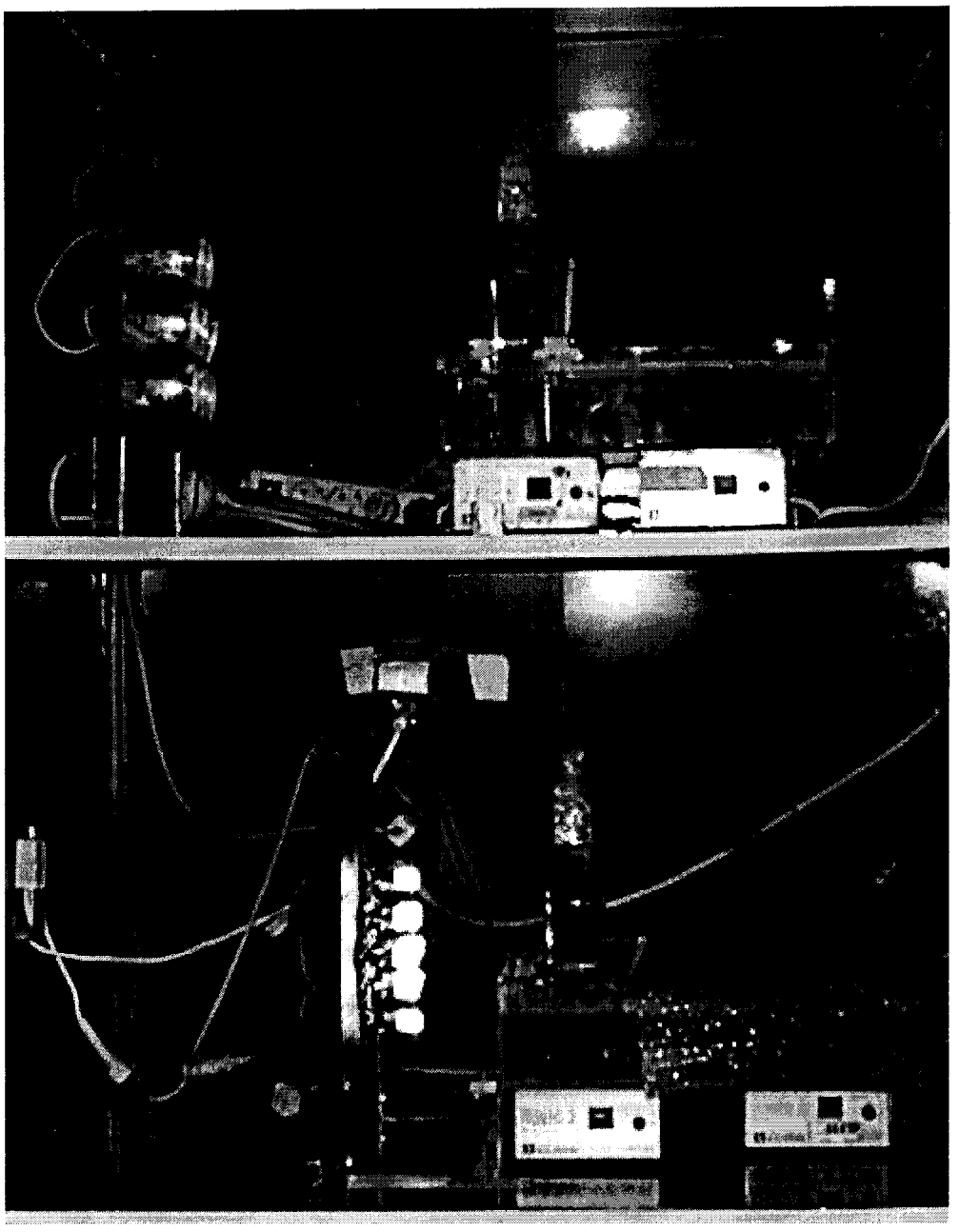

Figure 2: General view of the experimental set-up

III. EXPERIMENTAL RESULTS AND DISCUSSION

A. Acetic acid tests

The first of the carbon and electron sources employed was acetic acid, due to the fact that it is the major carbon source expected to be in the influent medium to the II compartment. Three tests have been carried out in order to evaluate the precision of the results. In all of the experiments the physical variables (temperature and the light intensity) have been kept at a constant value as is explained in "Materials and Methods". The pH value presents a small fluctuation along the tests, without an appreciable effect on the kinetic traits.

In test 1 the cells used to inoculate the flask had been previously grown in malic acid as carbon source. In the case of the tests 2 and 3 the biomass from the previous tests done under the halogen lamps, was used as the innoculum source. In all cases the volume of innoculum used was about 400 mL, due to the need to obtain a high initial biomass and, therefore a small lag phase.

The data obtained from these tests are collected in Appendix 6 and are represented in the following figures.

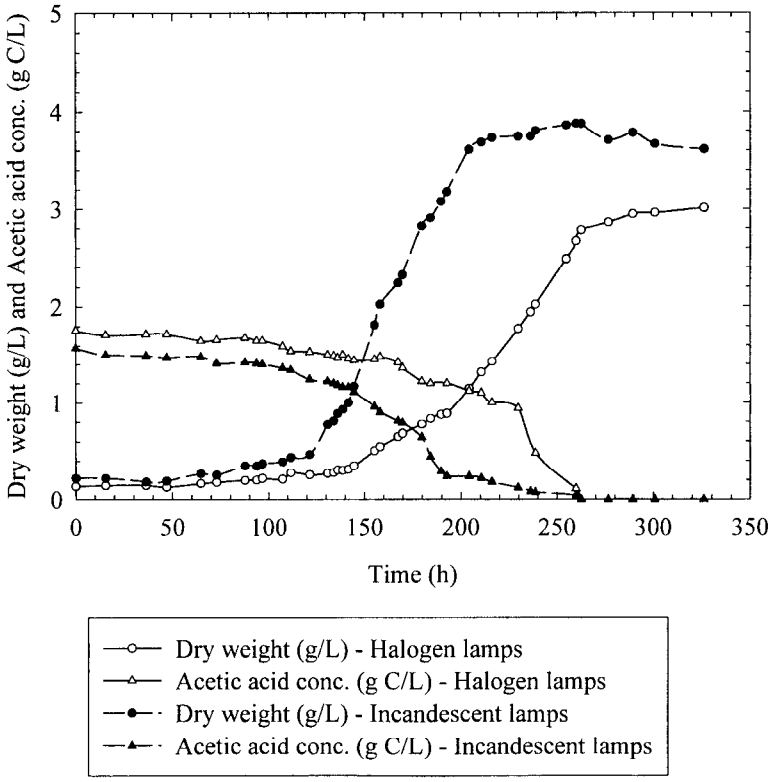

Figure 3: Test 1. Growth of *R. rubrum* cells in batch experiment with acetic acid as carbon source and the different kind of lamps, halogen and incandescent.

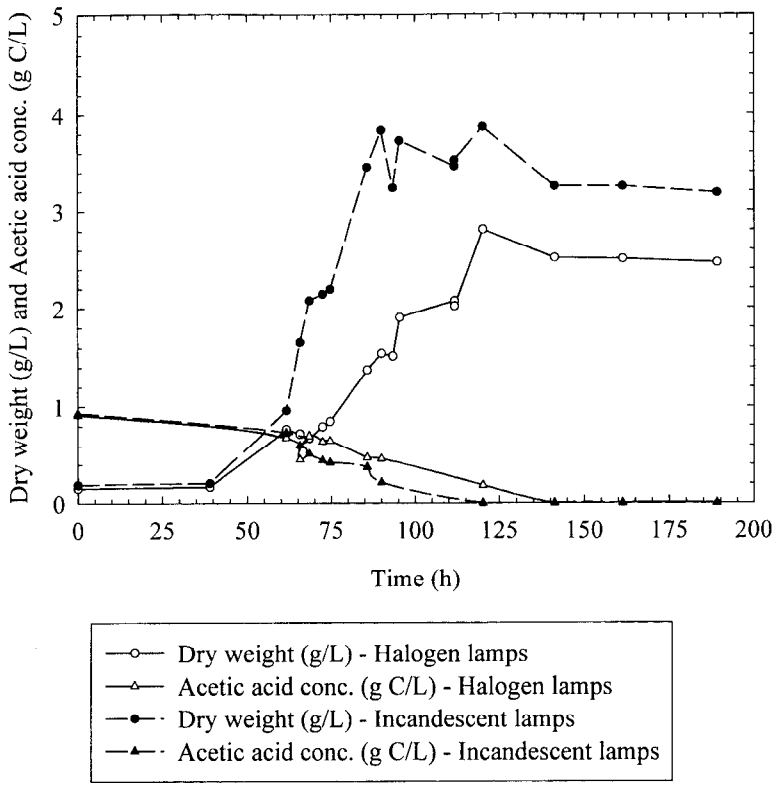

Figure 4: Test 2. Carbon source: Acetic acid

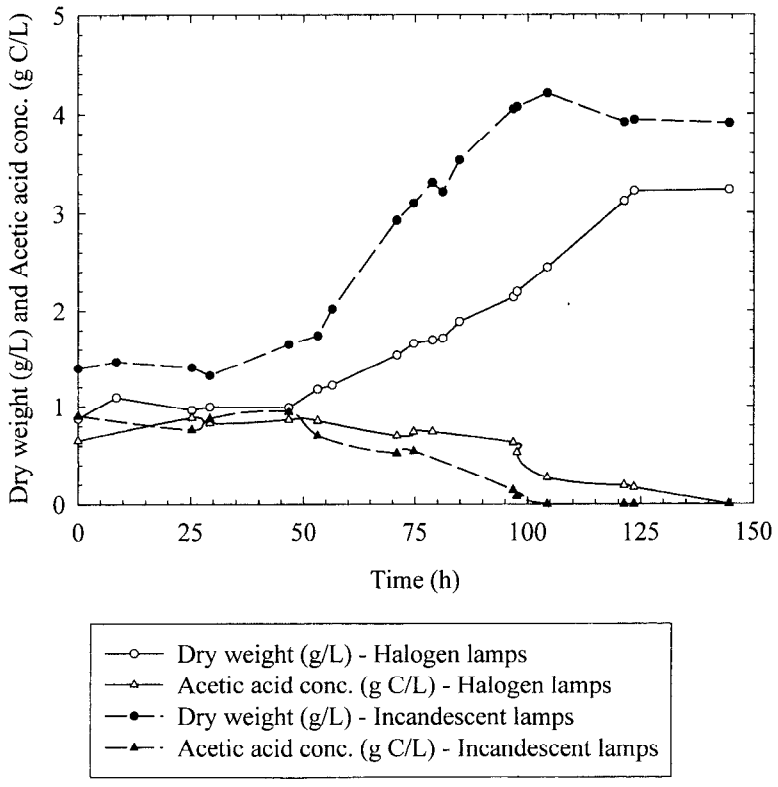

Figure 5: Test 3. Carbon source: Acetic acid

N° test	Lamp type	Increase Dry weight (g/L)	Initial Acetic acid conc.(g C/L)	Global yield (g D.W./g C)	Time (h)	Final pH value
1	Halogen	2.88	1.76	1.63	263	7.78
	Incandesc.	3.40	1.57	2.17	216	7.89
2	Halogen	2.31	0.91	2.54	120	7.46
	Incandesc.	3.01	0.93	3.22	90	7.61
3	Halogen	2.36	0.88	2.68	104	7.72
	Incandesc.	2.51	0.90	2.79	124	7.75

Table 2: Global yield results

Evaluation of the volumetric biomass productivity

In figure 6 the experimental results with the halogen lamps have been represented. Comparison of the results is done only in the linear phases of growth were growth rate is mainly governed by light limitation and were any acceleration or adaptation phases have ended. The same treatment is done with the incandescent lamp results, in figure 7.

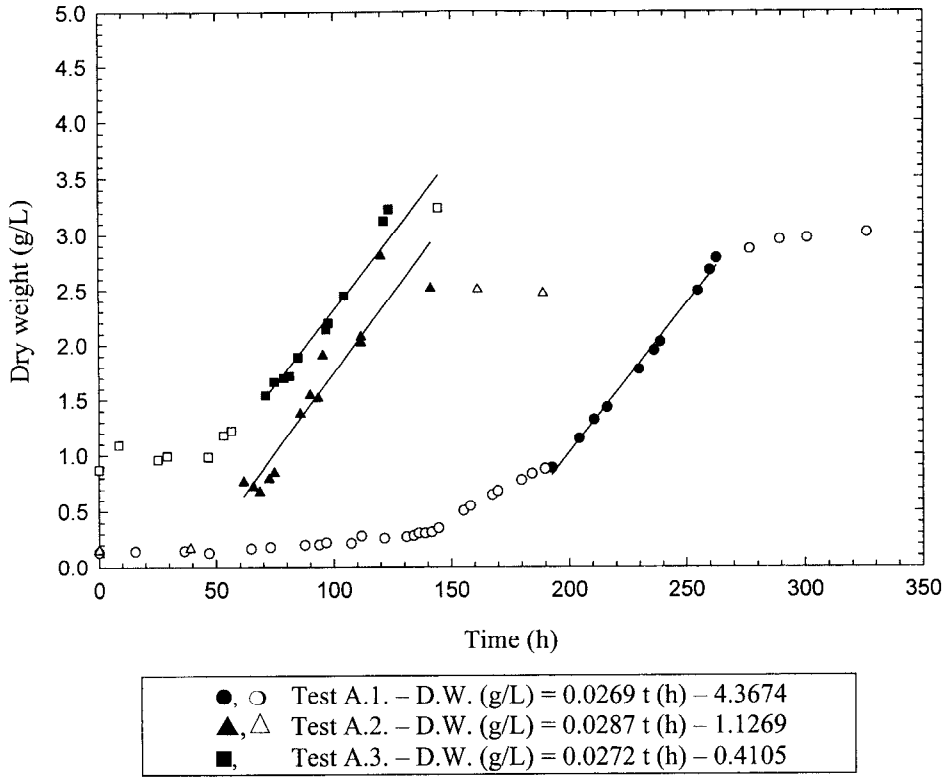

Figure 6: Halogen lamp tests. Carbon source: acetic acid
Filled symbols: data used for linear regression

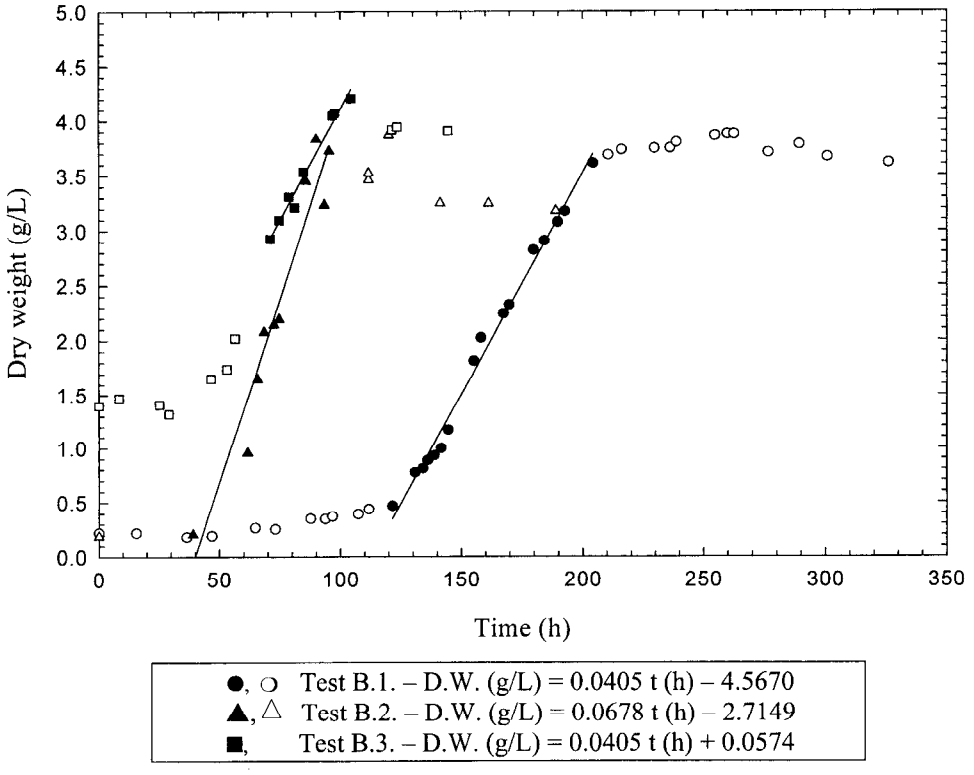

Figure 7: Incandescent lamp tests. Carbon source: acetic acid.
Filled symbols: data used for linear regression

In general it can be appreciated that the volumetric biomass productivity measured for each experiment is very similar for the tests carried out using the same type of lamp, in all of the three experiments done, presenting a low standard deviation. However, in the case of incandescent lamps illumination test number 2, it shows a high level of dispersion of the results, which does not allow its comparison with the rest of the data. Therefore it is considered that all the other 5 runs are consistent with the illumination conditions used in each one and are representative of the behaviour under the culture conditions used. Nevertheless, if an average of all the data from test 2 is done, the volumetric biomass productivity calculated, is comparable to the one obtained from the linear parts of tests 1 and 3, as can be seen in figure 7.

In figures 6 and 7 it can be observed that the lag phase is longer in test 1 than in tests 2 and 3. This fact is probably due to the different source of the cells used in the innoculum. In the first case the innoculum was pre-grown using culture medium containing malic acid as a carbon source while in the other cases it contained acetic acid.

Type of lamp	Halogen	Incandescent
Average volumetric biomass productivity ($g \cdot L^{-1} \cdot h^{-1}$)	0.0276	0.0496
Standard error	$5.57 \cdot 10^{-4}$	$9.1 \cdot 10^{-4}$

Table 3: volumetric biomass productivity for both types of lamps. Acetic acid as a carbon source.

Calculated volumetric biomass productivity values for each type of lamp are shown in table 3.

B. Propionic acid tests

In order to observe the influence of the carbon and electron source on the growth rate when different kind of lamps are used several tests were carried out using propionic acid as carbon source and electron donor. In this case two tests have been performed. In these experiments, as in the case of acetic acid tests, the temperature and the light intensity have been kept constant along time as is explained in "Materials and Methods". The pH value presents a small fluctuation along the tests, without an appreciable effect on the kinetics traits. In both cases the volume of innoculum used was about 400 mL, as in the acetic acid tests.

The data obtained from these experiments are collected in Appendix 6 and are represented in the following figures.

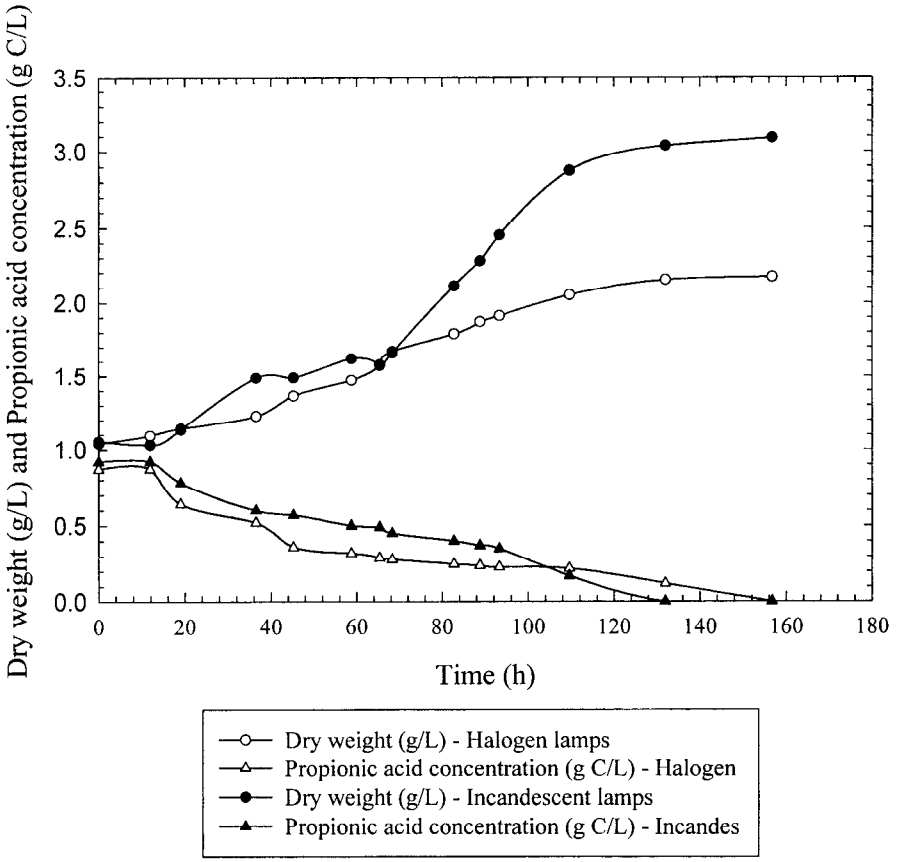

Figure 8: Test 4. Growth of *R.rubrum* cells in batch experiments with propionic acid as carbon source and different kind of lamps, halogen and incandescent.

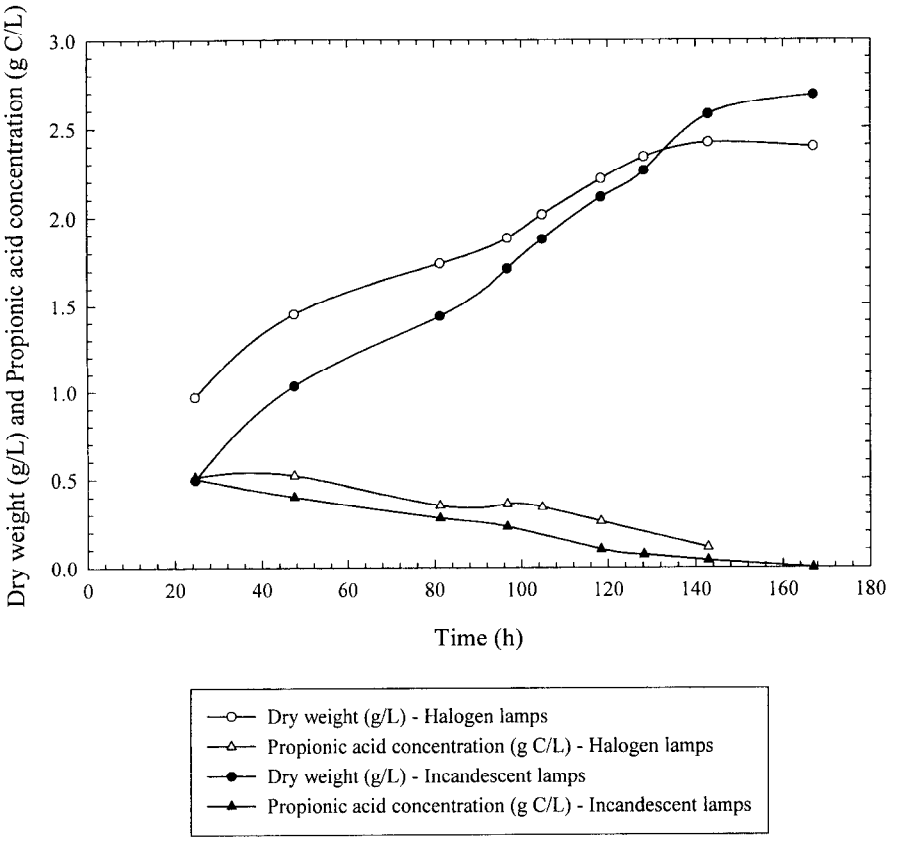

Figure 9: Test 5. Propionic acid

N° test	Lamp type	Increase Dry weight (g/L)	Initial Acetic acid conc.(g C/L)	Global yield (g D.W./g C)	Time (h)	Final pH value
4	Halogen	1.12	0.87	1.29	130	7.62
	Incandesc.	2.04	0.92	2.22	140	7.80
5	Halogen	1.42	0.51	2.78	90	7.68
	Incandesc.	2.19	0.50	4.38	150	7.73

Table 4: Global yield results

Evaluation of the volumetric biomass productivity

In figure 10 the experimental results with the halogen lamps have been represented. Comparison of the results is done only in the linear phases of growth were growth rate is mainly governed by light limitation and were any acceleration or adaptation phases have ended. The same treatment is done with the incandescent lamp results, in figure 11.

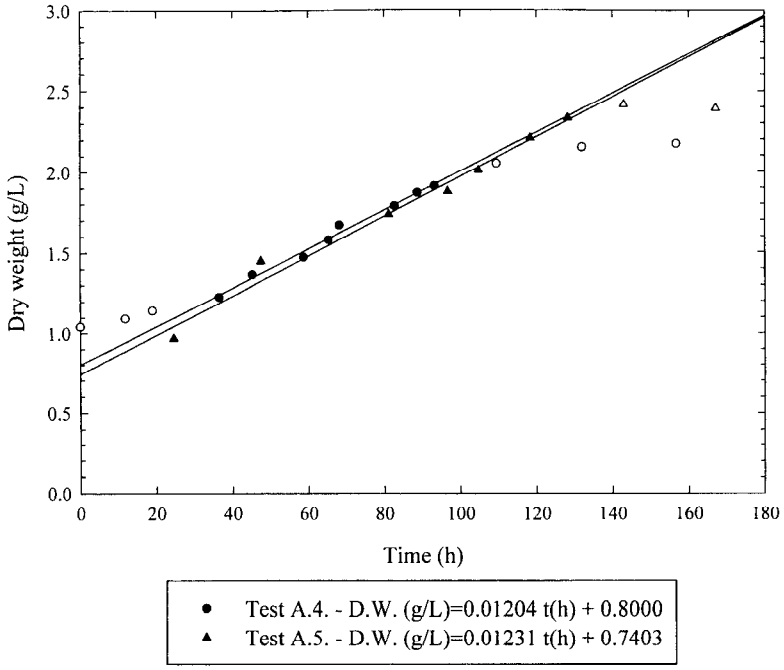

Figure 10: Halogen lamp tests. Carbon source: propionic acid. Filled symbols: data used for linear regression

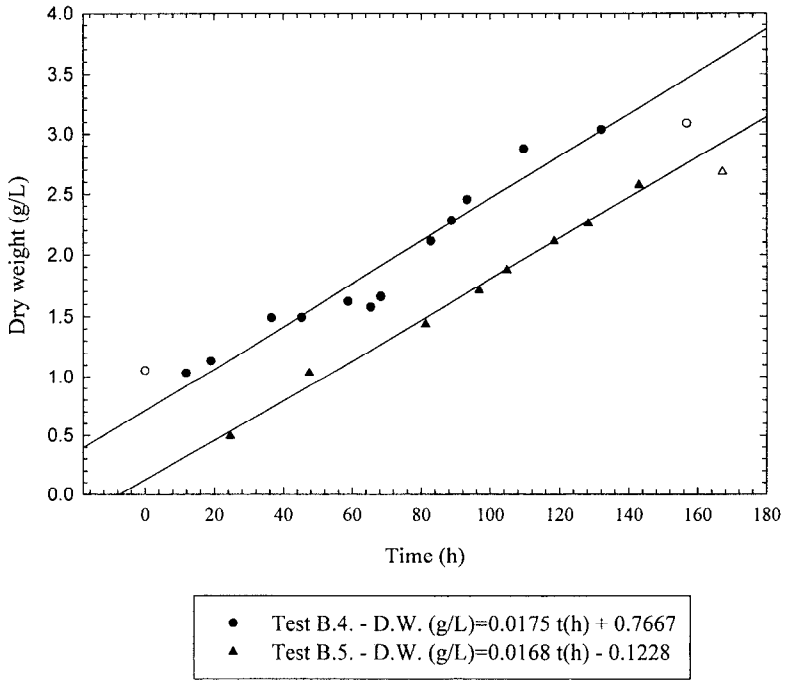

Figure 11: Incandescent lamp tests. Carbon source: propionic acid. Filled symbols: data used for linear regression

In figures 10 and 11, as in the acetic acid tests, it can be observed that the volumetric biomass productivity has a small experimental error for each type of lamps. In both figures only exponential phase points have been taking into account and lag phase and steady phase points have been discarded. In general, the tests present a low standard deviation except for test B.4., which despite the fact that has an experimental error higher than in the other tests, the average slope in very close to the one of the test B.5.

Calculated average volumetric biomass productivity values for each kind of lamp are shown in table 5.

Type of lamp	Halogen	Incandescent
Average volumetric biomass productivity ($g \cdot L^{-1} \cdot h^{-1}$)	0.0122	0.0172
Standard error	$1.35 \cdot 10^{-4}$	$3.50 \cdot 10^{-4}$

Table 5: volumetric biomass productivity for both types of lamps. Propionic acid as a carbon source.

The experimental results obtained with acetic acid and propionic acid show that the cells are able to grow at a higher growth rate under incandescent light sources, at the same illumination level measured by the Licor sensor (measurement range 400-700nm). This fact can be explained taking into account the fact that the incandescent lamps emit also radiation in the range between 700 and 950 nm, which is barely present in the halogen lamps, just at the wavelengths where it can absorbed by the chlorophylls. It can be concluded then that the cells can take advantage of this fraction of radiation, not available in the case of the halogen lamps.

On the other hand, the total amount of energy emitted by the incandescent lamps in the range 350-950 nm is higher than the one emitted by the halogen lamps under the 350-950 nm range. Given the fact that these cells can use the light energy available in the range of 700-950 nm, it appears therefore a logical consequence the fact that, under incandescent light illumination, the cells grow at a higher growth rate.

In order to evaluate if the culture is able to grow faster under incandescent ilumination as a result of the fact that the total amount of energy emitted between 350 and 950 nm is higher or because the *R. Rubrum* cells present a higher efficiency growing under this kind of radiation it was proposed to perform a test where the total amount of radiation emited between 350 and 950 nm was the same in both kind of lamps.

So, the light intensity of the incandescent lamps in the range 350-950 nm was readjusted to 13.91 ± 0.34 W/m^2 and the light intensity of the halogen set-up was maintained at 13.98 ± 0.25 W/m^2. The carbon and electron source choosen was acetic acid due to the fact that the growth rate is higher and *R. Rubrum* cells grow very well in this medium.

Data obtained in test 6 are depicted in figure 12.

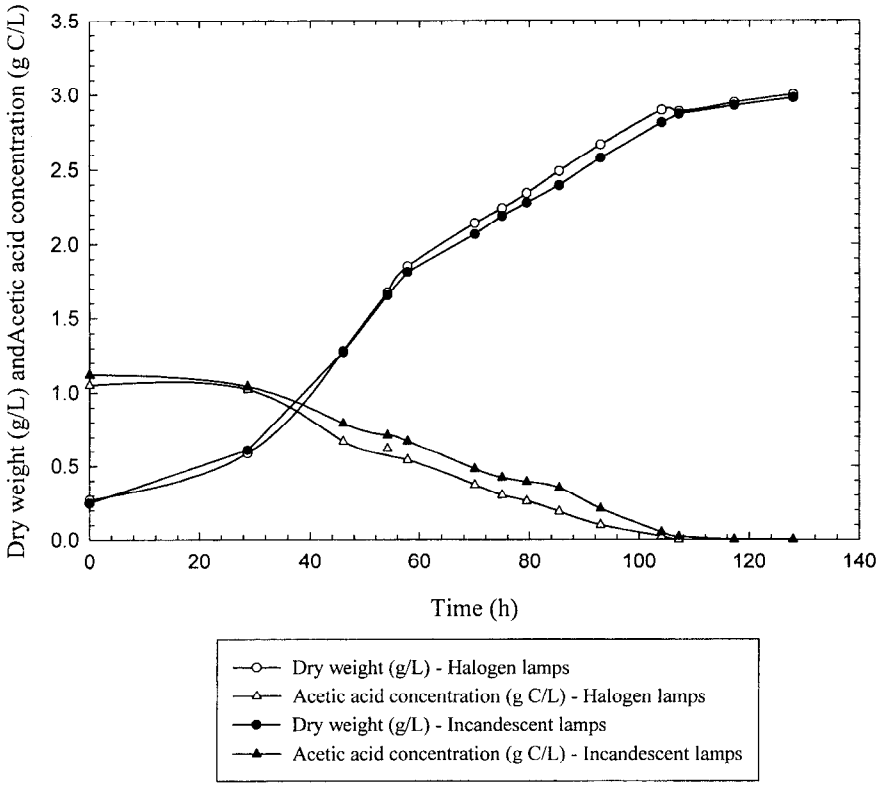

Figure 12: Test 6. Experiment with the same total amount of energy emitted in the range 350-950 nm in both kind of lamps.

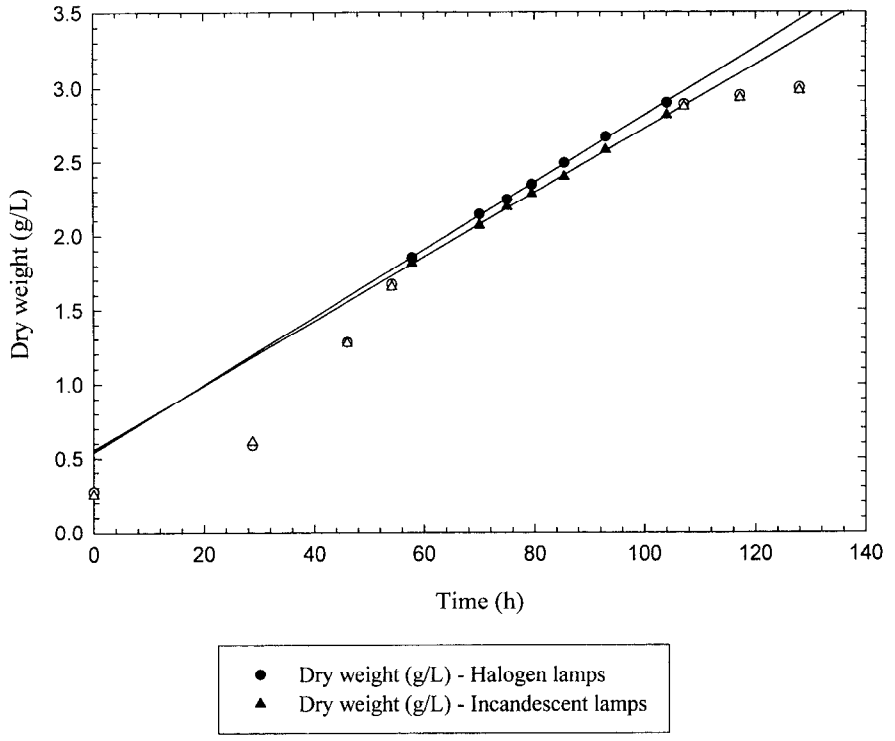

Figure 13: Evaluation of the average volumetric biomass productivity under light limitation for both type of lamps. Filled symbols: data used for linear regression.

Type of lamp	Halogen	Incandescent
Light intensity in the range 350-950 nm (W/m^2)	13.98 ± 0.25	13.91 ± 0.34
Average volumetric biomass productivity ($g \cdot L^{-1} \cdot h^{-1}$)	0.023	0.022

Table 6: volumetric biomass productivity for both types of lamps. Acetic acid as the carbon source.

N° test	Lamp type	Increase Dry weight (g/L)	Initial Acetic acid conc.(g C/L)	Global yield (g D.W./g C)	Time (h)
6	Halogen	2.73	1.05	0.41	105
	Incandesc.	2.73	1.12	0.39	105

Table 7: Global yield results

In figure 13 and in table 6 it can be observed the fact that the *R. rubrum* cells are able to grow at the same growth rate either halogen or incandescent lamps because the differences between halogen and incandescent volumetric biomass productivity are smaller than the experimental error. Therefore, although it has been described a difference in efficiency of the light collected at 520 nm or 880 nm, it appears that the cells can adapt themselves to else light from both sources with a maxima efficiency probably by modification of their composition in photosyntethic pigments of each kind. An indication of this effect could be the fact that the calibration courbes of absorbance versus dry weight are different for each type of lamp. As a consequence, in practice terms, the global eficiency of light energy utilization by the cells under the illumination of both kind of lamps are very close. It is interesting to take into account the fact that the volumetric biomass productivity obtained in this test is very close to the one attained in experiments 1, 2 and 3.

IV. CONCLUSIONS

Incandescent lamps have been tested for the growing of *R. rubrum* and with the obtained results it can be concluded that this kind of light source is very appropriate for its use in compartment II. The growth rate obtained using incandescent lamps has been notably larger than using halogen lamps when the energy emitted between 400 and 700 nm is the same. Also, the lag phase duration has diminished and the global yield has increased.

On the other hand, when lamps are regulated so as to emit the same amount of energy between 350-950 nm, no significance difference was observed.

On the other side, several factors such as the lamp energetic efficiency, the far infrared emission, the heat generated, the degree of penetration of the light into the reactor, etc. have to be considerate for the use of incandescent lamps in the pilot plant reactor.

HYDROGEN CONSUMPTION TESTS PROPOSAL

I. INTRODUCTION

The purpose of the second compartment is the metabolisation of the products of the degradation activities achieved in the first compartment. The main components at the outlet of the first compartment are expected to be fatty acids (acetic, propionic, butyric, isobutyric, valeric, isovaleric, caproic, isocaproic), some alcohols, aminoacids, amines, hydrogen, carbon dioxide, hydrogen sulphide, etc. To process these products into biomass, in anaerobic conditions, anoxygenic phototrophic bacteria were chosen.

Purple non-sulphur bacteria are capable of photoheterotrophic growth on a mixture of organic carbon sources and some have also been reported as being capable to grow photoautotrophically with H_2 as an electron donor and CO_2 reduction (Yoch, 1978). It was therefore suggested to split compartment II into IIa and IIb, both colonised with the same type of bacteria. Compartment IIa would be for the photoheterotrophic growth and its task is to handle the soluble effluent. Compartment IIb would be devoted to the autotrophic growth on the gas phase of compartment I and a minimum of soluble effluent from compartment IIa. This division (splitting) in principle is necessary because H_2 consumption is inhibited by organic compounds.

On the other hand it would be interesting the possibility to combine subcompartment IIa and IIb if the *Rhodospirillum rubrum* cells were capable of consuming the volatile fatty acids at the same time than fixing the CO_2 with the H_2 as the electron donor source under certain conditions. Therefore, in the present TN a study of the feasibility to combine subcompartment IIa and IIb is studied.

II. EXPERIMENTAL SET-UP

From the safety point of view, experimental tests using H_2 have to be carried out following safety measures due to the risk of explosion. This fact will restrict the use of standard materials and equipment and the performance of the tests.

On the other hand, taking into account the grade of the precision of the results that have to be provided there is a limited number of experimental set-up possible. In the present TN two different possibilities are studied.

A. Continuous culture experiments

From the point of view of the quality of information to obtain from an experiment and for symilarity with the operational conditions to be used in the MELISSA loop, the best option would be to perform the cultures in a continuous stirred tank reactor (CSTR). In this case, the experiment would consist in a continuous culture in heterothophic conditions under carbon source limitation, using a volatile fatty acid as carbon source such as acetic acid. Once the steady state would be reached, a mixture of hydrogen and carbon dioxide, similar to the one already used in other autotrophic experiments with photosynthetic bacteria (80% hydrogen, 20% CO_2), would be fed into the reactor. If exists an increase in the dry weight steady state value, this must be due to the capability of the cells to fix CO_2 using H_2 as the electron donor. An alternative possibility is only feeding with H_2, but in this case the increase in dry weight value, would depend on the CO_2 produced from the volatile fatty acid being consumed. This is either small or is instead consumed, depending on the reductance degree of the carbon source used. Measurements of the outlet liquid and the outlet gas will show the level of metabolisation of the volatile fatty acid and the concomitant hydrogen consumption. The hydrogen partial pressure in the gas phase should be, in this case, determined.

However, this alternative has several disadvantages. On one hand, conventional equipment (reactor and auxiliary instrumentation) can not be used in this tests, due to the explosive risk of the H_2. It has to be used in a pressurised glass vessel (in order to be able to provide the light to the cells), in the same way as it is done in hydrogenation reactions. Using the same type of reactor peripherals like proven spark free lamps and stainless pipes and connections. On the other hand, the H_2 measurement in the output gas is not easily done and the hydrogen partial pressure monitorization is complex.

Hydrogen calibrated flowmeters and two pumps are also required, all of them leak tested for hydrogen.

Moreover, this experimental set-up has to be installed in a well aerated zone (even outside the laboratory) or in a spark free laboratory, due to the risk of existence of H_2 leaks. However, the risk of a possible deflagration is not discarded because the vessel is surrounded by the halogen lamps, therefore an ignition source is always available.

B. Batch experiments

Another more simple alternative, that could be a good compromise between the results obtained and safety requirements, is to carry out the experiments in flask bottles. This choice is cheaper than the previous strategy and more feasible, at least for evaluation experiment.

This alternative could be carried out in several flask bottles. A half of them would contain the standard media as a reference, using the volatile fatty acid as a carbon and electron source. The second half would contain a certain partial pressure of H_2 (or 80% hydrogen, 20% CO_2) in addition to the culture media used in the reference bottles. All the bottles would have to be located at the same distance of a light source, having thus the same light intensity. During the experiment, one bottle among reference ones and another one from the H_2 containing ones, would be taken at different time intervals, so as to follow the evolution of the culture. This would avoid perturbations in the culture due to sampling such as a modification in the hydrogen partial pressure when the sample (liquid and gas phases) would be extracted. Therefore, the number of required flasks is the same as the number of necessary samples. At those time intervals, the change in H_2 partial pressure and the biomass and volatile fatty acid concentrations would be measured. If the *Rhodospirillum rubrum* cells are capable of consuming the hydrogen and the volatile fatty acid at the same time, in the flasks that would contain hydrogen and CO_2 an increase of biomass yield related to the reference bottles should be expected, for an equal degree of consumption of the carbon source. That is, the increase should be seen along the whole culture and not as a final dry weight increase once the volatile fatty acid is exhausted. This would allow to differentiate among the two electron sources being consumed simultaneously or sequentially.

In this batch culture alternative a special flask bottle is required due to the fact that it has to be hydrogen leak tested and also has to maintain the axenic conditions through all the process of inoculation and gas sparging. This flask should be designed specially for this task and a first attempt is presented in figure 10. The material of the tubing and the valves it would be stainless steel (AISI-314).

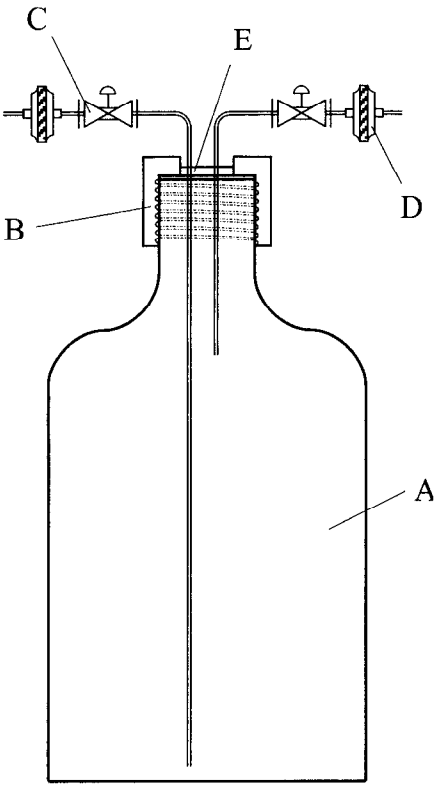

Figure 8: Flask bottle. A: Standard bottle. B: Screw-top. C: Ball valve. D: 0.22 μm filter. E: Special joint (hydrogen leak tested)

Clearly this second alternative is simpler than the continuous culture, because there is no H_2 in the culture media flow and no control and instrumentation is required. Actually, if light intensity is not high, the temperature could be maintained by air convection around the bottles and the pH could be maintained by means of a buffer solution. Addequate mixing could be achieved by using a multiple magnetic stirrer.

Moreover, the problem of the hydrogen partial pressure measurement in the outlet gas is avoided because it is only necessary to measure the pressure at the end of the life of each flask. However this fact involves the use of a high number of flasks.

III. REFERENCES

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- TN 37.81: LENGUAZA B. *Scientific tests for R. rubrum. Growth on different C sources. Part I.* MELISSA Contract 11549/95/NL/FG

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APPENDIX 1: THE CULTURE MEDIA COMPOSITION

- ***Acetic acid media***

Reactant	Concentration
CH_3COOH	2.502 g/L
NH_4Cl	0.761 g/L
Na_2SO_4	0.538 g/L
EDTA-Na·2 H_2O	0.020 g/L
$MnCl_2$·4 H_2O	0.010 g/L
$FeSO_4$ · 7 H_2O	0.020 g/L
KH_2PO_4	0.200 g/L
K_2HPO_4	0.300 g/L
MOPS	21.000 g /L
$NaHCO_3$	0.250 g/L
$MgSO_4$·7 H_2O	0.200 g/L
$CaCl_2$·2H_2O	0.050 g/L
Trace elements *	1.00 mL/L
Biotin *	1.00 mL/L

- ***Propionic acid media***

Reactant	Concentration
CH_3CH_2COOH	2.058 g/L
NH_4Cl	0.761 g/L
Na_2SO_4	0.538 g/L
EDTA-Na·2 H_2O	0.020 g/L
$MnCl_2$·4 H_2O	0.010 g/L
$FeSO_4$ · 7 H_2O	0.020 g/L
KH_2PO_4	0.200 g/L
K_2HPO_4	0.300 g/L
MOPS	21.000 g/L
$NaHCO_3$	0.670 g/L
$MgSO_4$·7 H_2O	0.200 g/L
$CaCl_2$·2H_2O	0.050 g/L
Trace elements *	1.00 mL/L
Biotin *	1.00 mL/L

- **** Trace elements***

Reactant	Quantity
Fe citrate	0.3 g
$MnSO_4 \cdot H_2O$	0.002 g
H_3BO_3	0.001 g
$CuSO_4 \cdot 5H_2O$	0.001 g
$(NH_4)_6Mo_7O_{27} \cdot 4H_2O$	0.002 g
$ZnSO_4$	0.001 g
EDTA-Na	0.05 g
$CaCl_2 \cdot 2\ H_2O$	0.02 g
Distilled water	100 mL

- **** Biotin***

Reactant	Quantity
Biotin	0.015 g
Distilled water	1 L

APPENDIX 2: CARBON DETERMINATION

This protocol can be used for the analysis of either carbohydrates, volatile fatty acids (ex. acetic, propionic, butyric, isobutyric, valeric) and ethanol.

The range of concentrations that can be determined depends of the compound analysed. For the compounds being analysed in the present case they are 0-10 g/L for the acetic acid and 0-4 g/l for the propionic acid. For the analysis, 20 μL of sample are used. Samples have to be analysed three times.

Instrumentation

- Liquid chromatograph Hewlett Packard 1050
- Refraction index detector Hewlett Packard 1047A
- Integration software Millenium 2.15.10

Mobile phase

- Sulphuric acid (0.015 M) prepared with Milli-Q water (resistance 18.2 Ω), adjusted at pH=3.00 with diluted acetic acid and filtered through a 0.45 μm membrane.

Analysis physical conditions

- Column: ionic exchange Aminex HPX-87H from Bio Rad
- Flow rate: 0.6 mL/min, isocratic
- Injection volume: 20 μL
- Analysis time: approximately 30 minutes
- Temperature: 25 ºC

Sample preparation:

- Centrifuge (12000 g, 10 min, 8 ºC)
- Filter (0.22 μm membrane)

Calibration

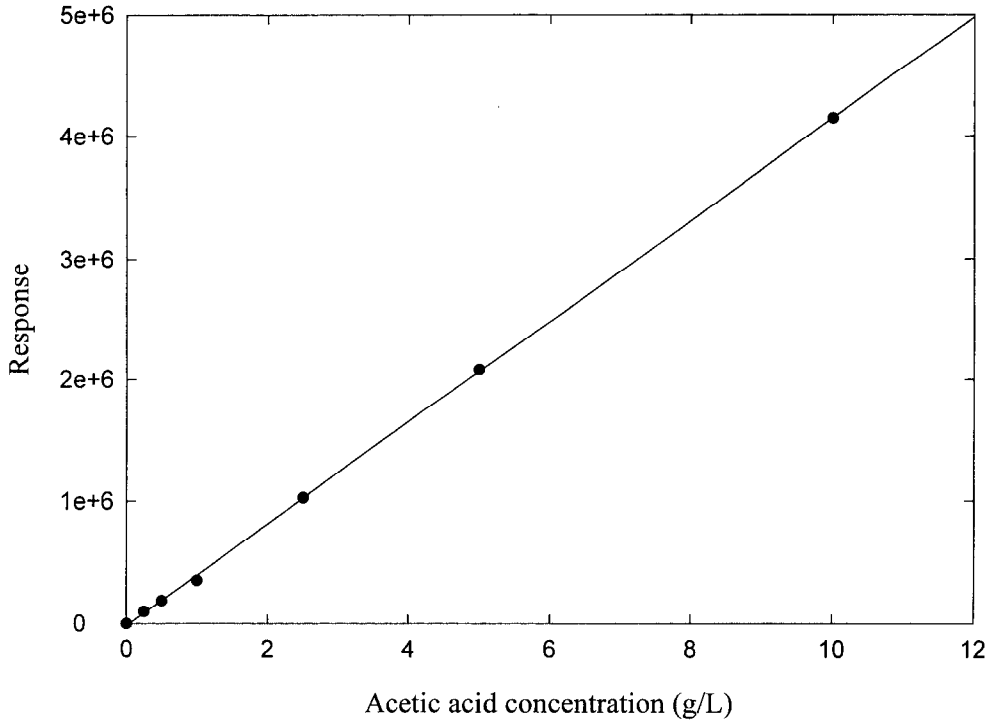


Figure 9: Acetic acid calibration

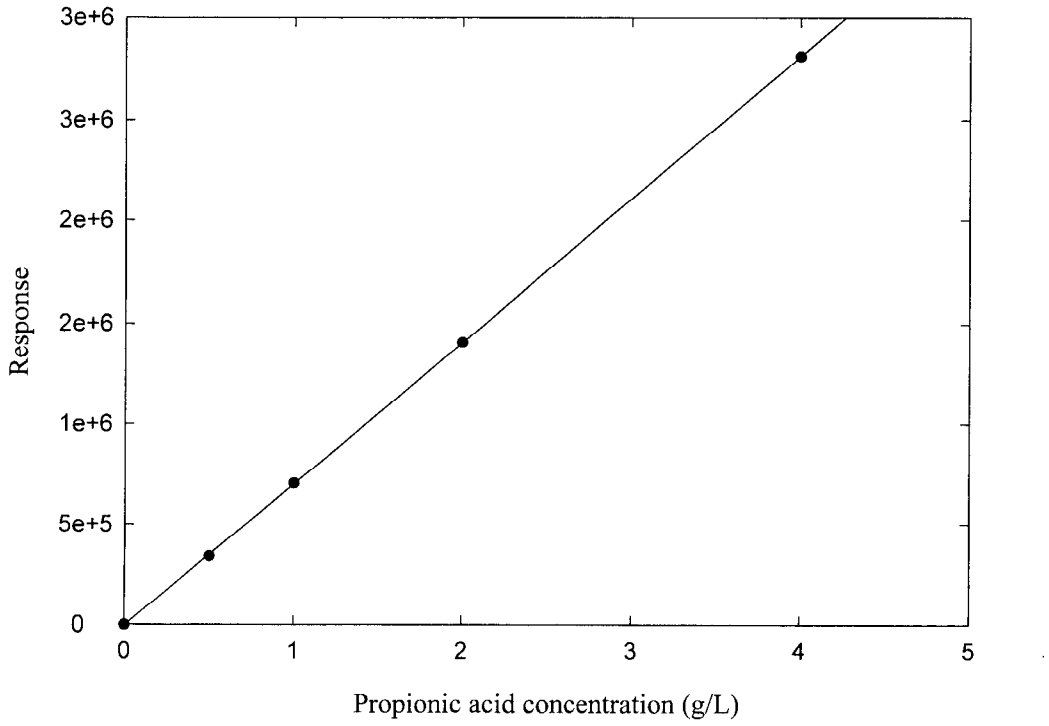


Figure 10: Propionic acid calibration

APPENDIX 3: LIGHT INTENSITY DISTRIBUTION

Halogen lamps

	-5	-4	-3	-2	-1	0	1	2	3	4	5
10	27.86	29.92	31.99	30.96	33.02	34.05	33.02	34.05	34.05	34.05	31.99
9	30.96	34.05	34.05	35.08	35.08	37.15	36.11	37.15	37.15	37.15	36.11
8	33.02	36.11	37.15	37.15	38.18	39.21	39.21	39.21	40.24	40.24	38.18
7	33.02	37.15	38.18	39.21	40.24	41.27	41.27	41.27	42.31	41.27	39.21
6	36.11	39.21	41.27	42.31	44.37	44.37	44.37	44.37	45.4	43.34	41.27
5	38.18	42.31	44.37	45.4	47.46	47.46	47.46	47.46	47.46	46.43	44.37
4	40.24	44.37	47.46	48.5	49.53	49.53	49.53	49.53	49.53	47.46	46.43
3	41.27	45.4	48.5	48.5	49.53	51.59	50.56	52.62	52.62	51.59	48.5
2	41.27	46.43	48.5	49.53	51.59	51.59	51.59	50.56	50.56	49.53	46.43
1	41.27	44.37	47.46	48.5	50.56	51.59	50.56	50.56	50.56	48.5	46.43
-3	22.7	23.73	24.76	26.83	26.83	27.86	27.86	27.86	26.83	26.83	25.8
-4	28.89	31.99	36.11	36.11	39.21	40.24	41.27	41.27	40.24	40.24	37.15
-5	35.08	37.15	40.24	42.31	44.37	44.37	44.37	44.37	42.31	41.27	40.24
-6	31.99	34.05	38.18	39.21	40.24	41.27	41.27	41.27	39.21	38.18	36.11
-7	30.96	31.99	33.02	35.08	35.08	36.11	37.15	37.15	36.11	34.05	31.99
-8	26.83	27.86	28.89	29.92	29.92	33.02	30.96	33.02	33.02	30.96	29.92
-9	26.83	28.89	30.96	33.02	33.02	35.08	35.08	33.02	31.99	30.96	29.92

Table 5: Light intensity (W/m^2) measured at the surface of the roux flask

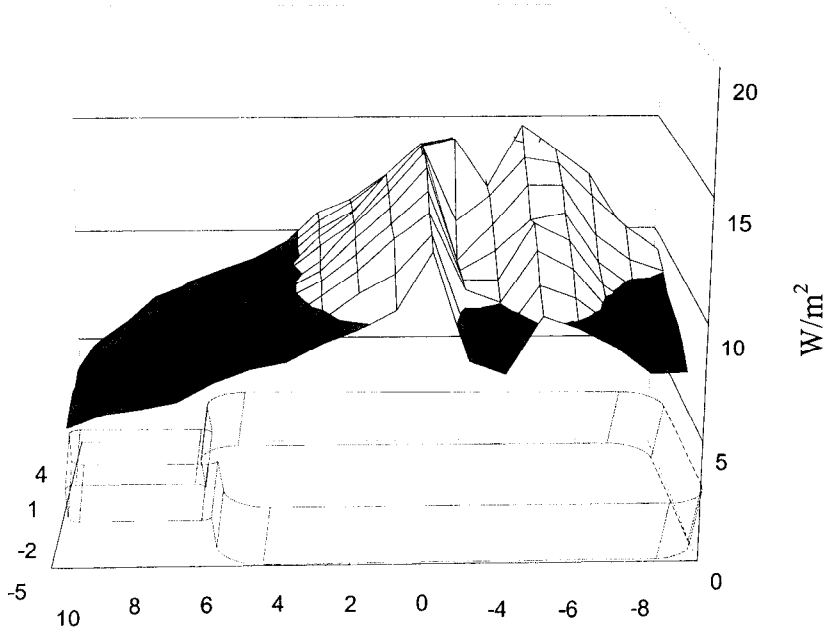


Figure 11: Light intensity distribution along the roux flask

Incandescent lamps

	-5	-4	-3	-2	-1	0	1	2	3	4	5
10	27.86	29.92	31.99	30.96	33.02	34.05	33.02	34.05	34.05	34.05	31.99
9	30.96	34.05	34.05	35.08	35.08	37.15	36.11	37.15	37.15	37.15	36.11
8	33.02	36.11	37.15	37.15	38.18	39.21	39.21	39.21	40.24	40.24	38.18
7	33.02	37.15	38.18	39.21	40.24	41.27	41.27	41.27	42.31	41.27	39.21
6	36.11	39.21	41.27	42.31	44.37	44.37	44.37	44.37	45.4	43.34	41.27
5	38.18	42.31	44.37	45.4	47.46	47.46	47.46	47.46	47.46	46.43	44.37
4	40.24	44.37	47.46	48.5	49.53	49.53	49.53	49.53	49.53	47.46	46.43
3	41.27	45.4	48.5	48.5	49.53	51.59	50.56	52.62	52.62	51.59	48.5
2	41.27	46.43	48.5	49.53	51.59	51.59	51.59	50.56	50.56	49.53	46.43
1	41.27	44.37	47.46	48.5	50.56	51.59	50.56	50.56	50.56	48.5	46.43
-3	22.7	23.73	24.76	26.83	26.83	27.86	27.86	27.86	26.83	26.83	25.8
-4	28.89	31.99	36.11	36.11	39.21	40.24	41.27	41.27	40.24	40.24	37.15
-5	35.08	37.15	40.24	42.31	44.37	44.37	44.37	44.37	42.31	41.27	40.24
-6	31.99	34.05	38.18	39.21	40.24	41.27	41.27	41.27	39.21	38.18	36.11
-7	30.96	31.99	33.02	35.08	35.08	36.11	37.15	37.15	36.11	34.05	31.99
-8	26.83	27.86	28.89	29.92	29.92	33.02	30.96	33.02	33.02	30.96	29.92
-9	26.83	28.89	30.96	33.02	33.02	35.08	35.08	33.02	31.99	30.96	29.92

Table 6: Light intensity (W/m^2) measured at the surface of the roux flask

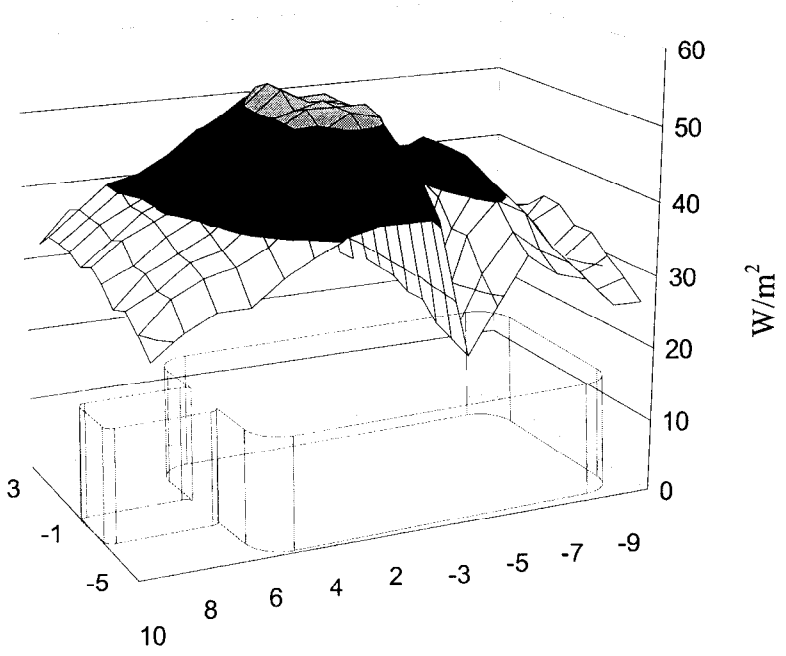


Figure 12: Light intensity distribution along the roux flask

APPENDIX 4: INTEGRATION OF THE LAMP SPECTRAL DATA

Conversion of quantum sensor output in $\mu mol \cdot s^{-1} \cdot m^{-2}$ (400-700 nm) to radiometric units in $W \cdot m^{-2}$ (400-700 nm) is complicated. The conversion factor will be different for each light source, and the spectral distribution curve of the radiant output of the source (W_λ; $W \cdot m^{-2} \cdot nm^{-1}$) (figure 13) must be known in order to make the conversion. The accurate measurement of W_λ is a difficult task, which should not be attempted without adequate equipment and calibration facilities. The radiometric quantity desired is the integral of W_λ over the 400-700 nm range, or:

$$W_T = \int_{400}^{700} W_\lambda \, d\lambda \qquad (1)$$

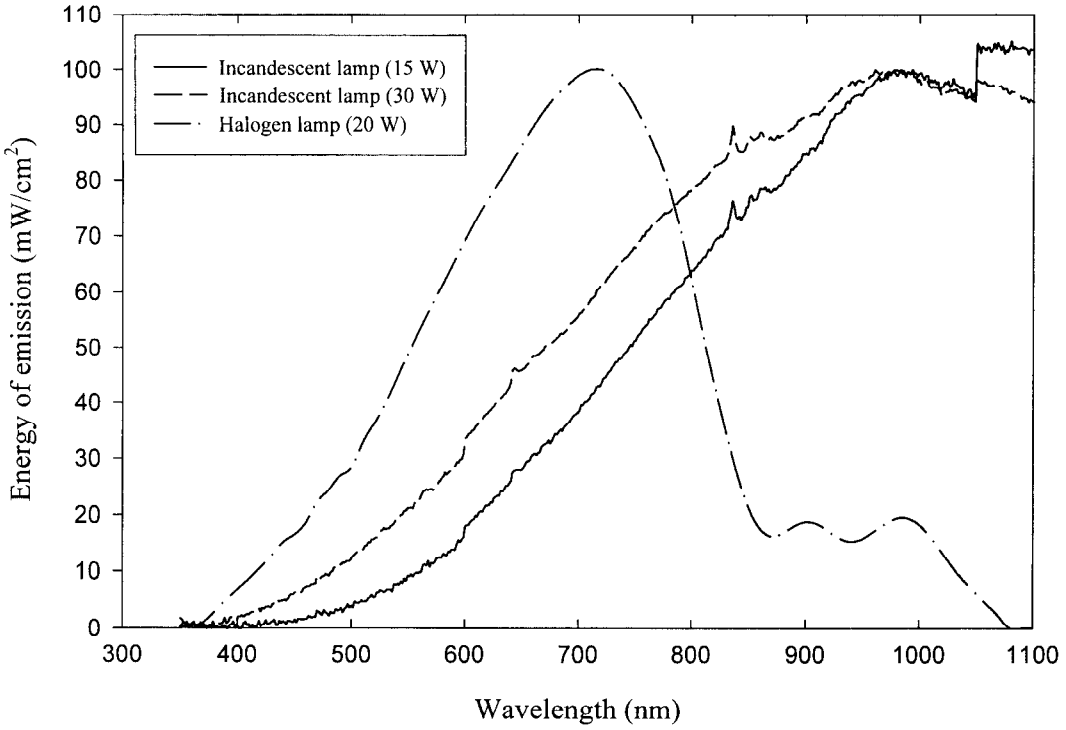


Figure 13: Spectral distribution curve of the radiant output of the source. It has been measured for three different lamps: halogen, incandescent 15W and incandescent 30W.

At a given wavelength λ, the number of photons per second is

$$\text{photons } s^{-1} = \frac{W_\lambda}{hc/\lambda} \qquad (2)$$

where $h=6.63\cdot10^{-34}$ J·s (Plank's constant), $c=3.00\cdot10^{8}$ m·s^{-1} (velocity of light) and λ is in nm. hc/λ is the energy of one photon. Then, the total number of photons per second in the 400-700 nm is:

$$\int_{400}^{700} \frac{W_\lambda}{hc/\lambda} d\lambda \qquad (3)$$

This is the integral that is measured by the sensor. If R is the reading of the quantum sensor in μmol·s^{-1}·m^{-2} (1 μmol·s^{-1}·m^{-2} = $6.022\cdot10^{17}$ photons s^{-1} m^{-2}), then

$$6.022\cdot10^{17}\cdot(R)=\int_{400}^{700} \frac{W_\lambda}{hc/\lambda} d\lambda \qquad (4)$$

Combining eq. (1) and eq (4) gives

$$W_T = 6.022\cdot10^{17}\,(Rhc)\frac{\int_{400}^{700} W_\lambda\, d\lambda}{\int_{400}^{700} \lambda\, W_\lambda\, d\lambda} \qquad (5)$$

To achieve the two integrals, discrete summations are necessary. Also, since W_λ appears in both the numerator and the denominator, the normalised curve N_λ may be substituted for it. Then

$$W_T = 6.022\cdot10^{17}\,(Rhc)\frac{\sum_i N_{\lambda,i}\,\Delta\lambda}{\sum_i \lambda_i N_{\lambda,i}\Delta\lambda} \qquad (6)$$

where $\Delta\lambda$ is any desired wavelength interval, λ_i is the centre wavelength of the interval and N_λ is the normalised radiant output of the source at the centre wavelength. In final form this becomes

$$W_T(400-700\,\text{nm}) \approx 119.8R\,\frac{\sum_i N_{\lambda,i}}{\sum_i \lambda_i N_{\lambda,i}} \qquad (7)$$

where R is the reading in μmol·s^{-1}·m^{-2}.

Conversion of the lamp emitted energy between (400-700 nm) range to (350-950 nm) is made using eq. (8)

$$W_T(350-950\,nm) = \frac{W_T(400-700\,nm)\cdot I(350-950\,nm)}{I(400-700\,nm)} \qquad (8)$$

where I is the value of the area under the spectral distribution curve between the intervals 400 and 700 nm or 350 and 950 nm.

Therefore,

$$W_T(350-950\,nm) \approx 119.8\,R\,\frac{\sum_i N_{\lambda,i}\cdot I(350-950\,nm)}{\sum_i \lambda_i N_{\lambda,i}\cdot I(400-700\,nm)} \qquad (9)$$

Taking into account the spectral distribution curve of both kinds of lamps and doing the appropriate calculations the conversion parameters can be calculated.

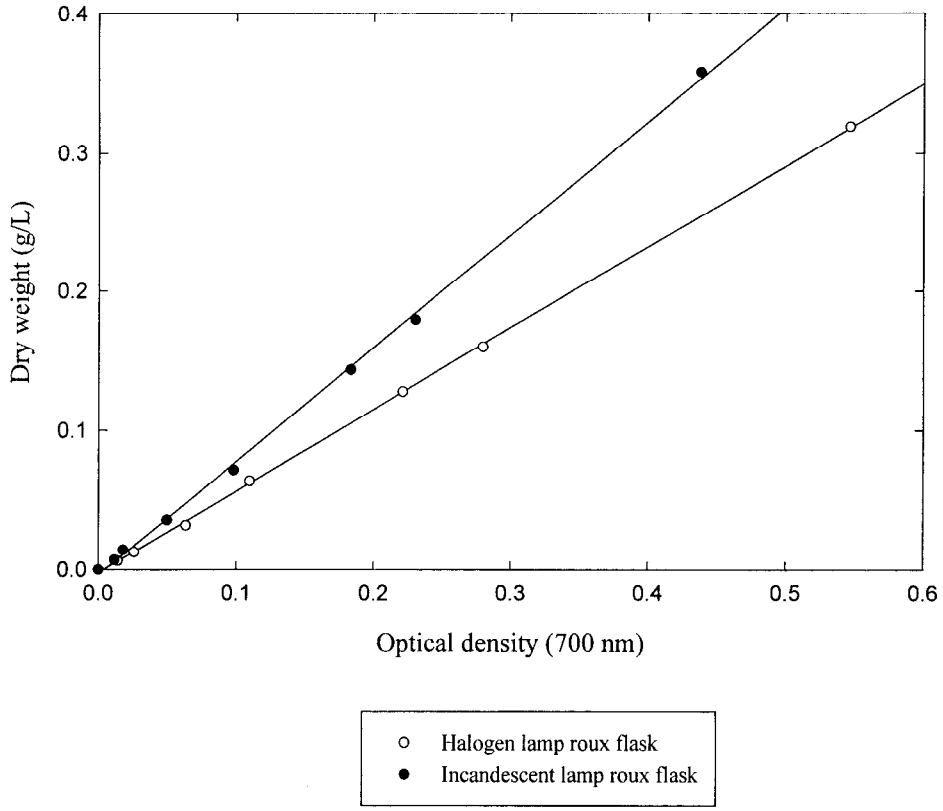
Halogen lamps	Incandescent lamps	
0.368	1.032	$\frac{W/m^2\ (350-950\,nm)}{\mu mol\cdot m^{-2}\cdot s^{-1}\ (400-700\,nm)}$

Table 7: Conversion parameters from $\mu mol\cdot m^{-2}\cdot s^{-1}$ to $W\cdot m^{-2}$ units

APPENDIX 5: BIOMASS DRY WEIGHT CALIBRATION

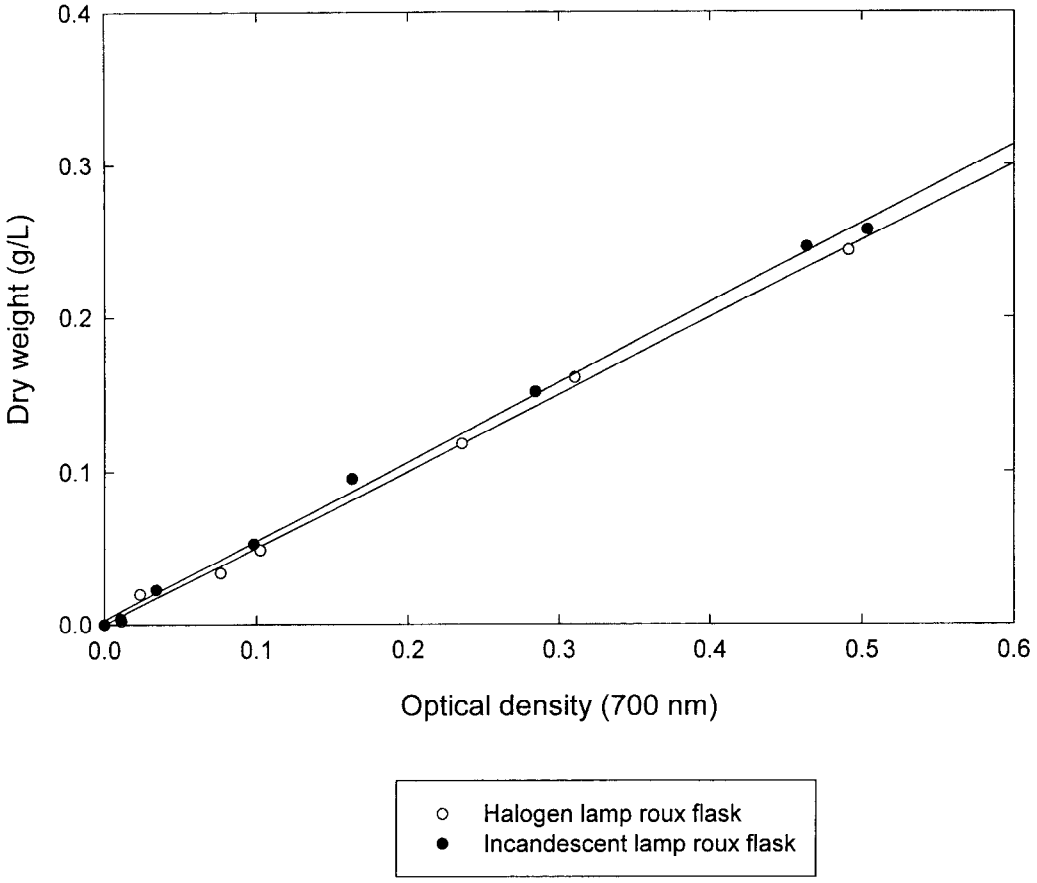
With the purpose of know the dry weight from the optical density measurement a calibration between absorbance and dry weight have been done. Dry weight was measured using biomass from the both cultures filtered using Millipore 0.22 μm filters and dried at 100 ºC until constant weight.

- **Acetic acid tests**



- Halogen lamps: D.W. (g/L)= $-3.6597 \cdot 10^{-3} + 0.8152 \cdot$ O.D. (700 nm)
- Incandescent lamps: D.W. (g/L)= $-2.1277 \cdot 10^{-3} + 0.5859 \cdot$ O.D. (700 nm)

- **Propionic acid tests**



- Halogen lamps: D.W. (g/L)= 0.0001 + 0.5005 · O.D. (700 nm)
- Incandescent lamps: D.W. (g/L)= 0.0028 + 0.5173 · O.D. (700 nm)

APPENDIX 6: EXPERIMENTAL DATA

- Data obtained in test 1 (depicted in figure 3):

Time (h)	Halogen lamps		Incandescent lamps	
	Dry weight (g/L)	Acetic acid conc. (g C/L)	Dry weight (g/L)	Acetic acid conc. (g C/L)
0.00	0.1334	1.76	0.2198	1.57
15.50	0.1424	1.71	0.2190	1.50
36.50	0.1448	1.72	0.1823	1.49
47.00	0.1276	1.72	0.1909	1.47
64.67	0.1635	1.65	0.2661	1.48
72.92	0.1762	1.66	0.2525	1.41
87.58	0.1979	1.68	0.3497	1.42
93.70	0.2004	1.65	0.3475	1.41
96.67	0.2189	1.65	0.3713	1.40
107.33	0.2122	1.59	0.3905	1.36
111.67	0.2766	1.54	0.4373	1.34
121.50	0.2566	1.53	0.4656	1.24
130.83	0.2703	1.50	0.7715	1.22
133.95	0.2799	1.49	0.8095	1.20
136.08	0.3043	1.47	0.8851	1.18
138.75	0.3028	1.50	0.9290	1.16
141.58	0.3112	1.46	0.9984	1.16
144.53	0.3481	1.44	1.1686	1.11
155.17	0.5020	1.45	1.8119	0.96
158.07	0.5446	1.48	2.0209	0.90
167.42	0.6464	1.42	2.2381	0.81
169.75	0.6805	1.36	2.3218	0.79
179.75	0.7755	1.22	2.8230	0.64
184.25	0.8325	1.20	2.9072	0.44
189.67	0.8736	—	3.0790	0.29
192.67	0.8852	1.20	3.1762	0.24
204.20	1.1433	1.12	3.6109	0.24
210.50	1.3146	1.10	3.6855	0.22
216.08	1.4258	1.00	3.7319	0.18
229.75	1.7726	0.94	3.7449	0.12
236.25	1.9399	—	3.7474	0.08
238.83	2.0189	0.48	3.8029	0.07
254.80	2.4841	—	3.8594	—
259.87	2.6686	0.11	3.8756	0.04
262.58	2.7809	0.00	3.8736	0.00

Time (h)	Halogen lamps		Incandescent lamps	
	Dry weight (g/L)	Acetic acid conc. (g C/L)	Dry weight (g/L)	Acetic acid conc. (g C/L)
276.58	2.8591	0.00	3.7077	0.00
289.33	2.9458	0.00	3.7823	0.00
300.83	2.9600	0.00	3.6657	0.00
326.08	3.0085	0.00	3.6117	0.00

- Data obtained in test 2 (depicted in figure 4):

Time (h)	Halogen lamps		Incandescent lamps	
	Dry weight (g/L)	Acetic acid conc. (g C/L)	Dry weight (g/L)	Acetic acid conc. (g C/L)
0.00	0.1506	0.91	0.1881	0.93
39.08	0.1659	—	0.2034	—
61.58	0.7615	0.68	0.9553	0.73
65.67	0.7189	0.45	1.6487	0.60
68.42	0.6731	0.70	2.0741	0.51
72.42	0.7874	0.64	2.1395	0.44
74.67	0.8423	0.65	2.1896	0.42
85.75	1.3705	0.47	3.4497	0.37
90.00	1.5394	0.46	3.8330	0.21
93.42	1.5118	—	3.2354	—
95.42	1.9018	—	3.7268	—
111.67	2.0694	—	3.4607	—
111.67	2.0166	—	3.5234	—
120.08	2.8078	0.18	3.8695	0.00
141.25	2.5126	0.00	3.2501	0.00
161.33	2.5014	0.00	3.2475	0.00
189.00	2.4620	0.00	3.1785	0.00

- Data obtained in test 3 (depicted in figure 5):

Time (h)	Halogen lamps		Incandescent lamps	
	Dry weight (g/L)	Acetic acid conc. (g C/L)	Dry weight (g/L)	Acetic acid conc. (g C/L)
0.00	0.8725	0.65	1.3968	0.90
8.50	1.0903	—	1.4673	—
25.25	0.9592	0.88	1.4055	0.76
29.25	0.9912	0.83	1.3195	0.87
46.67	0.9832	0.86	1.6534	0.94
53.17	1.1751	0.85	1.7409	0.70
56.42	1.2184	—	2.0160	—
70.92	1.5387	0.70	2.9249	0.52
74.67	1.6615	0.74	3.0968	0.54
78.83	1.6960	0.74	3.3085	—
81.17	1.7150	—	3.2131	—
84.83	1.8869	—	3.5348	—
96.83	2.1384	0.63	4.0477	0.14
97.67	2.1923	0.53	4.0699	0.08
104.33	2.4432	0.27	4.2050	0.00
121.33	3.1199	0.19	3.9145	0.00
123.50	3.2240	0.17	3.9412	0.00
144.50	3.2365	0.00	3.9051	0.00

- Data obtained in test 4 (depicted in figure 8):

Time (h)	Halogen lamps		Incandescent lamps	
	Dry weight (g/L)	Propionic acid conc. (g C/L)	Dry weight (g/L)	Propionic acid conc. (g C/L)
0.00	1.0409	0.87	1.0532	0.92
11.83	1.0934	0.87	1.0325	0.92
19.00	1.1427	0.64	1.1340	0.78
36.50	1.2266	0.52	1.4887	0.60
45.25	1.3650	0.36	1.4907	0.57
58.75	1.4727	0.32	1.6205	0.50
65.33	1.5803	0.29	1.5742	0.49
68.25	1.6706	0.28	1.6594	0.45

Time (h)	Halogen lamps		Incandescent lamps	
	Dry weight (g/L)	Propionic acid conc. (g C/L)	Dry weight (g/L)	Propionic acid conc. (g C/L)
82.75	1.7890	0.25	2.1129	0.40
88.75	1.8713	0.24	2.2797	0.37
93.25	1.9133	0.23	2.4529	0.35
109.58	2.0523	0.22	2.8753	0.17
132.00	2.1524	0.12	3.0372	0.00
156.75	2.1721	0.00	3.0901	0.00

- Data obtained in test 5 (depicted in figure 9):

Time (h)	Halogen lamps		Incandescent lamps	
	Dry weight (g/L)	Propionic acid conc. (g C/L)	Dry weight (g/L)	Propionic acid conc. (g C/L)
24.58	0.9675	0.51	0.4938	0.50
47.50	1.4479	0.52	1.0323	0.40
81.25	1.7378	0.35	1.4339	0.28
96.75	1.8786	0.36	1.7077	0.23
104.83	2.0111	0.34	1.8720	—
118.42	2.2098	0.26	2.1093	0.10
128.25	2.3341	—	2.2554	0.07
142.92	2.4169	0.11	2.5748	0.04
167.08	2.3920	—	2.6843	0.00

- Data obtained in test 6 (depicted in figure 12):

Time (h)	Halogen lamps		Incandescent lamps	
	Dry weight (g/L)	Acetic acid conc. (g C/L)	Dry weight (g/L)	Acetic acid conc. (g C/L)
0.00	0.2689	1.05	0.2488	1.12
28.75	0.5857	1.02	0.6084	1.04
46.00	1.2767	0.67	1.2710	0.79
54.17	1.6693	—	1.6502	0.71
57.83	1.8482	0.54	1.8104	0.67
70.00	2.1379	0.37	2.0640	0.48
75.00	2.2382	0.30	2.1870	0.42

Time (h)	Halogen lamps		Incandescent lamps	
	Dry weight (g/L)	Acetic acid conc. (g C/L)	Dry weight (g/L)	Acetic acid conc. (g C/L)
79.50	2.3400	0.26	2.2760	0.39
85.42	2.4866	0.19	2.3917	0.35
93.00	2.6634	0.10	2.5739	0.21
104.08	2.8930	0.02	2.8090	0.05
107.17	2.8844	0.00	2.8659	0.02
117.25	2.9429	0.00	2.9240	0.00
128.00	2.9954	0.00	2.9736	0.00