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Bench scale loop tests using compartment I outlet medium

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Table of Contents

1 INTRODUCTION

The MELISSA project (Microbiological Ecological Life Support System Alternative) of the European Space Agency (ESA) is devoted to the development of a biological life support system to be used during Manned Space Missions. In order to achieve this purpose the project proposes the connection between five compartments, four of which contain microbial organisms and one higher plants and algae.

The liquefying compartment, or compartment I, is responsible for the biodegradation of human faecal material and other wastes generated by the crew. The volatile acids, ammonium, gases and soluble components produced during the fermentation are fed into the second compartment.

The anoxygenic phototrophic compartment II metabolises some of the compounds produced in the anaerobic liquefying compartment, with edible biomass generation. To achieve this goal *Rhodospirillum rubrum* and *Rhodobacter capsulata* are cultured in an anaerobic environment, either in photoheterotrophic or in photoautotrophic conditions.

The objective of compartment III (nitrifying compartment) is to transform the ammonium ions present in the exit stream from compartment II into nitrate, the most appropriate nitrogen source assimilated by the cells cultured in compartment IV. It consists in a packed-bed reactor with cells of two bacterial strains (*Nitrosomonas europaea* and *Nitrobacter winogradskyi*) immobilized onto polystyrene beads (Biostyr).

Compartment IVa has as its main task the carbon dioxide removal and supply of oxygen for the crew respiration generating at the same time edible biomass as food supply. This compartment is currently implemented in airlift reactors where *Spirulina platensis* is cultivated. This cyanobacteria presents a high nutritional value and contains all the essential amino acids, besides cysteine, in the adequate concentrations according to the FAO proposed standards.

The higher plants compartment is the basic food supplier for the crew. This compartment is being preliminary designed and some studies are being done with the candidate crops.

To assure the satisfactory operation of the system, it is important to study the connection between these bioreactors not only at optimal conditions but also taking into account possible deviations in the behaviour of any of them.

Dynamic operation of a loop of bioreactors will result in transitory states, especially when the system is driven from one steady state to another one or due to an eventual malfunction. In these cases, incomplete consumption of substrates may result and the effect of those on the subsequent bioreactors must be well characterized in order to foresee their behaviour and ensure the proper operation of the loop.

Once this study has been successfully done in the connection of compartments II, III and IVa using acetic acid or a mixture of acetic, propionic and butyric acids as carbon source (Creus et al., 1999; Creus et al., 2001; Creus et al., 2002a, Creus et al., 2002b), a closer approach to the future operation of the whole connection of the loop had to be carried out.

The operation of the liquid connection of compartments I, II, III and IVa is presented in this work. Once the previous tests demonstrated that the connection of compartments II, III and IVa using artificial medium operates properly, the more complete loop liquid connection using real outlet medium from compartment I is done. If the complete loop of microbial bioreactors can be operated satisfactorily under these conditions, then the feasibility of the connection of the MELISSA loop at liquid phase level will be demonstrated.

2 SET-UP AND MATERIALS AND METHODS

In order to perform the next step in the liquid connection of the loop, compartments I, II, III and IVa, are required. Since at the moment of the realisation of this work compartment I is being developed by EPAS, one of the MELISSA partners in Gent (Belgium), and in order to have a highly realistic approach, the outlet medium from compartment I reactor, operated at Gent, was autoclaved and transported to Barcelona pilot plant, where it was used as feed medium to the rest of the loop. A brief description of each compartment and the different steps required in their connection are given in this section.

2.1 SET-UP

2.1.1 Compartment I

The feed composition of compartment I, for this study, has been a mixture of human faeces, non-edible biomass of plants and some *S.platensis* and *R.rubrum* biomass. This feed is analogous to the future MELISSA feed once all the compartments will be interconnected. The ratio of each compound added to compartment's I feed is the same as the ratio of each waste produced per person and day (Hermans and Demey, 2001).

A person, having a MELISSA-HPC diet produces approximately, depending on the chosen diet, per day: (Poughon, 1997)

- 486 g DW non-edible parts of higher plants
- 30 g DW faecal material
- 25 g *Spirulina platensis*
- 100 g *Rhodospirillum rubrum*

The demonstration reactor, operated at the University of Gent/EPAS (Hermans and Demey, 2001), has a working volume of 1 L and the temperature is set at 55ºC. The reactor is stirred with a magnetic stirrer and the pH fluctuates around 8. The hydraulic retention time is about 16 days. Using these conditions the organic material is converted up to 54%, if the conversion efficiency is calculated based on the organic material fed to

the reactor and the organic material converted. A picture of this compartment is found in figure 2.1.

Figure 2.1.- Picture of compartment I (Hermans and Demey, 2001)

2.1.2 Connection between compartment I and compartments II, III and IVa

The outlet of compartment I, once filtered and autoclaved, was sent to Barcelona. There, it is centrifuged (BECKMAN J2-21 M/E) (10000rpm, 4ºC, 20min) and after centrifugation it is filtered using liquid filters (MILLIPORE OPTICAP^{TM} 4") of 0.22μm.

The experimental set up of compartments II, III and IVa, placed in Barcelona, and the necessary separation units to connect them are shown in figure 2.2.

2.1.3 Compartment II

Cultures corresponding to the second compartment are done in a 0.5 L tank bioreactor (BIOSTAT Q, Braun Biotech International). A pH control unit (CRISON pHrocon 18) regulates the pH of the culture media at 6.9 by addition of acid (HCl 1M) or base (NaOH 1.5M). A magnetic stirrer, set at 600 rpm, is used to ensure the perfect mixing of the culture.

A constant CO_2 flow, varying from 0.75-2.24 mL/min, depending on the cultures conditions, is bubbled to the culture providing anaerobic conditions and CO_2-carbon

source, which is required in order to degrade all the volatile fatty acids present in the incoming media. The temperature, set at 30ºC, is controlled by an external water jacket.

Illumination of the bioreactor is obtained using 18 halogen lamps (*SYLVANIA* Halogen –Professional de luxe-, 12V, 20W, ∅ 50mm.) distributed, radially around the external wall, in 6 supports containing, each one, 3 lamps. One 15V–40A power supply provides the necessary electrical power. Changing the voltage supplied to the lamps, the light intensity is modified. A calibration of the light intensity in W/m^2 at the surface of the reactor as a function of the voltage is given in Appendix I. A picture of this compartment is provided in figure 2.3.

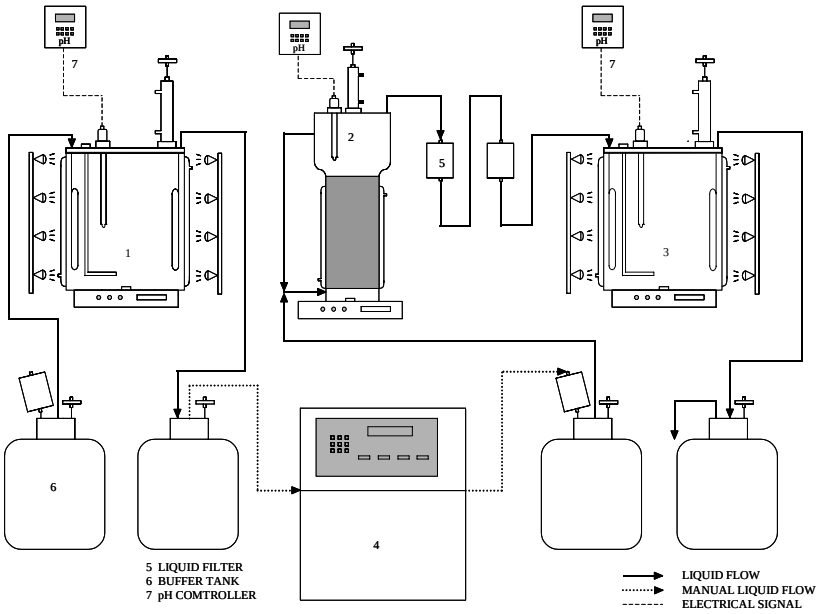

Figure 2.2. - Diagram of the experimental set up for the liquid connection of compartments II, III and IVa of MELISSA loop.

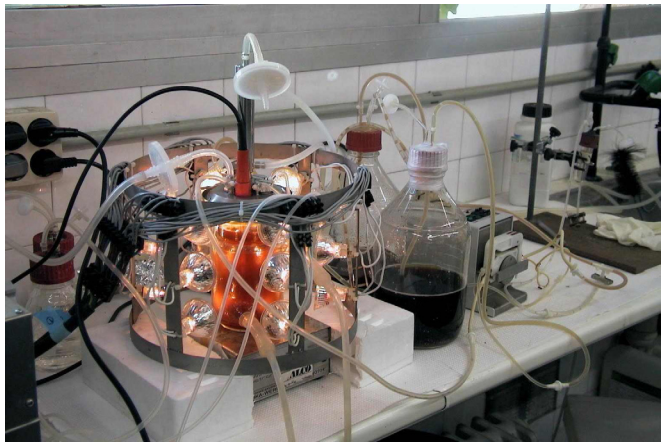

Figure 2.3.- Picture of compartment II. MELISSA effluent of compartment I is used as incoming media.

2.1.4 Connection between compartment II and III

Due to the fact that the outlet of compartment II contains biomass, a biomass removal step is necessary. To this purpose a centrifugation step is introduced in the liquid effluent of compartment II previous to the transfer operation to compartment III. The volumes managed during the continuous runs at bench scale, are too small to use a continuous centrifuge. Thus, a batch centrifuge (BECKMAN J2-21 M/E) (10000rpm, 4ºC, 20min) is used. As the centrifugation operation is a discontinuous process, two buffer tanks, one for the outlet of compartment II and the other for the inlet of compartment III are required. Then the collection of the liquid effluent for centrifugation and the storage of the biomass free medium after the centrifugation step are allowed. This centrifugation step is done daily as the quality of *Rhodospirillum rubrum* decreases significantly when it is stored during a longer period.

To avoid contamination of the centrifuged media, sterilization before introducing it to the input storage tank of the third compartment is required. To this purpose a filtering step, using liquid filters (MILLIPORE OPTICAP^{TM} 4") of 0.22µm, is introduced.

2.1.5 Compartment III

Implementation of compartment III is done using a packed-bed column, as described previously in TN 37.510 (Pérez *et al.*, 1997).

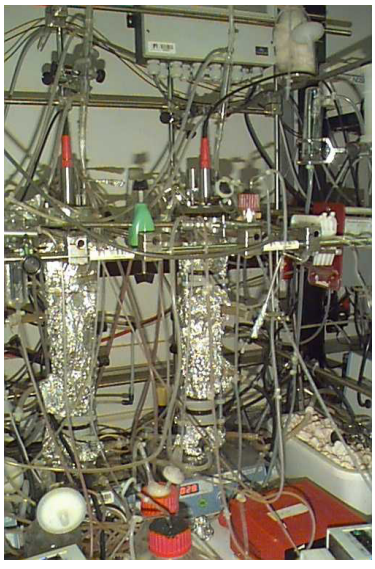

Figure 2.4.- Picture of compartment III.

The pH is controlled by an autonomous controller (CRISON pH/mV 252). Regulation of the pH at 8.4, is achieved by means of acid (CO_2) or base (Na_2CO_3 40g/L) addition. Temperature is controlled at 30ºC by an external water jacket. Aeration is done through a gas sparger. Part of the effluent of the bioreactor is recirculated in order to achieve a higher conversion. A magnetic stirrer is used to ensure the perfect mixing between the inlet and the recirculation. A picture of this compartment is found in figure 2.4.

2.1.6 Connection between compartments III and IVa

These two compartments can be directly connected. However, due to the fact that the outlet of compartment III can contain some biomass (basically: cells leaked out from the biofilm structure), two filtering steps are included. Filtration is done through liquid filters (MILLIPORE OPTICAP^{TM} 4") of 0.22µm located one in the output of compartment III and another one at the input of compartment IV. In this way, the two compartments are isolated allowing a disconnection in case of a potential malfunction of one of them.

2.1.7 Compartment IVa

The same equipment used in compartment II is used in compartment IVa. A calibration of the light intensity in W/m^2 at the surface of the reactor as a function of the voltage is given in Appendix II. A picture of this compartment is found in figure 2.5.

Figure 2.5.- Picture of compartment IVa.

2.2 MATERIALS AND METHODS

2.2.1 Strains and inoculum

The strain of *Rhodospirillum rubrum* used (ATCC 25903) is obtained from the American Type Culture Collection. It is revived and the subcultures are done using their recommended medium. The inoculum volume is fixed as the 10% of the working volume.

The strains of *Nitrosomonas europaea* and *Nitrobacter winogradsky*i used are obtained from the American Type Culture Collection: *Nitrosomonas europaea* (ATCC 19718) and *Nitrobacter winogradsky*i (ATCC 25391). A co-culture (*Nitrosomonas europaea* and *Nitrobacter winogradsky*i) obtained from the operation of a Biostat B reactor (Pérez *et al*., 1997) is used to inoculate the reactor.

The *Spirulina platensis* strain used is obtained from the Pasteur Institute*: Arthospira platensis* (PCC 8005). *S. platensis* is revived and the subcultures are done using their recommended medium. The inoculum's volume is fixed as the 10% of the working volume.

2.2.2 Analytic procedures

2.2.2.1 Cell concentration

- Dry weight

S. platensis and *R. rubrum* dry weight are determined by filtering through a 1.2μm and 0.22μm respectively pre-weighted filters, dried until constant weight in a microwave (20 min., 150 W) and cooled down in a desiccators.

- Optical density

The optical density measured at 750nm and 700nm is a direct measurement respectively of the *S. platensis* and *R.rubrum* concentration. Polysaccharides do not absorb at these wavelengths. Thus, these measurements reflect only the diffusion of the light produced by the presence of the microorganisms, fact that is directly related with biomass concentration. The spectrophotometer used is a Kontron Instrument, Uvikon 941, Italy.

2.2.3 Ammonium, nitrite and nitrate concentrations

- Ammonium

Ammonium was measured using UV measurement determinations by means of LCK 305 ammonium analysis kits (Dr. Lange Nitrax).

BASIS: Ammonium ions react with the hypochloride and salicylate ions in presence of nitroferrocyanide. Nitroferrocyanide acts as a catalyser (pH=12.6) forming iodophenol blue. Iodophenol blue is quantified measuring the absorption at 694nm.

- Nitrate

Nitrate is measured using UV measurement determinations by means of LCK 339 nitrate analysis kits (Dr. Lange Nitrax).

BASIS: Nitrate ions, in presence of sulphuric or phosphoric acid, react with 2,6-dimethylphenol forming 4-nitro-2,6-dimethylphenol which is quantified measuring the absorption at 370 nm.

- Nitrite

Nitrite is measured using UV measurement determinations by means of LCK 341 nitrite analysis kits (Dr. Lange Nitrax).

BASIS: Nitrite ions, in acid solutions, react with primary aromatic amines to form diazone salts which are quantified measuring the absorption at 524 nm.

2.2.4 Acetic, butyric and propionic concentrations

Acetic, butyric and propionic acids concentrations are determined either by:

high resolution liquid chromatography (HPLC) (Hewlett Packard 1050 with an Aminex HPX-87H ionic exchange column and an HP 1047 index refraction detector.

or gas chromatography (Hewlett Packard 5890).

2.2.5 Axenicity control

The fermentors broth and the media are checked for bacterial contamination by optical microscopy (ZEISS AXIOSKOP).

2.2.6 Metals concentration determination

- Solid samples

A sample of 0.3 g is pre-digested during 12 h using 6 mL of HNO_3. Then the digestion takes place in an analytic microwave first heating the sample to 170ºC. The heating is done gradually and the desired temperature is reached after 4 minutes. Then a second gradual temperature increase, that takes 3.5 minutes, is done up to 180ºC. This temperature is maintained 10 more minutes. The obtained solutions are diluted with Milli-Q water up to 100mL and then they are filtered. 5 elements (Na, K, Mg, Ca and P) are directly analysed from these solutions using induced coupled plasma emission spectroscopy (ICP-OES). The other elements (B, Mn, Fe, Ni, Cu and Zn) are analysed from 2/10 (2/100 in case of Fe) dilutions using induced coupled plasma mass spectroscopy (ICP-MS). External standards are used in order to quantify the samples.

- Liquid samples

Liquid samples are filtered previously to their analysis. Na, K and Ca are analysed from 1/10 or 1/100 dilutions using ICP-OES and the other elements (Mg, B, P, Mn, Fe, Ni, Cu and Zn) are analysed using ICP-MS after diluting 1/100 the sample. External standards are used in order to quantify the samples.0

3 RESULTS AND DISCUSSION

3.1 Culture media

The culture media outcoming from compartment I, once centrifuged and filtered in Barcelona, was analysed. The volatile fatty acids and the ammonium concentrations are found in table 3.1.

Volatile fatty acids	g/L
Acetic acid	0.77
propionic acid	0.27
Butyric acid	0.10
isobutyric acid	0.15
Valeric acid	0.17
Isovaleric acid	0.15
Nitrogen source	N-ppm
Ammonium	414

Table 3.1.- Volatile fatty acids and ammonium concentrations of the outlet media of compartment I.

Thus, in optimal conditions, when all the volatile fatty acids are consumed in compartment II, the *R. rubrum* produced per Litre of incoming media can be calculated following the stoichiometric equations described in TN45.4 (Favier-Teodorescu, L. *et al.*, 1999), as it is found in table 3.2.

- **Acetic acid** + 0.4115 NH_3 + 0.0299 H_3PO_4 + 0.0066 H_2SO_4 $\rightarrow$ 1.8550 $CH_{1.6004}O_{0.3621}N_{0.2218}S_{0.0036}P_{0.0161}$ + 0.1450 CO_2 + 1.1843 H_2O

- **Propionic acid** + 0.7201 NH_3 + 0.0523 H_3PO_4 + 0.0116 H_2SO_4 + 0.2462 CO_2 $\rightarrow$ 3.2462 $CH_{1.6004}O_{0.3621}N_{0.2218}S_{0.0036}P_{0.0161}$ + 1.5726 H_2O

- **Butyric acid** + 1.0287 NH_3 + 0.0748 H_3PO_4 + 0.0165 H_2SO_4 + 0.6374 CO_2 $\rightarrow$ 4.6374 $CH_{1.6004}O_{0.3621}N_{0.2218}S_{0.0036}P_{0.0161}$ + 1.9609 H_2O

- **Iso-butyric acid** + 1.0287 NH_3 + 0.0748 H_3PO_4 + 0.0165 H_2SO_4 + 0.6374 CO_2 $\rightarrow$ 4.6374 $CH_{1.6004}O_{0.3621}N_{0.2218}S_{0.0036}P_{0.0161}$ + 1.9609 H_2O

- **Valeric acid** + 1.3373 NH_3 + 0.0972 H_3PO_4 + 0.0215 H_2SO_4 + 1.0286 CO_2 $\rightarrow$ 6.0286 $CH_{1.6004}O_{0.3621}N_{0.2218}S_{0.0036}P_{0.0161}$ + 2.3491 H_2O

- Iso-valeric acid + 1.3373 NH_3 + 0.0972 H_3PO_4 + 0.0215 H_2SO_4 + 1.0286 CO_2 → 6.0286 $CH_{1.6004}O_{0.3621}N_{0.2218}S_{0.0036}P_{0.0161}$ + 2.3491 H_2O

Volatile fatty acids	g/L	*R.rubrum* produced (DW) g/L
Acetic acid	0.77	0.55
propionic acid	0.27	0.27
Butyric acid	0.10	0.12
isobutyric acid	0.15	0.18
Valeric acid	0.17	0.23
Isovaleric acid	0.15	0.20
TOTAL		1.55

Table 3.2.- Theoretical R.rubrum production from each VFA per Liter of incoming media, at optimal conditions.

In table 3.3, the concentrations of the different elements of the outcoming media of compartment I are shown and can be compared with their concentration in the inlet media of compartment I, given by EPAS N.V:, Eco Process Assistance, University of Gent, (Belgium), and the artificial incoming media used previously, to proceed with the connection of compartments II, III and IVa in UAB.

	Outlet Compartment I (ppm)		**Artificial medium (ppm)**		**Inlet Compartment I**			
					(•) Treated (ppm)		**(•) Dissolved (ppm)**	
	mean	***stddev***	**mean**	***stddev***	**mean**	***stddev***	**mean**	***stddev***
Na	12	15	314	13	50.63	*8.98*	40.8	*12.76*
K	688	3	378	3	-	-	-	-
Mg	26.6	0.5	113.3	0.5	144.38	*9.64*	99.58	*19.38*
Ca	24.5	1.5	25.3	1.5	715	*96.63*	210.25	*80.63*
B	2.2	<0.5	0.7	0.1	1.41	*0.43*	0.84	*0.64*
P	7.2	<1	175.2	1.2	523.13	*102.60*	139.53	*34.77*
Mn	<0.5	0.74	3.6	0.2	2.77	*0.66*	0.17	*0.02*
Fe	<1	<0.1	5.2	0.1	12.01	*3.71*	1.24	*0.19*
Ni	0.74	0.22	<0.3	-	0.471	*0.48*	0.26	*0.23*
Cu	<0.1	-	<0.1	-	1.13	*0.18*	0.10	*0.05*
Zn	0.22	0.07	0.31	0.7	11.35	*2.49*	9.91	*9.38*
S	-	-	-	-	67.69	*17.38*	62.55	*24.43*

Table 3.3.- Analysis of different elements concentrations of the outcoming media of compartment I and comparison with their concentration in the inlet media of compartment I, given by EPAS N:V:, Eco Process Assistance, University of Gent, (Belgium), and the artificial incoming media used previously to proceed with the connection of compartments II, III and IVa in UAB. (•) One group of the samples is treated with nitric acid and the other group is centrifuged and filtered

If the different element concentrations are compared, several observations can be done:

- There is a difference between the concentration of elements in the inlet media of compartment I and their concentrations in its outlet media. This difference is too big to be attributed to biomass formation alone. Thus, these elements have probably been retained in some of the separation steps between compartment I and II. This fact can be increased, as explained in materials and methods section, by the fact that the liquid output of compartment I was filtered in Gent and centrifuged and filtered again in Barcelona when the samples arrived. This was done due to the fact that the bottles that arrived at Barcelona were leaking and had to be filter sterilized again before its storage and use.

- The different elements concentration in the inlet media of compartment I is higher or similar than the one found in the artificial media for the connection of compartments II, III and IVa. Thus, assuming complete liquefaction, if these elements were not lost in the separation steps done in the outlet of compartment I it seems that the whole connection can be done without adding extra elements. Future tests will verify the present data. Na and Mn are the exception. Na and Mn are found in a lower concentration in the inlet and outlet media of compartment I than in the artificial media for the connection of compartments II, III and IVa. Na, however, is added in order to adjust the pH, thus it is added during the loop operation.

- Taking into account that only 6 L of outcoming media from compartment I were available, and that the purpose of this TN is to verify whether there are non identified compounds which interfere in the normal operation of the loop connection, different missing compounds have been added to the outcoming media from compartment I in order to achieve the same elements concentrations present in the artificial connection media of compartments II, III and IVa, used previously (Creus *et al.* 2002b). These compounds are found in table 3.4.

In table 3.4, the different compounds added to the media outcoming compartment I in order to use this media to connect compartment I to II, III and IVa are presented.

Na is not added because it is added when adding P and in order to adjust the pH in compartments II and IVa. The only precaution that has to be taken into account is that if the ratio K^+/Na^+ is >5 then, *S.platensis* growth is inhibited (Richmond 1986, Zarrouk 1966). However, it has been observed that during the loop operation, Na is added in excess and the ratio K^+/Na^+ is always < 5 (Creus, *et al*., 2001).

The media outcoming compartment I presents a dark-brown colour. Fact that has to be taken into account, as compartments II and IVa are photosynthetic. The absorption spectrum of the media as a function of wavelength is found in figure 3.1 and it is compared with the absorption spectrum of the artificial media used to connect compartments II, III and IVa.

	Outlet CI (ppm)	**Media CII, III, IVa (ppm)**		**Elements Requirement**	**Added to the outlet of CI**	
		measured	**calculated**		**Compounds**	**g/L**
Na	12	314	233	>200	*	*
K	688	378	362	-	-	-
Mg	26.6	113.3	118.4	91.7	$MgSO_4.7H_2O$	0.93
Ca	24.5	25.3	24.7	0.24	$CaCl_2.2H_2O$	0.001
B	2.2	0.7	0.52	-	-	-
P	7.2	175.2	221.6	214.4	Na_2HPO_4	0.98
Mn	<0.5	3.6	3.4	>3.1	$MnCl_2.2H_2O$	0.01
Fe	<1	5.2	6.6	>5.6	$FeSO_4.7H_2O$	0.03
Ni	0.74	<0.3	0.12	-	-	-
Cu	<0.1	<0.1	0.02	-	-	-
Zn	0.22	0.31	0.07	-	-	-

Table 3.4.- Element requirement and compounds added to the outcoming media of compartment I in order to emulate the artificial media used for the connection of compartments II, III and IVa and compounds added to the media. (*) Na is added adding P and in order to adjust the pH.

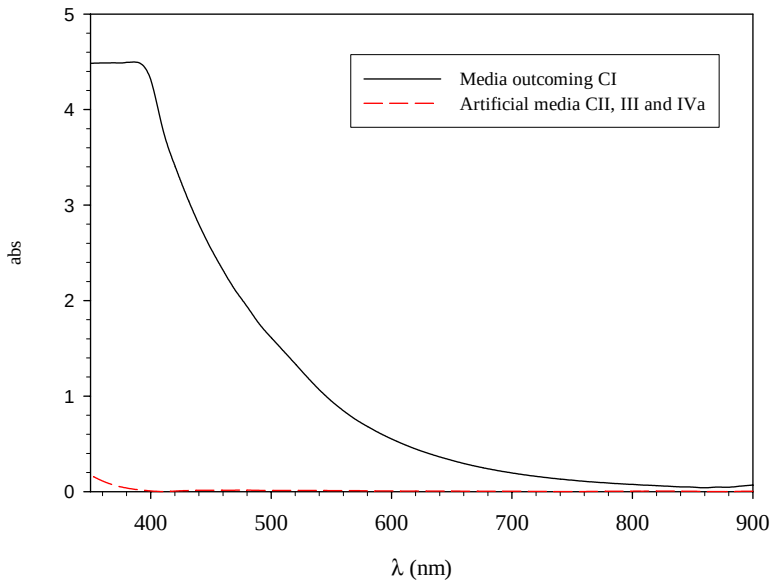

Figure 3.1.- Absorption spectrums of the outcoming media of compartment I and the artificial media used to connect CII, III and IVa

In order to see whether this higher absorption of the outcoming media of compartment I can directly affect the energy collecting centres of *S.platensis* and *R. rubrum*, the light absorption spectrums of these microorganisms are presented in figures 3.2 and 3.3.

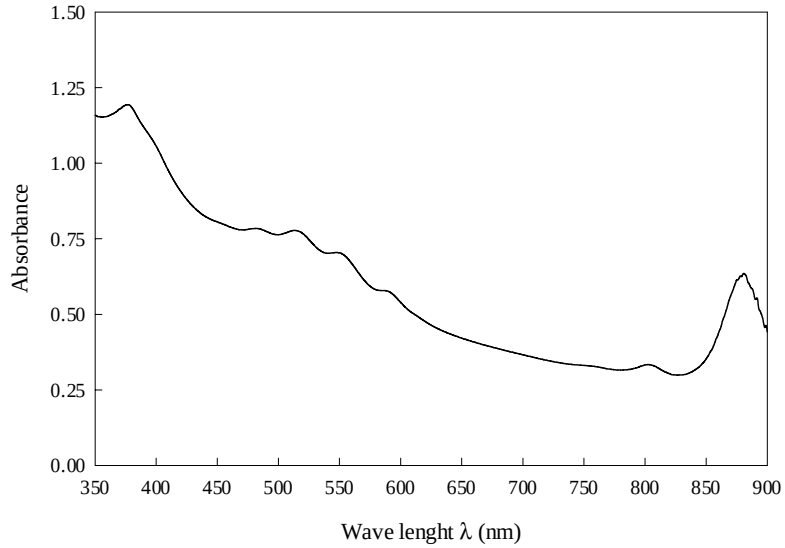

Figure 3.2- Light absorption spectrum of *R. rubrum* cells. The chlorophyill absorption peaks are found at 870, 805 and 360 nm and the carotenoids at 525 and 550nm.

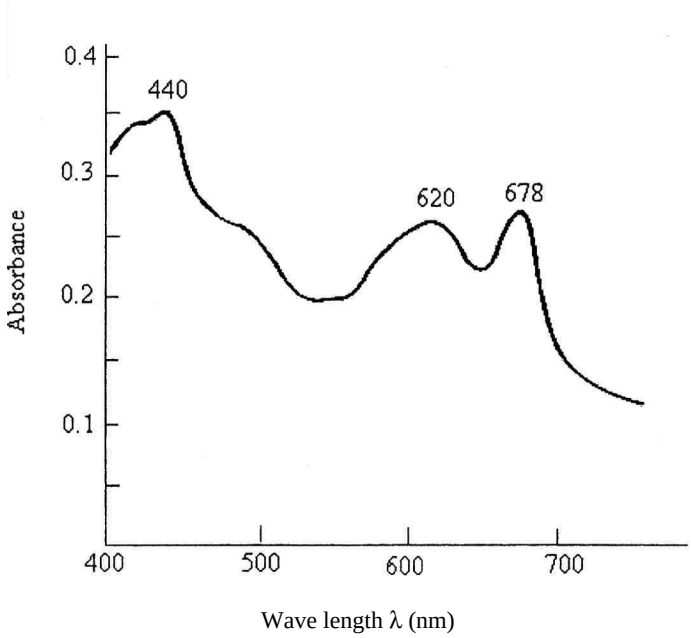

Figure 3.3- Light absorption spectrum of *S. platensis* cells (Mohanty *et al*., 1997). The chlorophyill absorption peaks are found at 440 and 678 nm and the allophycocyanines at 620.

As seen in figures 3.2 and 3.3, both microorganisms have absorption peaks at wavelengths below 650 nm, where the absorption spectrum of the media outcoming compartment I is significantly higher than the one of the artificial media of the connection of compartments II, III and IVa. Thus, the energy usable for the microorganism providing the same Fr is lower for the media outcoming compartment I than for the artificial media. This fact has to be taken into account.

3.2 Batch cultures using the complemented outcoming media of compartment I.

In order to see whether *R. rubrum* and *S. platensis* can grow in media outcoming from compartment I, different batch tests are carried out.

R. rubrum and *S. platensis* are grown in 250 mL bottles. 100 mL of media and 10 mL of inoculum are used. The experiments are done 3 times and in each set of experiments 5 different batch tests are run, under the following media conditions:

I. 100% of artificial medium, see section 3.1.

II. 75% of artificial medium and a 25% of medium outcoming from compartment I.

III. 50% of artificial medium and a 50% of medium outcoming from compartment I.

IV. 25% of artificial medium and a 75% of medium outcoming from compartment I.

V. 100% of medium outcoming from compartment I.

Samples are taken at the end of each batch experiment and the volatile fatty acids concentration and the axenicity of the culture are followed.

The experimental set-up is presented in figure 3.5.

Figure 3.5.- Experimental set-up of the *S. platensis* and *R. rubrum* batch-tests.

3.2.1 *R. rubrum* batch-tests

The batch tests results growing *R. rubrum* in media outcoming compartment I are presented in table 3.10.

test	I	II	III	IV	V
A	√	√	√	×C	×C
B	√	√	√	√	√
C	√	√	√	√	√

Table 3.10.- *R. rubrum* growing on media outcoming compartment I. I, II, III, IV and V are the different batch conditions, see section 3.2. √: *R rubrum* grown, ×: *R. rubrum* not grown, C: contaminated.

As it is seen in table 3.10 *R. rubrum* grows perfectly well in all the different media conditions tested, except in the first set of experiments where the bottles having a 100% and a 75% of media outcoming from compartment I got contaminated.

The length of each set of batch tests is not presented as it basically depends on the inoculum stage. However, during each set of batch experiments a faster growth of the bottles containing a higher amount of artificial medium is appreciated. This fact that could be attributed either to some inhibitory effect of the media outcoming compartment I or to the lower energy availability of the cells due to the light absorption of the media outcoming compartment I, as previously discussed, see figure 3.2.

The volatile fatty acids are totally consumed in each set of experiments.

3.2.2 *S. platensis* batch-tests

The batch tests results growing *S. platensis* in media outcoming compartment I are presented in table 3.11.

test	I	II	III	IV	V
A	√	√	√	√	√
B	√	√	√	√	√
C	√	√	√	√	√

Table 3.11.- *S. platensis* growing on media outcoming compartment I. I, II, III, IV and V are the different batch conditions, see section 3.2. √: *S. platensis* grown, ×: *S. platensis* not grown.

As seen in table 3.11, *S. platensis* grows in all the different batch conditions tested. Therefore there are no toxic elements that can impede the *Spirulina* growth. Nevertheless it must be mentioned that for the tests a non axenic strain was used. Therefore, although the low toxicity of the medium has been verified, the tests should be repeated in the future using an axenic strain.

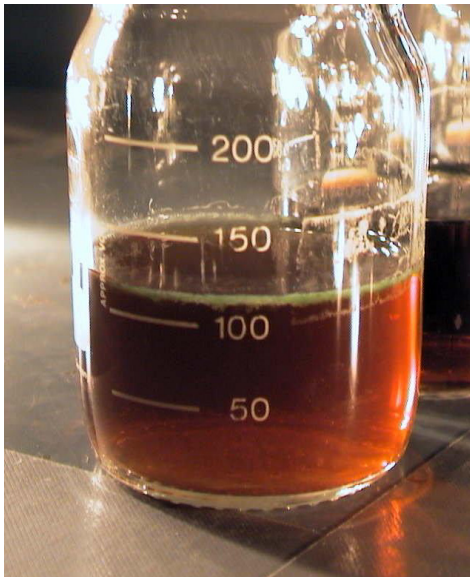

Figure 3.3.- *S.platensis* grown in media outcoming compartment I

3.3 Connection of compartments I, II, III and IVa .

Once seen that *R. rubrum* and *S. platensis* can grow in batch mode using the complemented media outcoming compartment I and once found the experimental conditions in which the connection of compartments II, III and IVa can be done using an inlet media similar to the outcoming media of compartment I, then the connection of compartments I, II, III and IVa has to be achieved.

As previously discussed compartment I is run in Belgium and the outlet of this compartment is send to Barcelona in order to proceed with its connection to compartment II, III and IVa.

The experimental conditions used to connect compartments II, III and IVa are presented in table 3.16. These conditions are similar to the ones used in section 3.3. However, taking into account the dilution rate of compartment II its light supply has been increased. Fact that has been done as non volatile fatty acids are wanted in the outlet of this compartment and the media outcoming compartment I has its own absorption, see figure 3.1.

The evolution of compartments II, III and IVa are presented in figures 3.14, 3.15 and 3.16.

	Compartment II	*Compartment III*	*Compartment IVa*
F_R *(W/m^2)*	137	-	94
Q_L *(mL/day)*	400	400	400
D (h^{-1})	0,042	0,035	0,042
ι (days)	1	0,84	1

Table 3.16.- Experimental conditions of compartments II, III and IVa during this experiment.

The evolution of compartments II, III and IVa are presented in figures 3.14, 3.15 and 3.16.

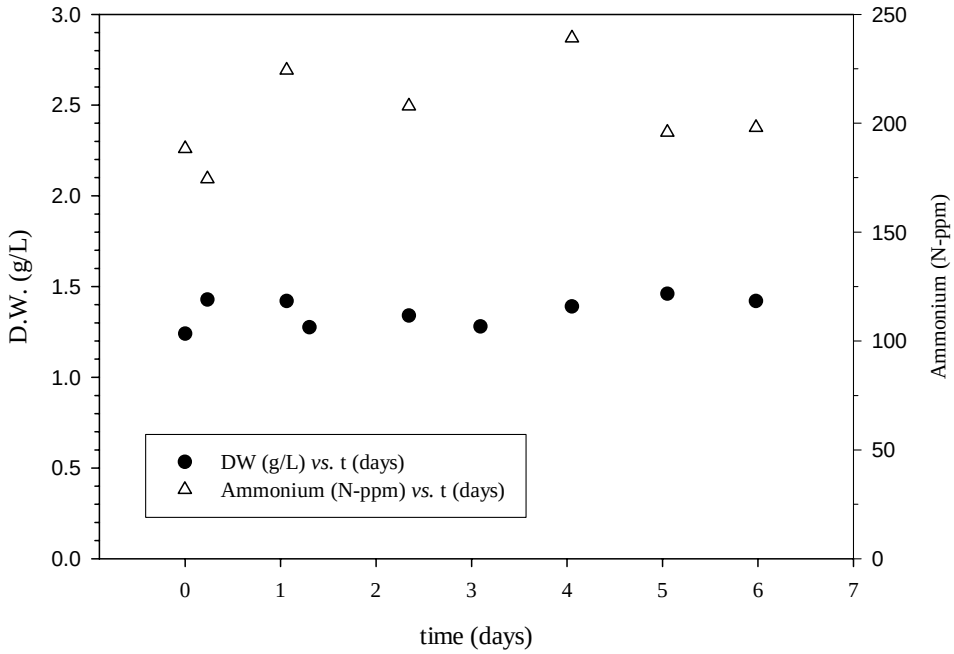

Figure 3.14.- Evolution of compartment II using the experimental conditions described in table 3.16

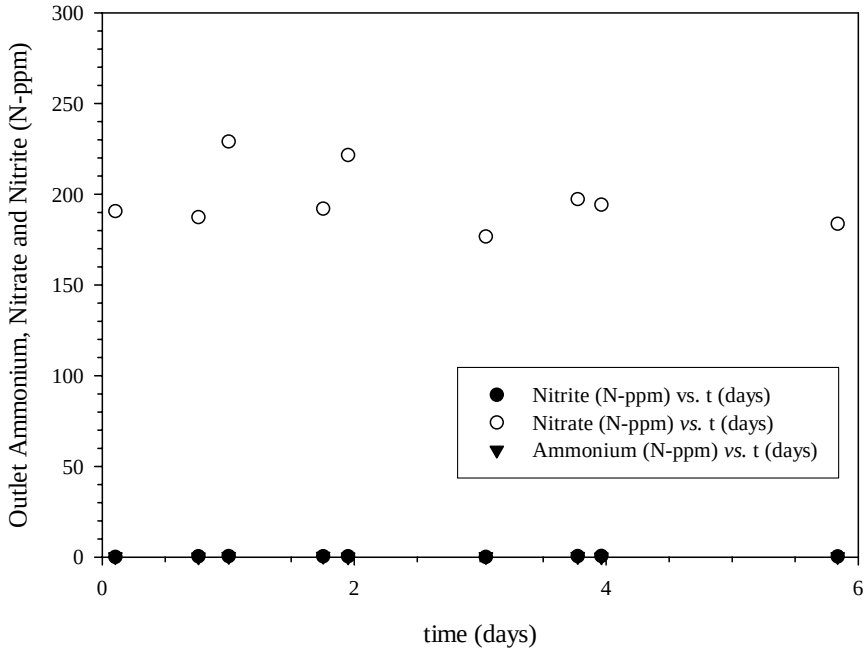

Figure 3.15.- Evolution of compartment II using the experimental conditions described in table 3.16

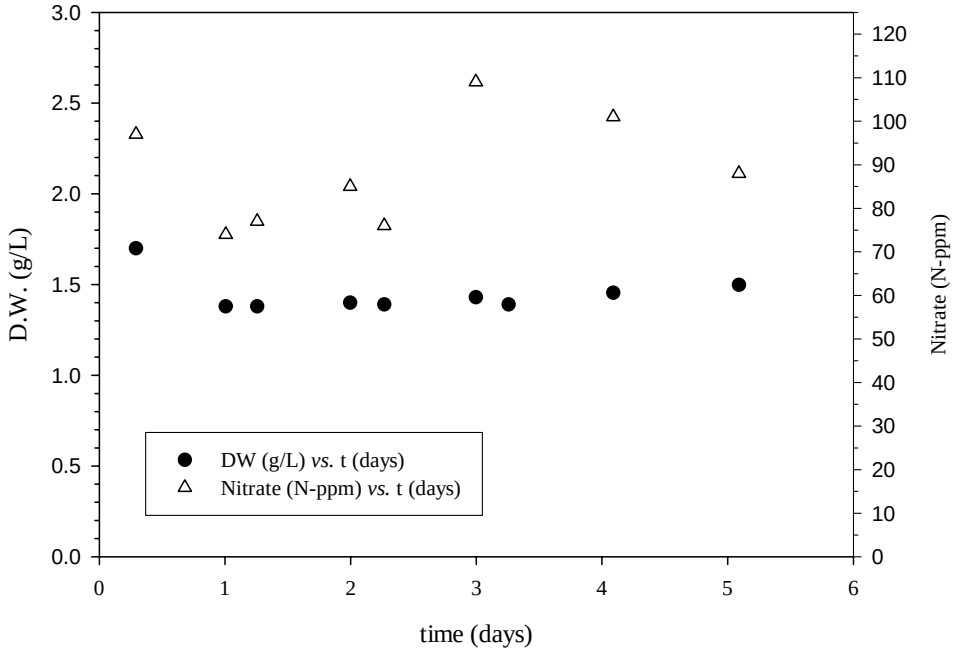

Figure 3.16.- Evolution of compartment II using the experimental conditions described in table 3.16

As can be seen in figures 3.14, 3.15 and 3.16, the connection of compartments I, II, III and IVa has been successfully done during more than 5 residence times. Thus, non-toxic effects have been observed when the media outcoming compartment I is used in compartments II, III and IVa successively.

As seen in figure 3.14, a steady state having a biomass concentration of about 1.45 g/L is obtained. Thus a 94 % of the expected biomass is formed taking into account that with this inlet media 1.55 g DW/L should be produced, see section 3.1. A production of 1.45 g DW/L of *R. rubrum* requires about 195 N-ppm if the *R. rubrum* elemental composition presented in section 3.1 is considered. The measured nitrogen consumption in compartment II during this experiment is of about 204 N-ppm as the inlet media contains 414 N-ppm and in the outlet, as it is seen in figure 3.14 about 210 N-ppm are found. Thus the nitrogen balance can also be considered closed. In figure 3.14, volatile fatty acids are not found as compartment II is working at optimal conditions and all the incoming volatile fatty acids are completely consumed.

Compartment III operates at its optimal conditions as seen in figure 3.15. Neither nitrite nor ammonium are found in the outlet, thus and all the incoming ammonium is converted to nitrate.

As it is seen in figure 3.16 compartment IVa has, also, a complete stable situation. A steady state at about 1.45 g DW/L is reached and non-toxic effects are observed.

The carbon balance of this compartment cannot be done, as the major carbon source is the CO_2 bubbled in the fermentor, which is introduced in excess and is not measured in the outlet.

Considering the *S.platensis* elemental composition: $CH_{1.650}O_{0.531}N_{0.170}S_{0.007}P_{0.006}$, (Cornet, 1992) in order to produce 1.45 g DW/L about 139 N-ppm are required. In figure 3.16 a nitrogen gap of about 115 N-ppm is found taking into account the nitrogen income of compartment IVa, figure 3.12. Thus, taking into account the intrinsic experimental error of the measures, the nitrogen balance can be considered closed.

4 CONCLUSIONS

- The oulet media of compartment I has been analysed and compared to the artificial media used in the connection of compartments II, III and IVa to see its requirements in order to proceed with the connection of compartments I, II, III and IVa.
- *R. rubrum* and *S. platensis* have grown in batch mode using media outcoming from compartment I without showing any sign of inhibition due to the medium effects.
- The connection of compartments I, II, III and IVa has been successfully performed, during more than 5 residence times, without neither presenting a major difficulty nor showing any toxic effect in any of the compartments.

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6 APPENDIX I. -LIGHT CALIBRATION OF COMPARTMENT II

The light availability determination is of key importance for the operation of photobioreactors. Once light intensity at the bioreactor's surface is known, the light availability at any point of the bioreactor can be determined.

The light intensity at the bioreactor's surface is obtained by measuring the light intensity at the axis of the bioreactor, using a spherical light sensor that integrates the light reaching its radial illuminated surface. Conversion of the light intensity measured by the spherical sensor to the light intensity at the surface of the bioreactor is done using the following equation:

$$Fr = \frac{Eb \cdot rb}{\pi \cdot Rb}$$

Where: Fr is the light flux at the bioreactor's surface, Eb is the light intensity measured by the sensor, rb is the sensor's radius (30mm) and Rb is the bioreactor's radius (31mm).

The available light measured by the sensor is given in mols/m2s. For the application of the previously developed light transfer mathematical models, it is necessary to convert the units of the sensor to W/m^2. The conversion coefficient used is 0.425, which has been previously calculated by J.F.Cornet by integration of the used lamps spectra in the range 350–950nm used by *Rhodospirillum rubrum*.

The Eb values are measured in the empty bioreactor with water circulating through the external jacket.

Light intensity measurements are done at different vertical positions and at different voltages supplied to the lamps. The results of these measurements are plotted in figure I.1.

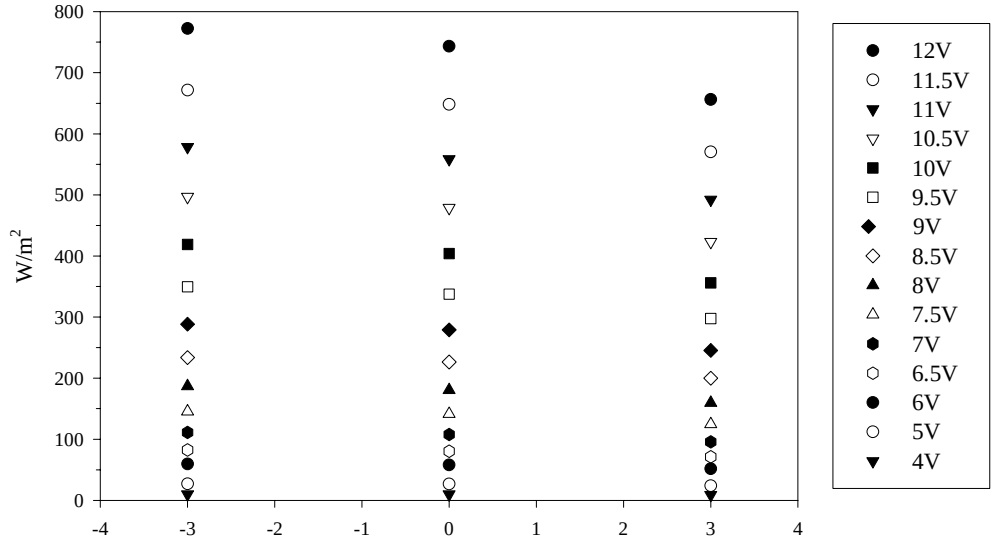

Figure I.1.- Light intensity at different vertical positions supplying different voltages (assuming 0 cm the centre of the reactor).

Averaging the measurements obtained at different vertical positions, the mean light intensity value for each voltage supplied to the lamps is obtained. The light intensity values measured by the sensor in mols/m2s, are converted to Fr values using the above mentioned formula and conversion factor. Figure I.2 shows the relationship between the voltage supplied to the lamps and the Fr of the bioreactor.

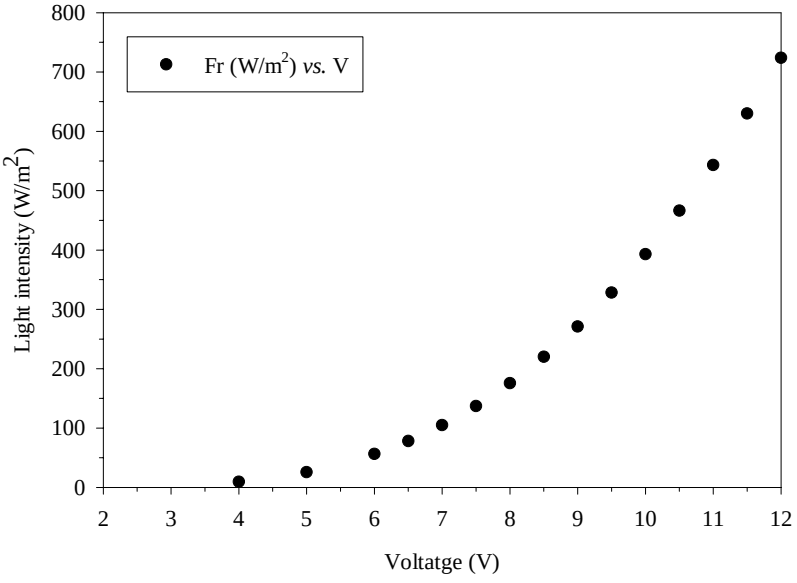

Figure I.2 .- Average light intensity supplying different voltages

7 APPENDIX II. -LIGHT CALIBRATION OF COMPARTMENT IVA

The determination of the light intensity at the bioreactor's surface is done as previously described in Appendix I . In this case, the Rb (radius of the bioreactor) is also 31mm. The conversion factor is 0.291, which has been calculated by J.F. Cornet by integration of the used lamps spectra in the range 350-750 nm used by *Spirulina platensis* cells. The results obtained by measuring light intensity at different vertical positions and supplying different voltages to the lamps are plotted in figure II.1.

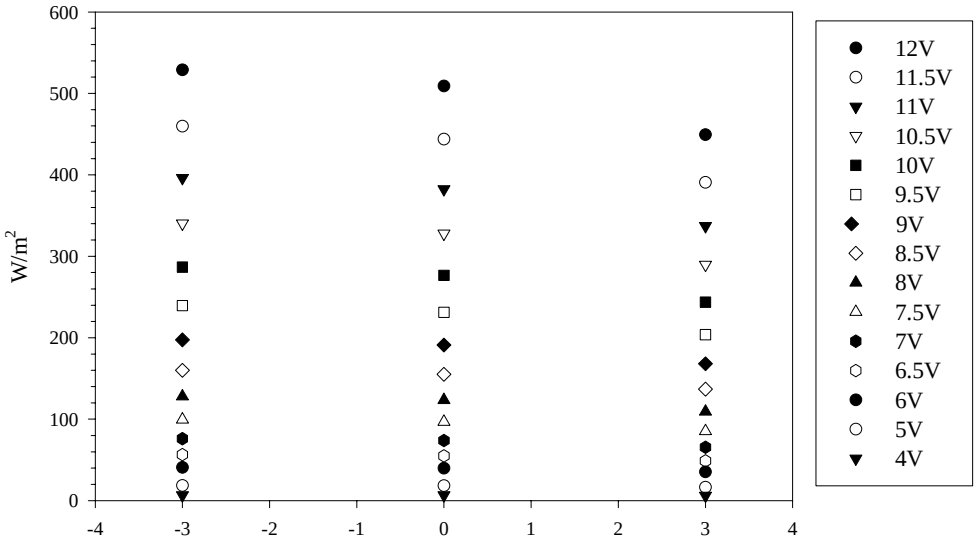

Figure II.1.- Light intensity at different vertical positions supplying different voltages (assuming 0cm the centre of the reactor)

The average relationship between the voltage supplied to the lamps and the Fr of the bioreactor obtained is presented in figure II.2.

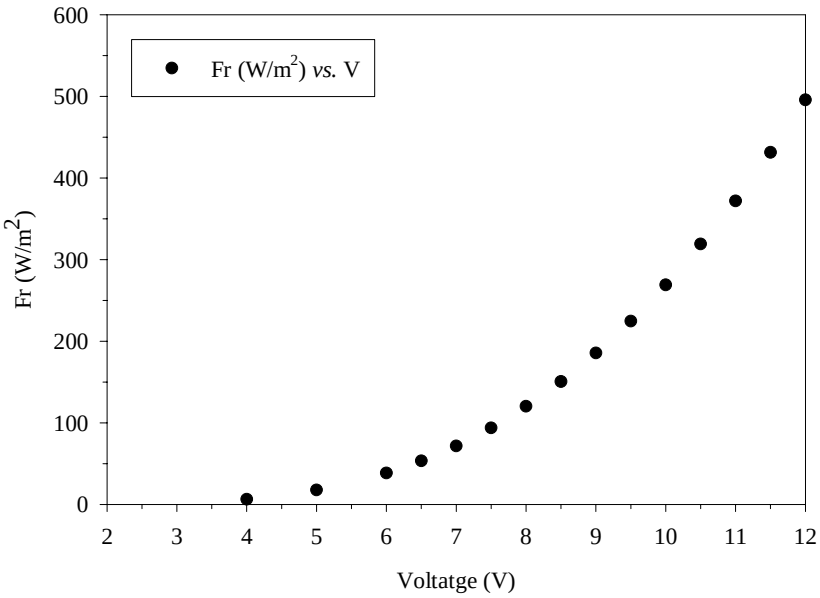

Figure II.2.- Average light intensity