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Redesign of Compartment II Pilot Reactor

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1. Introduction

In the present technical note the changes introduced in compartment II of the MELISSA Pilot Plant laboratory are reported. The main modification carried out affects the mixing system, although other control loops have also been changed. In its previous configuration, the photobioreactor was a gas-lift vessel, i.e. the culture medium was mixed by means of gas injection in its bottom part. The continuous operation of this gas-lift reactor with *R. rubrum* cells have faced serious operational problems, reported below, and finally it has been necessary to change the complete stirring system from the gas-lift to mechanical stirring, as it will be described in this technical note.

Indeed, it was observed several times the fact that when the gas-lift photobioreactor was working in continuous mode during several residence times *R. rubrum* cells started to attach to the glass surface of the reactor. Thus, the available light inside the medium was attenuated.

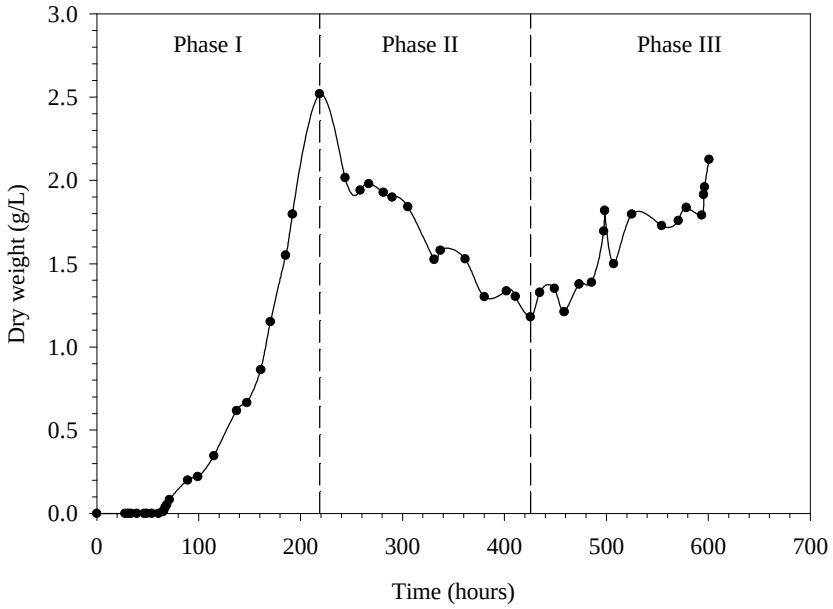

Figure 1: R. rubrum culture in compartment II reactor. Culture at the pilot bioreactor. Phase I: Batch, F_R=100 W/m^2. Phase II: Continuous mode (D=0.015 h^{-1}), F_R=100 W/m^2. Phase III: Continuous mode (D=0.015 h^{-1}), F_R=200 W/m^2. 1 g C/L in the fresh culture medium.

In figure 1, an example of this situation is provided. Phase I corresponds to the batch culture that could always be performed in a normal profile. In phase II, when the biomass concentration was significant (2.5 g/L), the continuous culture was started at a low dilution rate (D=0.015 h^{-1}). It was observed that after 40 hours of the beginning of the continuous culture mode, instead of evolve to a steady state, the cells started to

attach to the glass surface. In phase III the average incident light intensity was increased up to 200 W/m^2. The result was that the dry weight also augmented, but as there are cells attaching to the glass surface and cells detaching from it, the biomass profile was not constant at a steady level. At the end, the attached cells formed a eight-mm thick layer. This situation made the continuous controlled operation of a suspension culture of *R. rubrum* cells in this reactor completely unfeasible.

The internal glass surface of the bioreactor was treated with a hydrophobic agent, dimethyldichlorinesilane (Sigma), in order to avoid cell attachment. The result was an increased difficulty to attach the cells to the wall. However, once a first layer of cells was stuck, more cells would attach to it very quickly. Therefore, the result was not satisfactory.

In order to study the possible effect of the incident light intensity on the problem of the cell attachment more tests were carried out decreasing that parameter (figure 2).

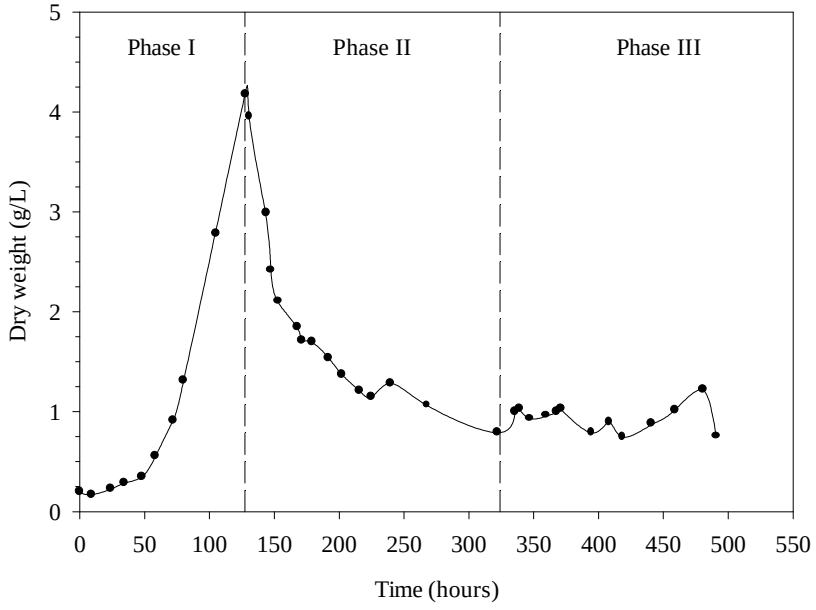

Figure 2: R. rubrum culture in compartment II reactor. Phase I: Batch, F_R=50 W/m^2. Phase II: Continuous mode (D=0.04 h^{-1}), F_R=50 W/m^2. Phase III: Continuous mode (D=0.04 h^{-1}), F_R=300 W/m^2. 2 g C/L in the fresh culture medium.

In phase I the cells grew in batch with a high carbon concentration (2 g acetic acid/L) and a low light intensity, 50 W/m^2. Once the carbon source was exhausted, the continuous mode was turned on. At first, biomass concentration seemed to tend to a steady state. However, 100 hours after starting the continuous mode, the biofilm attached to the glass wall was substantial. Then, in phase III, it was tested the effect of high incident light intensity (300 W/m^2) on that problem. No result was observed and the attached cells did not detach.

One possible explanation to the observed phenomenon is that under light limitation conditions and excess of carbon source the *R. rubrum* cells could synthesize an exopolysaccharide, which could make easy their attachment to the reactor surface. Therefore, more experiments were done in order to contrast that hypothesis.

Figure 3 gives de data from an experiment performed with low initial carbon source concentration, in order to force the cells to incorporate the slight carbon source as cell material and avoid exopolysaccharide formation.

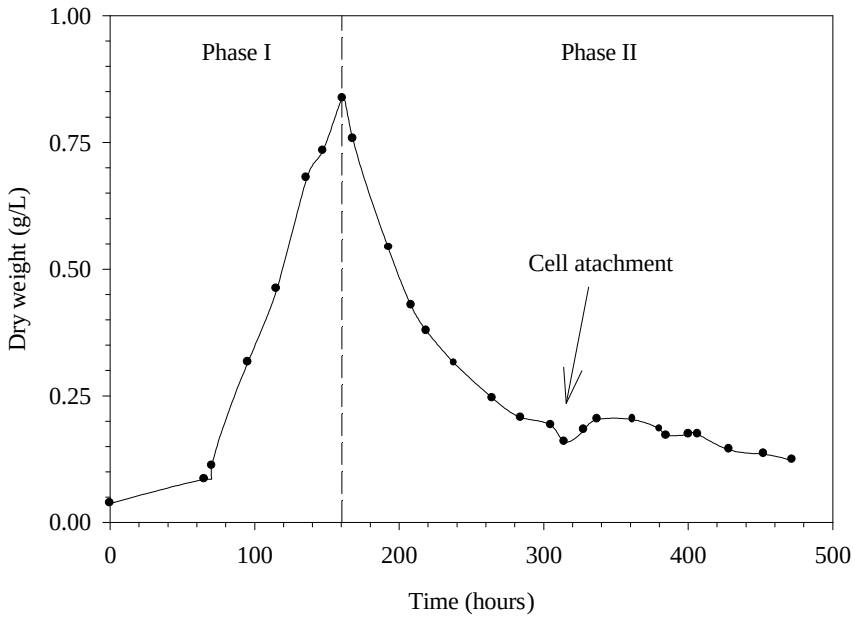

Figure 3: R. rubrum culture in compartment II reactor with low initial carbon concentration. Phase : Batch, F_R=50 W/m^2. Phase II: Continuous mode (D=0.035 h^{-1}), F_R=50 W/m^2. 0.50 g C/L in the fresh culture medium.

In that case, when the batch culture achieved the maximum theoretical biomass concentration and the continuous mode was started, the biomass concentration was decreasing following the typical wash-out profile until the steady state. After approximately 300 hours, it seemed to be stabilizing at a constant value. On that moment, the first layer of cells started to attach to the glass surface of the photobioreactor and the culture became unstable. From the moment at which the attached biofilm appeared until the end of the experiment, the cells did not detach.

In all of the previous experiments carried out it had been observed the fact that the first stuck cells always appeared just opposite the halogen lamp, drawing perfectly the lamp outline in the glass wall. That fact might be related with the possible existence of temperature micro-gradients on the glass surface of the reactor just opposite the lamp. In addition, the cells might be sensitive to those small temperature gradients and even they could have affinity for them. Thus, the cell attachment could be explained because of slight thermal gradients on the glass surface.

The temperature control of the reactor was done by means of an electric resistance to heat and a cool finger to cool down the reactor. They have been substituted for an external glass jacket, where the water circulation keep controlled the temperature of the culture medium in a efficient and more homogenous way at the same time that the thermal micro-gradients in the glass wall are avoided. When this modification was introduced in the hardware of the second compartment, new experiments were carried out (figure 4).

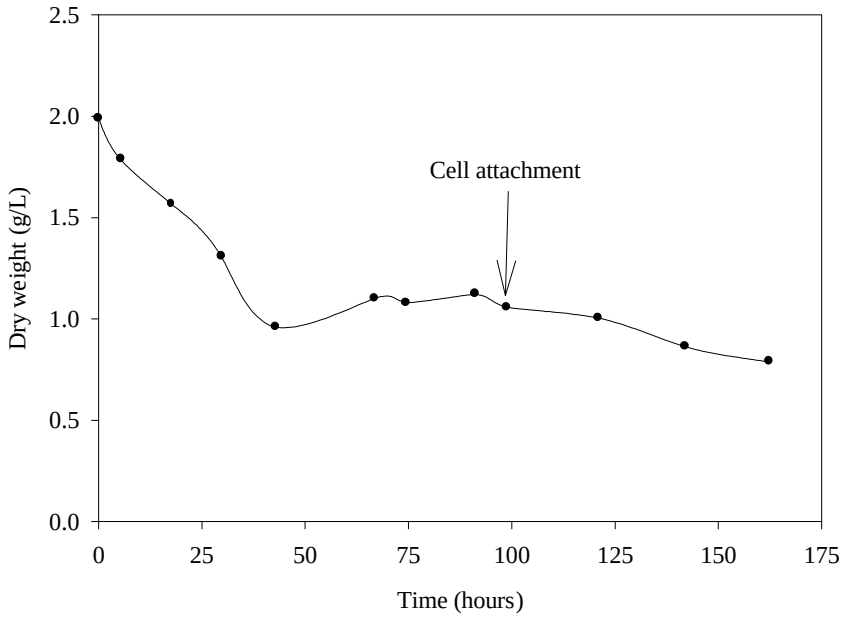

Figure 4: R. rubrum culture in compartment II reactor with temperature control through external glass jacket. Continuous mode (D=0.02 h^{-1}), F_R=180 W/m^2. 1.0 g C/L in the fresh culture medium.

In figure 4, where the initial time corresponds to the start of the continuous culture, it can be observed that the biomass concentration profile is right, even it seems to achieve a steady state, after 90 hours, when start to appear some cells attached to the glass wall. After that moment, the culture was unsteady.

In figure 5, it can be observed the pilot photobioreactor with the *R. rubrum* cells attached to the glass surface, with a layer of cells of 8 mm thickness.

In most of the previous experiments, it had been observed the fact that the cells, before attaching to the wall, had changed its physiology and morphology. When *R. rubrum* cells are growing in suspension with no nutrient limitations, usually during the batch culture or the beginning of the continuous culture, they are spiral shaped cells with observable motility (figure 6). Nevertheless, after two or three residence times in the continuous culture conditions they start to accumulate big granules inside, they loss some mobility and become longer. It seems as if they continue growing but could not divide normally (figure 7).

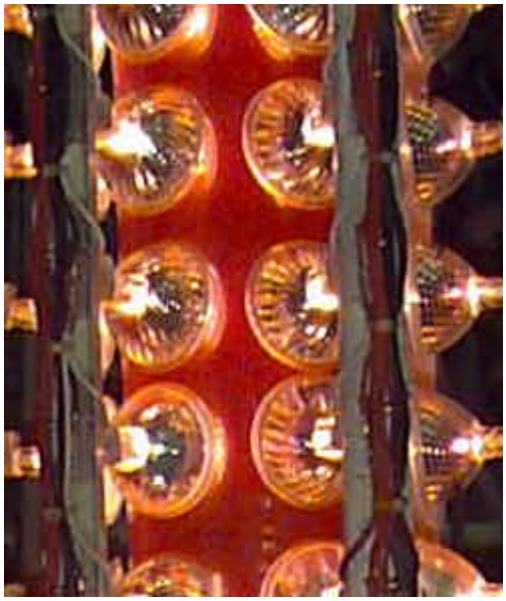

Figure 5: R. rubrum cells attached to the glass surface.

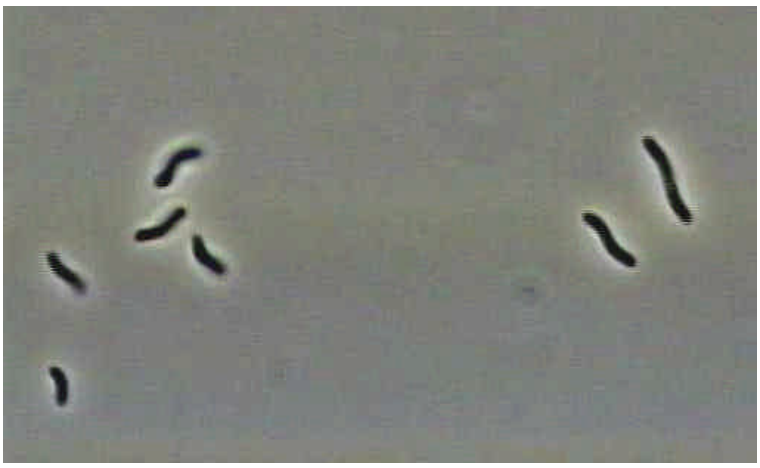

Figure 6: Normal morphology of R. rubrum cells growing successfully in suspension.

In order to find out the environmental conditions that could help the cells to grow properly, several factors that could affect the physiology and the morphology of the cells were considered. The parameters studied were the carbon concentration in the feed, the incident light intensity, the mixing gas flow rate and its composition, the presence of antifoam, and the exhaustion in the culture of an essential micronutrient.

An exhaustive experimental study was carried out in order to find out the possible effect due to both every single factor and all together. Unfortunately, the results seem not to follow a specific pattern. They are reported in table 1. In general terms, though, it is observed that cell attachment had occurred in all the continuous runs.

C CONC. (g C/L)	F_R (W/m^2)	AIR FLOW (L/min)	CO_2	Ar	MODE BATCH	MODE CONT.	LOOP OPEN	LOOP CLOSED	ANTI-FOAM	COOL JACKET	NUTRIENT OVERFEED	**CELLS NON AGREG.**
1	100	< 1	↑	↑↑	✓	×	✓	×	×	×	×	✓
1	100	< 1	×	↑↑	×	0.015 h^{-1}	✓	×	✓	×	×	×
1	200	< 1	×	↑↑	×	0.015 h^{-1}	✓	×	✓	×	×	×
1	50	< 1	↑	↑↑	✓	×	✓	×	×	×	×	×
1	50	< 1	×	↑↑	×	0.04 h^{-1}	✓	×	✓	×	×	×
2	50	< 1	↑	↑↑	✓	×	✓	×	×	×	×	✓
2	50	< 1	×	↑↑	×	0.04 h^{-1}	✓	×	✓	×	×	×
2	300	< 1	×	↑↑	×	0.04 h^{-1}	✓	×	✓	×	×	×
0.5	50	< 1	↑	↑↑	✓	×	✓	×	×	×	×	✓
0.5	50	< 1	×	↑↑	×	0.035 h^{-1}	✓	×	✓	×	×	×
0.5	50	< 1	×	↑↑	×	0.035 h^{-1}	✓	×	✓	×	×	×
2	300	< 1	↑	↑↑	✓	×	✓	×	×	×	×	✓
2	300	< 1	×	↑↑	×	0.015 h^{-1}	✓	×	✓	×	×	×
2	300	< 1	↑	↑↑	✓	×	✓	×	×	×	×	×
2	300	< 1	×	↑↑	×	0.015 h^{-1}	✓	×	✓	×	×	×
2	200	< 1	↑	↑↑	✓	×	✓	×	×	×	×	✓
2	200	< 1	×	↑↑	×	0.02 h^{-1}	✓	×	✓	×	✓	×
2	200	< 1	↑	↑↑	✓	×	✓	×	×	×	✓	×
1	180	< 1	↑	↑↑	✓	×	✓	×	×	✓	×	✓
1	180	< 1	×	↑↑	×	0.02 h^{-1}	✓	×	✓	✓	×	✓
1	300	< 1	×	↑↑	×	0.02 h^{-1}	✓	×	✓	✓	×	×
1	300	< 1	×	↑↑	×	0.02 h^{-1}	✓	×	×	✓	×	×
1	300	< 1	↑	↑↑	✓	×	✓	×	×	✓	×	×
0.5	150	< 1	↑	↑↑	✓	×	✓	×	×	✓	×	✓
0.5	150	< 1	×	↑↑	×	0.02 h^{-1}	✓	×	×	✓	×	×
0.5	150	< 1	↑	↑↑	×	0.02 h^{-1}	✓	×	×	✓	×	×
0.5	150	2	↑	↑	✓	×	×	✓	×	✓	×	✓/×
0.5	150	2	↑↑	×	×	0.02 h^{-1}	×	✓	✓	✓	×	✓
0.5	150	2	↑↑	×	×	0.02 h^{-1}	×	✓	✓	✓	×	×
0.5	150	2	↑	↑	✓	×	×	✓	✓	✓	×	×
0.5	150	2	×	↑	✓	×	×	✓	✓	✓	×	×

Table 1: Parameters studied in the several tests carried out. - : Small part of the total flow rate; - - : Big part of the total flow rate; ✓ : proper growth; × : cell attachment.

After this complete series of trial testing almost all the possible operational conditions in the air-lift reactor, it was considered that in the Melissa Pilot Plant Lab there is another photobioreactor (Applikon, 2.4 L) running with *R. rubrum* cultures where the cell attachment problem usually does not appear. That means that *R. rubrum* cells attach to the glass surface in the gas-lift reactor due to an environmental condition. Comparing both photobioreactors the main difference that can be observed is the

agitation system. In the gas-lift reactor the mixing is due to the gas flow, meanwhile the smaller reactor is provided of mechanic stirring. Therefore, a potential explanation for this difference could be that the higher shear stress provided by the mechanical stirring could prevent the phenomenon of cell attachment.

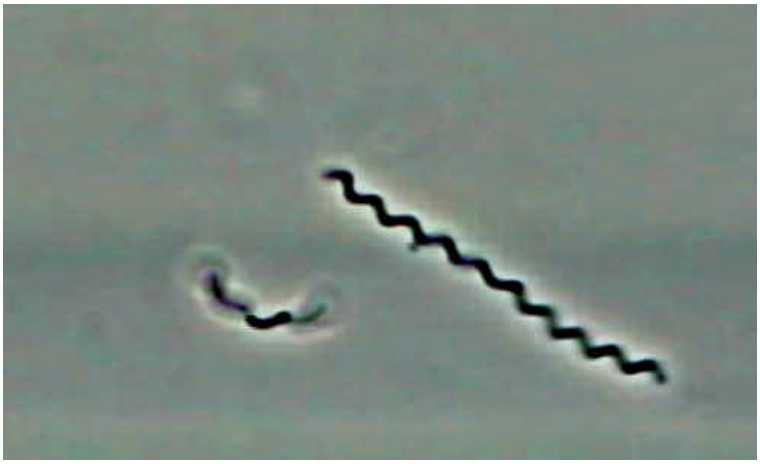

Figure 7: Microscopical observation of R. rubrum morphology. Left: normal mobile spiral shaped cell. Right: abnormal long non-mobile cell.

In order to check this hypothesis, an experiment was carried out in the 2 L Applikon reactor, shifting from the mechanical stirring normal situation to gas lift stirring. That test consisted on, once the culture was running successfully in continuous mode (D=0.02 h^{-1}), the mechanic stirring was switched off and an Argon flow rate was started to bubble the culture in order to mix it. The result can be observed in figure 8.

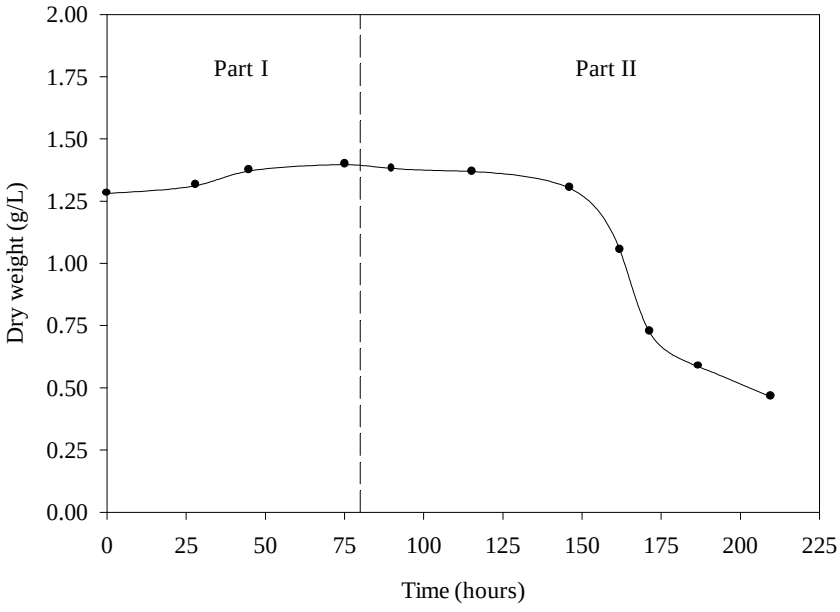

Figure 8: Test carried in a 2.4 L CSTR photobioreactor. The dash line represents the instant when the mechanic stirring was switched off and the gas bubbling switched on.

The results show that the biomass concentration was in a steady state and, after certain time of starting the argon flow, the cells started to wash out. They had stopped to divide cells and they had lost mobility. Watching the cells in the microscope the change of morphology was confirmed.

Because of these series of tests, it was decided to introduce modifications in the hardware of compartment II pilot scale reactor in order to replace gas-lift mixing by mechanical stirring.

Although in table 1 there are some tests that do not show it, parallel experiments demonstrate that *R. rubrum* cells present carbon source inhibition at moderate to high acetic acid concentrations. Thus, this topic will be more deeply addressed in future Technical Notes.

2. Modifications introduced on compartment II

The different modifications introduced in the 7 L Bioengineering reactor for compartment II to make feasible the performance of the pilot photobioreactor are documented in the part of this technical note. The mixing system, the gas and liquids loops and the temperature control loop suffered the most important variations.

1. Temperature control loop

Initially, the culture temperature was controlled by means of an electric heater and a cool finger using ethylenglicol (20 % v/v) at -10 ºC. Depending on the value measured by the Pt-100 probe, a PI controller switched on/off the heater or the electrovalve of the cooling circuit. This system had a successfully performance although it was somewhat unsteady in front of a disturbance, such as a step in the light intensity.

In order to solve the cell attachment problem, due to the need to avoid any possible temperature micro-gradients in front of the lamps on the glass surface, it was decided to build a new glass cylindrical vessel with a glass external jacket. By means of the MICON P-100 and the Pt-100 probe, the set-point of the thermostatic bath is varied in order to control the temperature of the water that flows through the glass jacket and, thus, to maintain the culture temperature at 30 ºC.

Because of having a glass jacket in the reactor, the relationship between the voltage supplied to the lamps and the incident light has been changed. Therefore, a new calibration of the incident light has been carried out (Appendix 2).

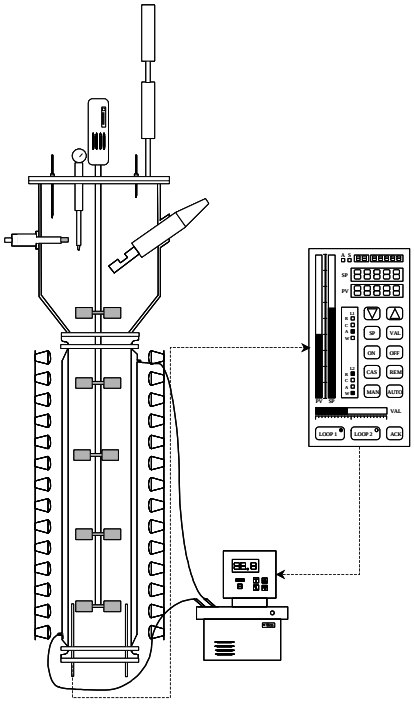

Figure 9: scheme of the temperature control loop of the pilot reactor.

2. Mixing system

As a result of checking that the gas flow through the culture has a negative effect from the physiology point of view and the fact that a mechanically stirred reactor showed a successful performance, it was decided to modify the gas-lift reactor into a mechanically stirred reactor. Therefore, several physical modifications were carried out, such as fitting a straight axis with several propellers on the lid of the reactor and removing the internal glass tube of the gas-lift system. This modification has the added advantage of eliminate gas consumption.

The stirring system comprises seven propellers are seven propellers. Its geometry is described in appendix 1 and it has been designed in order to provide a 80% axial mixing and a 20% radial mixing, because of the narrow shape of the photobioreactor.

3. Gas loop

With the change to the mechanical stirring, the gas loop in the reactor has been modified considerably, as now is only used to maintain a certain overpressure. The head of the reactor is connected after the ceramic filter to an Argon pipe, which maintains an overpressure in the headspace of 150 mbar, which decreases contamination risks.

4. Liquid loops

In order to avoid a possible bypass of the fresh medium some changes in the liquid loop have been produced. At present time, the fresh medium is fed in the reactor through the bottom lid, while the output medium is taken from the top part, maintaining the liquid level constant at eight litres. The acid and the alkali flows that control the culture pH are introduced, as the fresh medium, through the lower lid of the reactor.

3. Final configuration of compartment II

An important consequence of the removing of the internal glass tube is the increasing of the operating volume of the reactor, which has augmented from 7 L to 8 L. Therefore, as the dark volume has been maintained constant, the working illuminated volume has increased to the 61.5 % of the total volume.

In order to study the hydrodynamic behaviour of the reactor and verify whether it has a complete mixing, a RTD test has been carried out. Moreover, a continuous test has been done with the purpose of checking the performance of the pilot photobioreactor and that continue culture could be performed avoiding cell attachment.

RTD experiment

Material and methods

Blue dextran was chosen as the trace element due to several reasons. On one hand, its colour makes that its concentration could be easily followed by means of a spectrophotometer. On the other hand, at a pH value around seven, its colour at 618 nm does not vary significantly depending of the pH value.

In appendix 3 is shown the calibration between the absorbance at 618 nm and the blue dextran concentration.

A concentrated solution of blue dextran (16.0755 g/L) was prepared and a volume of 246.5 mL was injected in a very short time in the reactor through the lower lid. During the test, the liquid that had come out from the reactor was collected in a tank. Therefore, measuring its volume, it was possible to calculate the average flow rate: 0.171 L/h.

In order to find the coefficients that give the best fit between the theoretical profile and the experimental data, a least square procedure with a Marquardt-Levenberg algorithm has been used. The expected theoretical profile corresponding to a mass balance in a perfectly mixed tank in the experiment performed is (Levenspiel, 1979):

$$C = 0.438\, e^{-0.022 \cdot t}$$

Results and discussion

The experimental data obtained in the RTD test are depicted in figure 10. The fitting of a CSTR profile to the experimental data is also shown. In can be appreciated the fact that the difference between the experimental data and the CSTR profile is smaller than the experimental error.

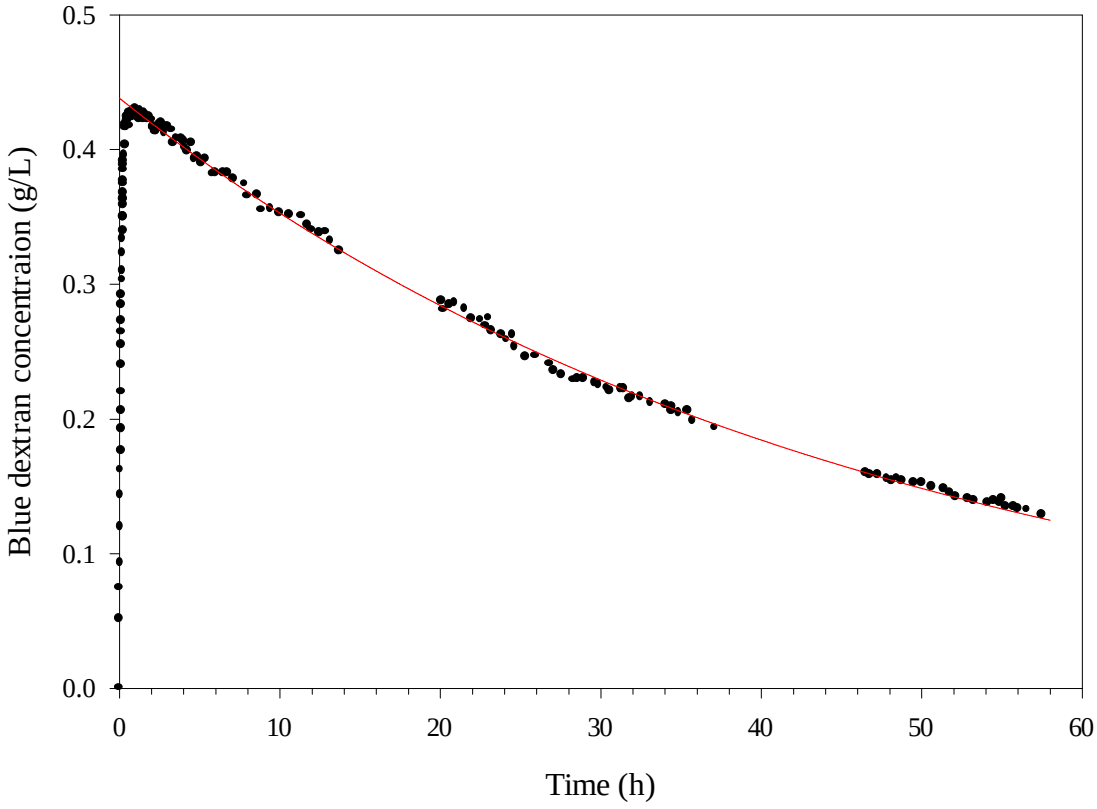

Figure 10: RTD test profile

The theoretical residence time used in the test was 46.66 hours, while from the fit to the experimental data the experimental residence time calculated is 46.21 hours. The relative error between both results is smaller than 1%. Therefore it can be concluded that the new mechanical stirring incorporated in the reactor provides a perfect mix behaviour.

Once the reactor is well characterized from the hydrodynamic point of view, it is necessary to check whether the cell attachment trouble has already disappeared.

Pilot reactor performance checking test

In order to check the performance of the new configuration of the pilot reactor a continuous test has been carried out. The reactor was inoculated with 1 L of inoculum. Once the *R. rubrum* cells were growing exponentially in the reactor with acetic acid as the only carbon source, at time equal to 94 hours, the continuous culture was started. The dilution rate was 0.02 h^{-1} and, approximately, at time equal to 160 hours, the steady state on the biomass concentration was achieved (0.69 g/L) and kept during more than 60 hours (1.2 residence times). During this period no cell attachment was observed, and the cell concentration profile was quite steady and the operation of the reactor satisfactory. Therefore, it can be concluded that the changes introduced enable to achieve a correct performance of this compartment that will be used in continuous runs to fully characterize this compartment. Experimental data are depicted on figure 11.

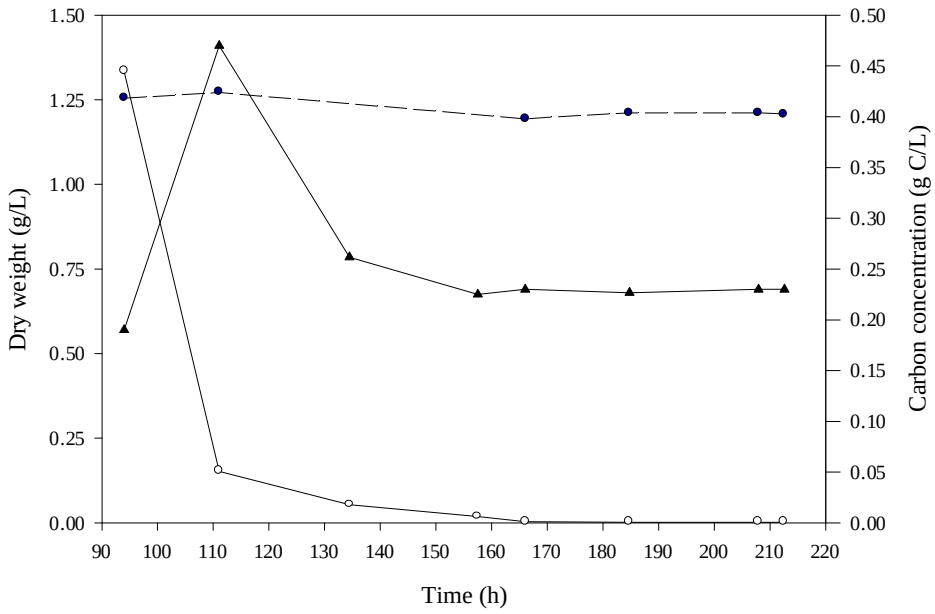

Figure 11: Continuous test using the 8 L pilot photobioreactor after hardware change to mechanical stirring. Its performance is checked successfully. Triangular points correspond to biomass dry weight and round points to carbon concentration (both in the fresh medium and in the culture).

4. Appendix

Appendix 1: Stirring system diagram

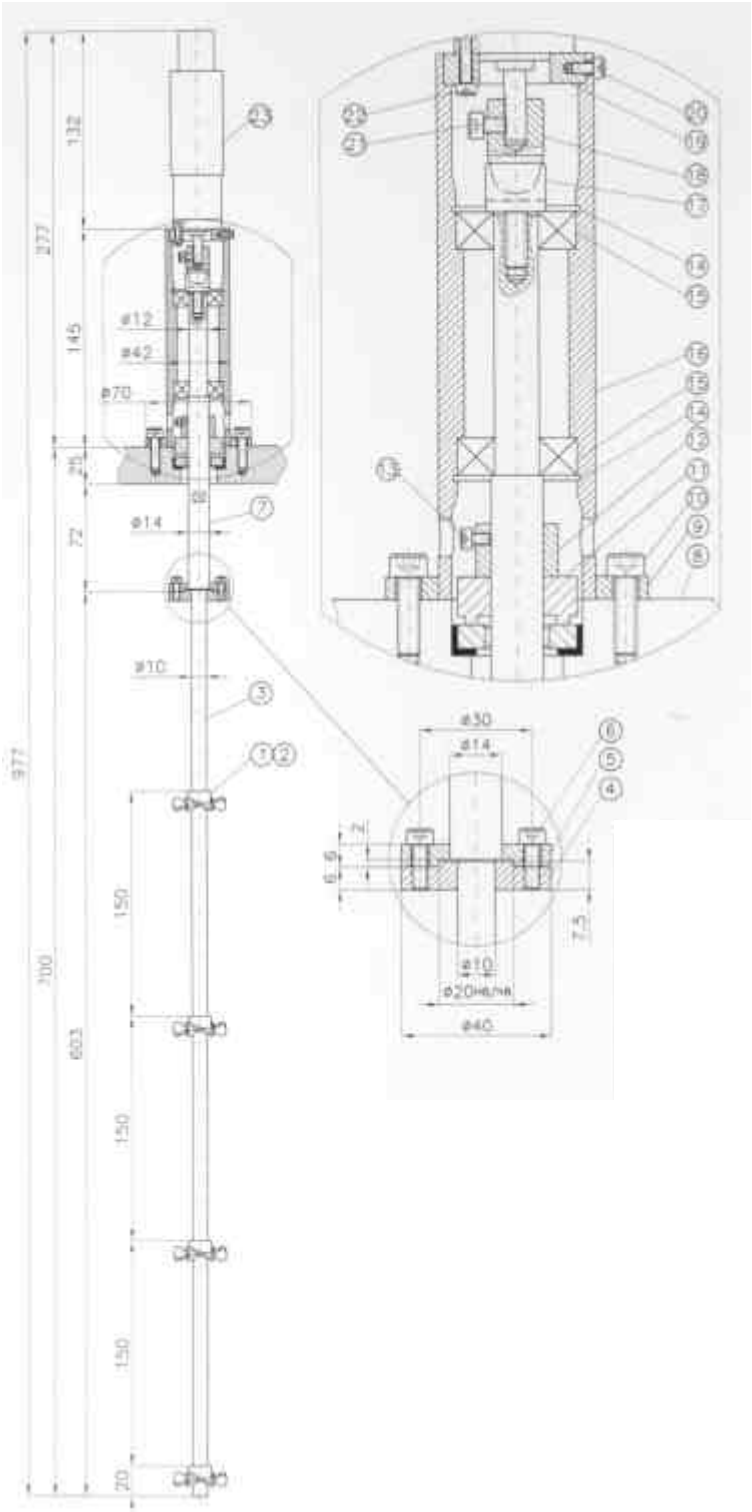

Figure 12: Scheme of the ball bearing and the stirring axis

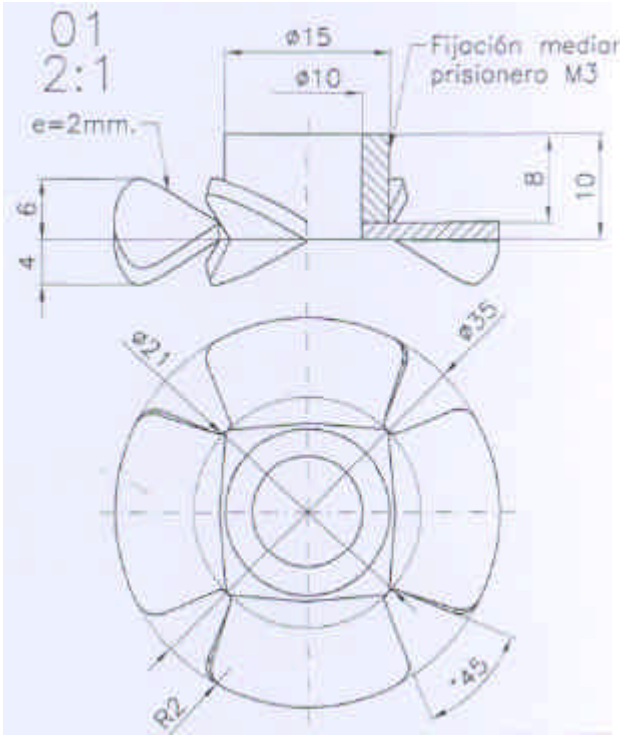

Figure 13: Scheme of the propeller used in the system

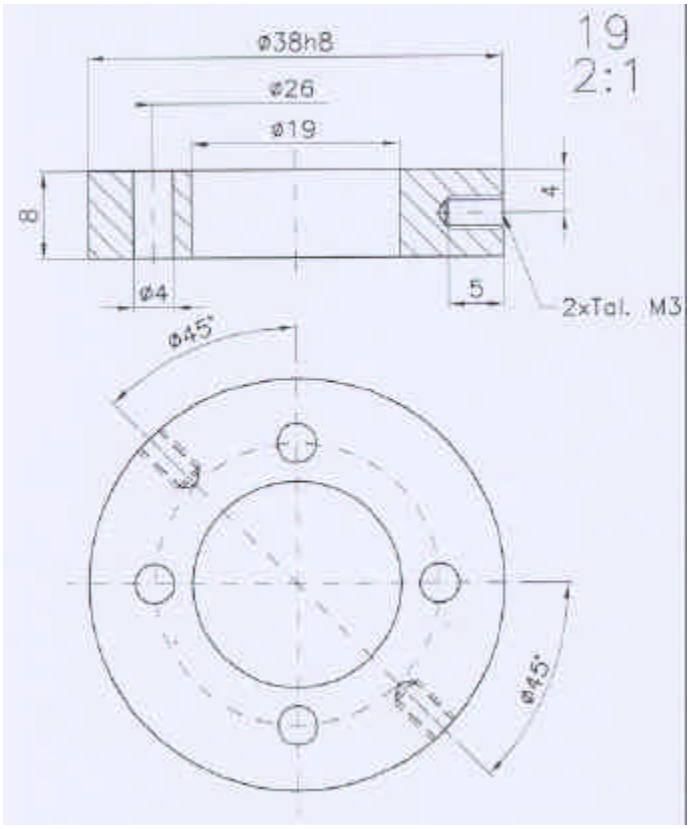

Figure 14: Scheme of the basis where is located the electrical engine.

Appendix 2: Incident light calibration

In order to measure the available light inside the photobioreactor it has been used a spherical quantum sensor (LI-193SA), attached to an amplifier LI-250 (Lincoln, NE, USA), which measures the incident light in all directions.

It is interesting to have a relationship between the voltage supplied to the lamps and the incident light at the surface of the reactor. Therefore, it was measured the incident light locating the spherical sensor in the centre of the vessel at several heights. The points chosen to measure the incident light had to be representative of the light distribution on the reactor along the vertical axis. Due to that reason, positions A, B, C and D were chosen, which located in relation to one lamp, all the possible positions are measured (figure 15). Therefore, an average value of the incident light intensity of these four positions will be a good average value at any point of the reactor on the axial axis.

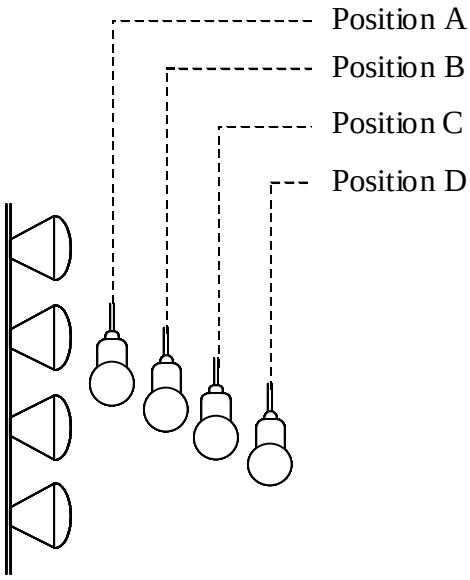

Figure 15: Positions of the sensor regarding to one lamp

In order to modify the voltage supplied to the lamps it was varied the analogical controller output, due to the need of using that relationship for control purposes. Actually, when the GPS will be working, it will be necessary to have a relationship between the analogical controller output and the light supplied to the culture.

In table 2 are listed the light intensity measured by the sensor in positions A, B, C and D and the average value depending on the analogical controller output.

% Controller output	Fr (W/m^2)				
	A	B	C	D	Average
50	1.63	2.39	1.91	1.58	1.88
55	4.29	6.19	5.18	4.23	4.97
60	9.76	14.20	11.88	9.70	11.38
63	15.13	22.04	18.39	15.10	17.66
65	19.85	28.93	24.16	19.84	23.20
67	25.62	37.33	31.18	25.66	29.95
70	36.53	53.17	44.48	36.67	42.71
72	45.58	66.51	55.57	45.82	53.37
74	56.24	82.00	68.01	56.59	65.71
76	68.59	99.97	83.67	69.04	80.32
78	82.64	121.03	101.54	83.75	97.24
80	98.47	143.13	121.90	99.42	115.73
82	117.65	170.95	145.25	119.11	138.24
84	139.25	202.43	171.83	141.15	163.66
86	163.48	237.57	201.84	166.04	192.23
87	176.66	256.97	218.10	184.86	209.15
88	190.64	277.47	235.59	193.28	224.25
89	205.43	299.07	251.85	208.65	241.25
90	218.75	318.10	272.71	224.76	258.58
91	236.33	342.26	293.58	241.60	278.44
92	253.90	367.81	314.51	256.17	298.10
93	272.20	394.46	337.14	276.74	320.13
94	291.01	421.33	360.93	296.87	342.54
95	310.93	450.54	385.75	317.37	366.15
96	331.79	480.85	408.52	338.60	389.94
97	354.71	505.96	436.70	356.90	413.57
98	377.84	543.23	465.62	381.65	442.09
98.5	390.22	560.58	480.26	395.34	456.60
99	402.44	579.39	495.57	408.15	471.39
99.5	427.19	615.71	510.50	420.60	493.50
100	456.47	653.12	526.02	433.04	517.16

Table 2: incident light measured on positions A, B, C and D and the average value.

In figure 16 is represented the average incident light, F_R in W/m^2, versus the analogical controller output. It can be seen that the relationship between the two variables is exponential and if the controller output is lower than 50% the lamps does not illuminate.

The experimental data have been fitted to a theoretical equation, providing an analytical relationship between the controller output and the average incident light supplied to the culture. This relationship is expressed by means of the following equation:

$$F_R\ (W/m^2) = -9.8423 + 1.3620 \cdot\ 10^{-10} \cdot\ (\%\ \text{controller output})^{6.2901}$$

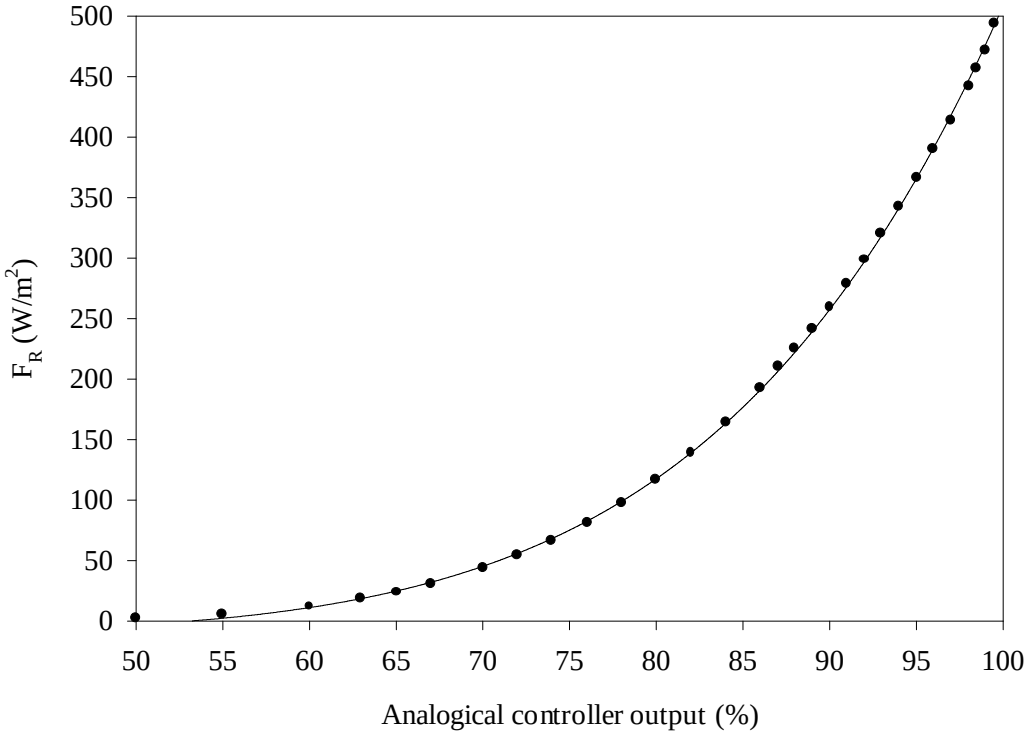

Figure 16: Relationship between the analogical controller output and the incident light supplied to the culture.

Appendix 3: RTD Optical density - concentration calibration

In order to be able to convert absorbance values into concentrations of blue dextran values a twenty points calibration was done.

In figure 17 it is shown the relationship between the absorbance at 618 nm and the blue dextran concentration, in g/L.

The results of the fitting are:

$$C\ (g/L) = -0.0112 + 1.0417 \cdot \text{Absorbance (618 nm)}$$

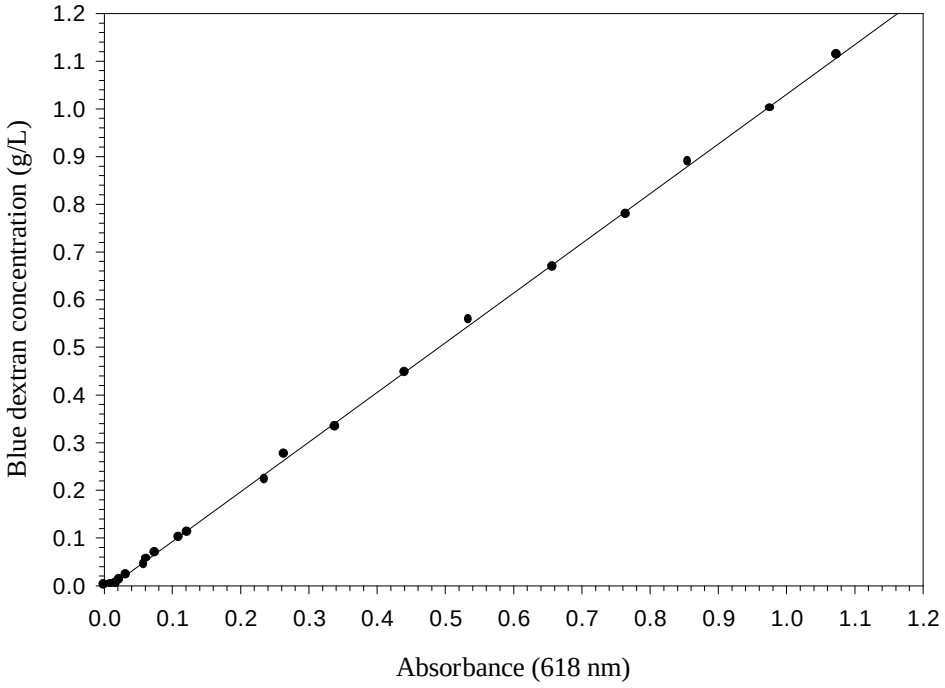

Figure 17: relationship between the absorbance at 618 nm and blue dextran concentration.