Universitat Autònoma de Barcelona
Dep. Enginyeria Química
08193 Bellaterra, Barcelona, Spain

MELISSA

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CARBON LIMITATION IN PHOTOSYNTHETIC COMPARTMENT

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VERNEREY A.; ALBIOL, J.; GODIA, F.

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Draft	0	10/04/2000	Preliminary Version
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Table of contents

1 Introduction

Carbon concentration mechanisms have been deeply described in cyanobacteria by numerous authors and there is a great number of bibliographic references dealing with k_La determination and modelling in airlift reactors. Nevertheless, only very few publications report the impact of CO_2-limitation on the culture of photoautotrophic micro-organisms in photobioreactors (Contreras et al, 1998 ; Camacho Rubio et al, 1999).

In T.N. 32.4 (Cornet et al, 1998), an analysis of carbon limitation in the *Spirulina* compartment was done by Cornet et al. The experiments described in this T.N were all performed in batch mode and had to be completed by continuous cultures. Moreover, a regime analysis, consisting in the comparison of the characteristics times of the process obtained by physical characterisation of the 77 l photobioreactor in the MELISSA pilot-plant, suggested that CO_2 supply could be a critical point for the operation of this compartment. For this reason, it was very interesting to investigate the behaviour of CO_2-limited continuous cultures in this reactor.

2 Materials and Methods

The reactor used in this study was a 77 l external loop airlift bioreactor which configuration is described in detail in TN 37.2 (Vernerey et al, 1998) and its physical characterisation appears in TN 43.110 (Vernerey et al, 1997).

For the realisation of these experiments, the gas-loop previously described in T.N. 37.2 was modified in order to allow the on-line measurement and control of gas composition, pressure and flow-rate in the entrance and the outlet of the reactor. The changes introduced in this circuit can be visualised on figure 1. In this configuration, the required mixtures in terms of CO_2 and air are obtained by mixing pressurised CO_2 and air. Three flow controllers/meters are used to realise the mixture and another flow controller/meter regulated the supply of gas into the reactor. The reactor is operated in open-gas circuit and a fourth gas flow-meter/controller is used in order to measure the

gas flow in the output of the reactor. The composition of the gas phase can be measured on-line either at the entrance or at the output of the reactor.

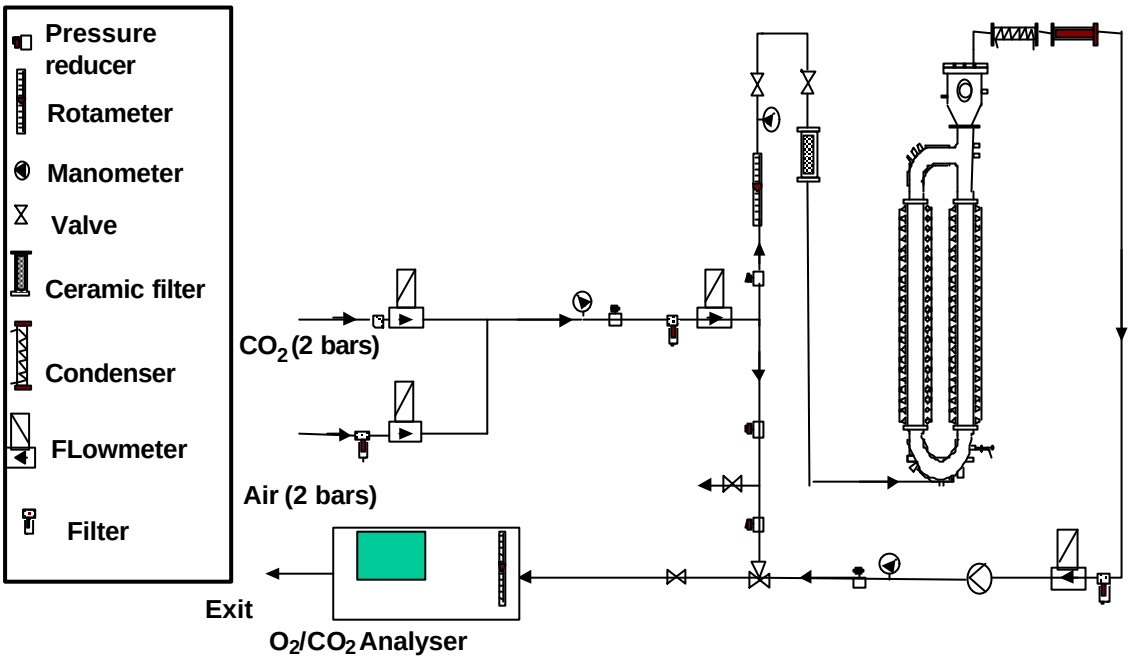

Figure 1 : Scheme of the gas-loop used for the CO_2 limitation experiments

Moreover, Zarrouck medium employed was free of carbonates and bicarbonates and CO_2 was introduced in the culture by the gas phase. The control loop used for pH regulation described in T.N 37.2 was also modified in order to regulate pH value by introduction of acid (figure 2).

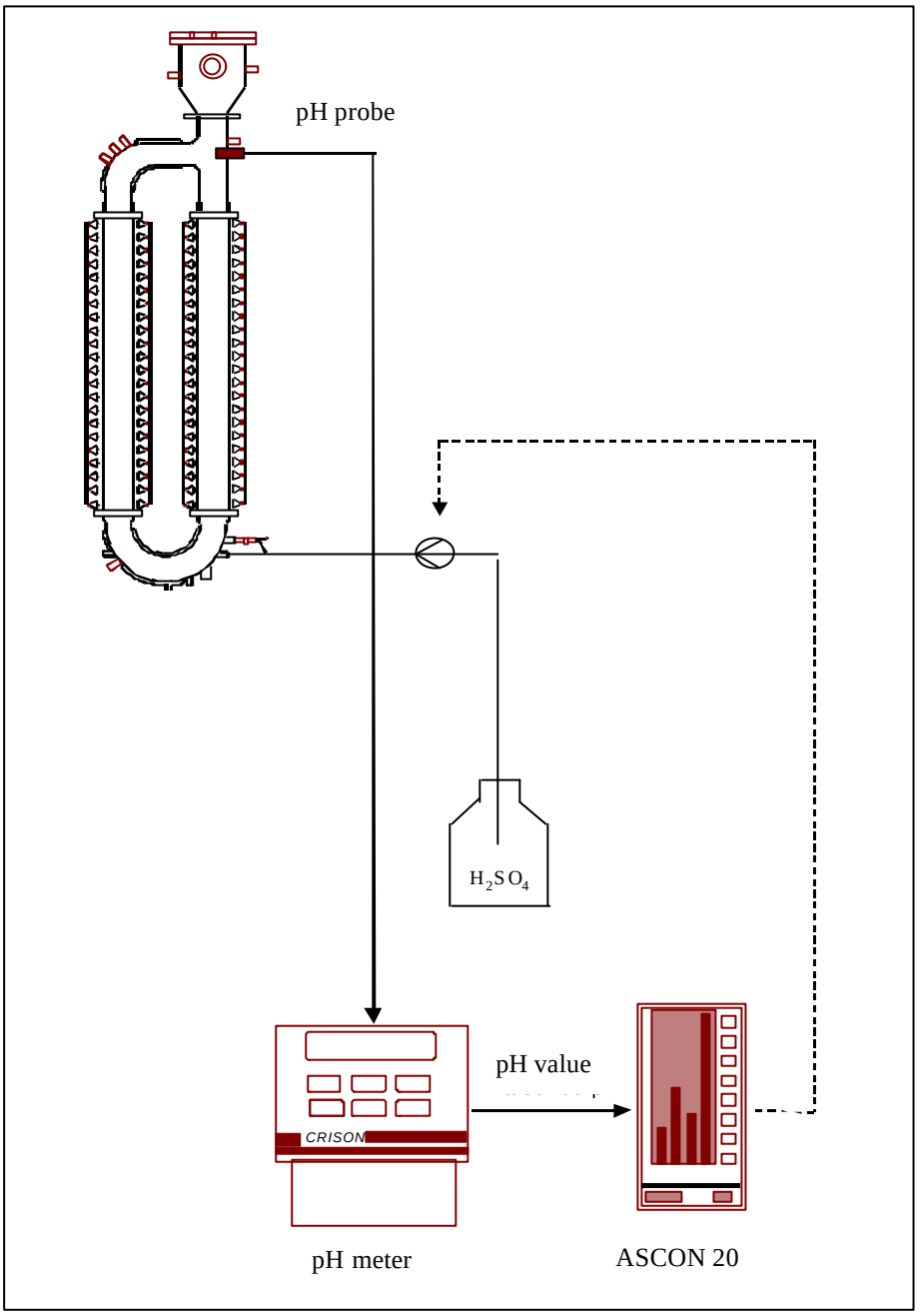

Figure 2 : Scheme of the configuration used for pH regulation.

Total biomass concentration was determined either by dry weight (Whatman Glass Microfibre Filters GF/F, 105 ºC, 24 h) or by spectrophotometry (750 nm). Total inorganic carbon in liquid medium was measured by a TIC-TOC analyser (O.I corporation, 700 TOC analyser, Texas, U.S). The determination is based on CO_2 liberation by acidification of the samples and then CO_2 detection by infrared measurement.

CO_2 concentration in the gas phase was determined by means of IR analysis in a Multor 610 gas analyser (Maihak, Multor 610, Hamburg, Germany).
Total sugars and proteins content of the biomass were also determined. Total sugars were analysed by the phenol method, described by Herbert and al., 1971. Protein content was determined by the modified Lowry method, also described by Herbert et al.

Elemental composition of the biomass was analysed for C, H, O, N, P, S at the Service de Microanalyses, CNRS (Gif-Sur-Yvette, France).

3 Experimental conditions

All the experiments were realised in continuous mode and four steady states were obtained, corresponding to two different values of Fr (155 $W.m^{-2}$ and 225 $W.m^{-2}$) and two values of CO_2 concentration at the entrance of the reactor (1% and 0.5%). As explained in previous Technical Notes, the material of which the illuminated parts of the photobioreactor were done did not allow the use of high gas flow-rates for agitation of the culture and CO_2 supply. The reason being that these parts are made of a material subject to deformation (Polyamid Tripan), that could be damaged or deformed if high gas flow rates are used.

Hence, gas flow rate at the entrance of the reactor was fixed to a value of 3 $l.min^{-1}$ because it had been previously demonstrated that, in these conditions sufficient mixing degree and gas-liquid mass transfer value (k_La) could be achieved (Vernerey et al, 1999). In these conditions of gas introduction (0.04 v.v.m), the carbon concentration in the gas phase was chosen to guarantee limiting conditions and in order to provide a quantity of CO_2 per unit of reactor volume of the same order than the ones used by Cornet et al (1998) during batch cultures. The operating conditions corresponding to each of the steady states are summarised on table 1.

Table 1 : Experimental conditions used for the carbon limitation experiments in the 77 l pilot reactor (*Spirulina* compartment).

Experiment	CO_2-1	CO_2-2	CO_2-3	CO_2-4
D (h^{-1})	0.026	0.026	0.026	0.026
Fr ($W.m^{-2}$)	155	223	155	223
Q gas ($l.min^{-1}$)	3	3	3	3
CO_2 E (%)	1	1	0.5	0.5

4 Results

During each experiment, total biomass concentration, gas phase composition at the inlet and at the outlet of the reactor and residual carbon concentration inside the reactor were measured and registered.

These results are depicted on figures 2 to 10 and the values of biomass concentration obtained, and of the rates of biomass synthesis are summarised on table 2. It is important to note that, during experiment CO_2-1, a fraction of *Spirulina* biomass remained attached on the walls of the bioreactor in the lower part of the downcomer. For this reason, the value of biomass concentration depicted in figure 2 was probably inferior to the real value. For this reason, at the end of this steady state, the reactor was washed and the same culture conditions were established again. In these conditions, a biomass concentration of 0.6 $g.l^{-1}$ was finally obtained (see table 2). This new value was in accordance with the mass balance corresponding to the experimental conditions tested.

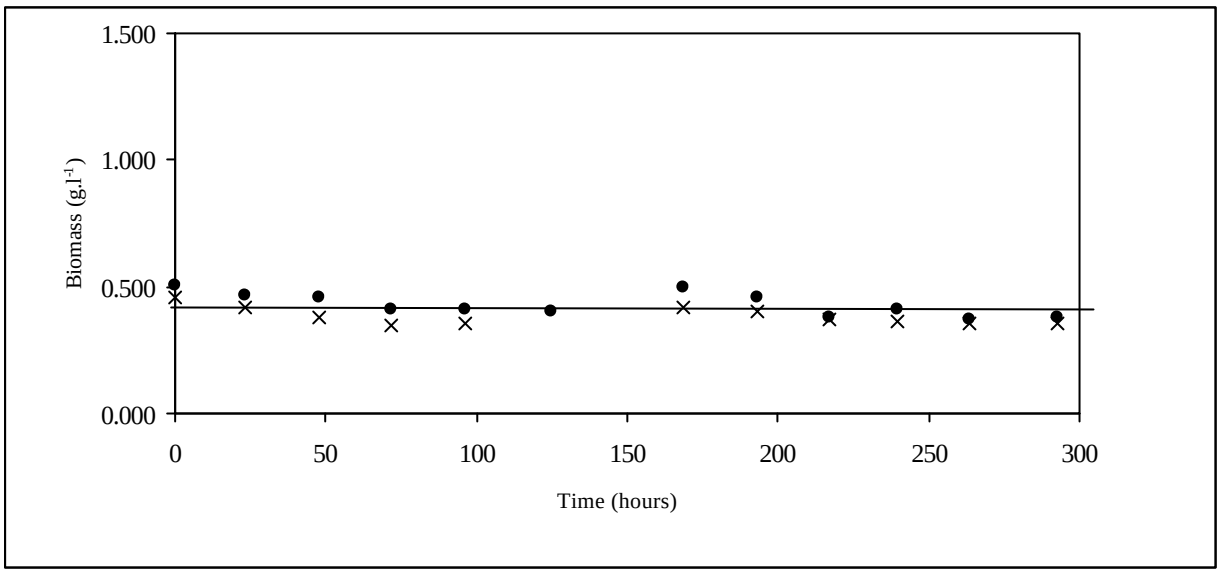

Figure 3 : Evolution of total biomass concentration during experiment CO_2-1. D = 0.026 h^{-1}, [CO_2]in = 1% ; Fr = 155 W/m^2. (x : biomass concentration determination by dry weight ; ● : biomass concentration determination by spectrophotometry).

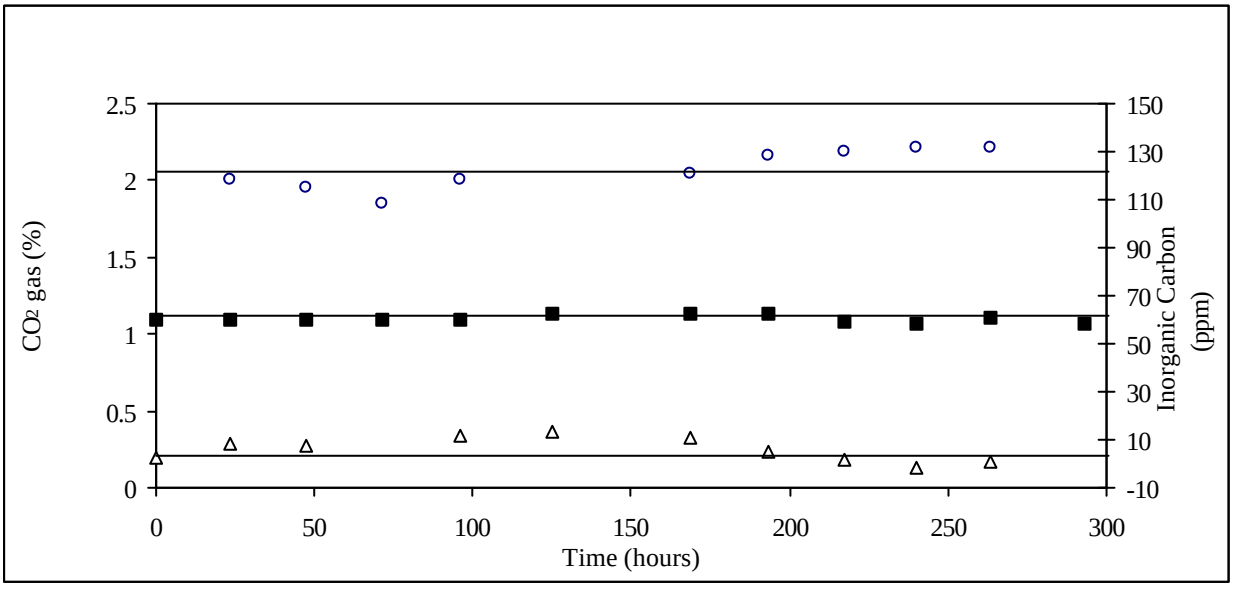

Figure 4 : Evolution total inorganic carbon concentration in liquid phase (O) , CO_2 concentration in the gas phase in the inlet (■) and the outlet (Δ) of the photobioreactor during experiment CO_2-1. D = 0.026 h^{-1}, Fr = 155 W/m^2.

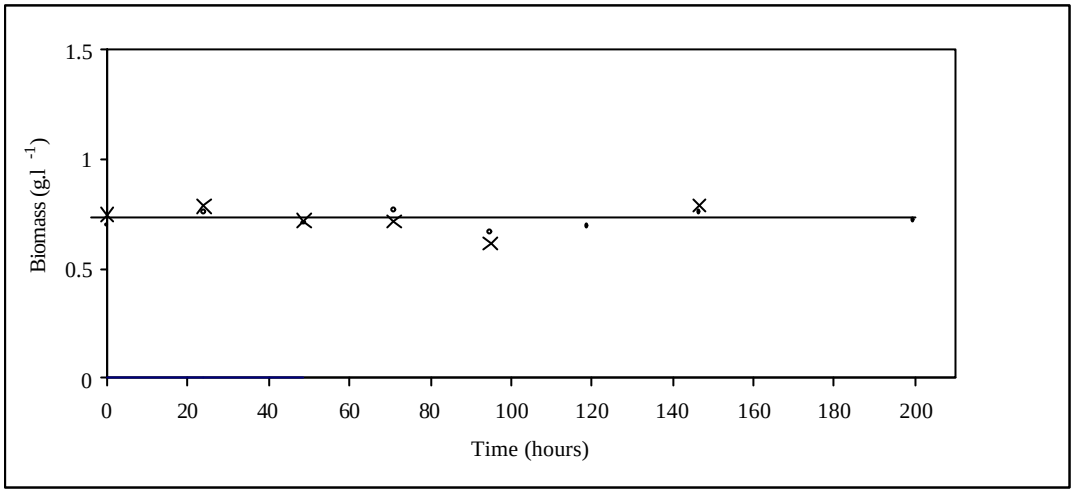

Figure 5 : Evolution of total biomass concentration during experiment CO_2-2. D = 0.026 h^{-1}, [CO_2]in = 1% ; Fr = 305 W/m^2. (x : biomass concentration determination by dry weight ; ● : biomass concentration determination by spectrophotometry).

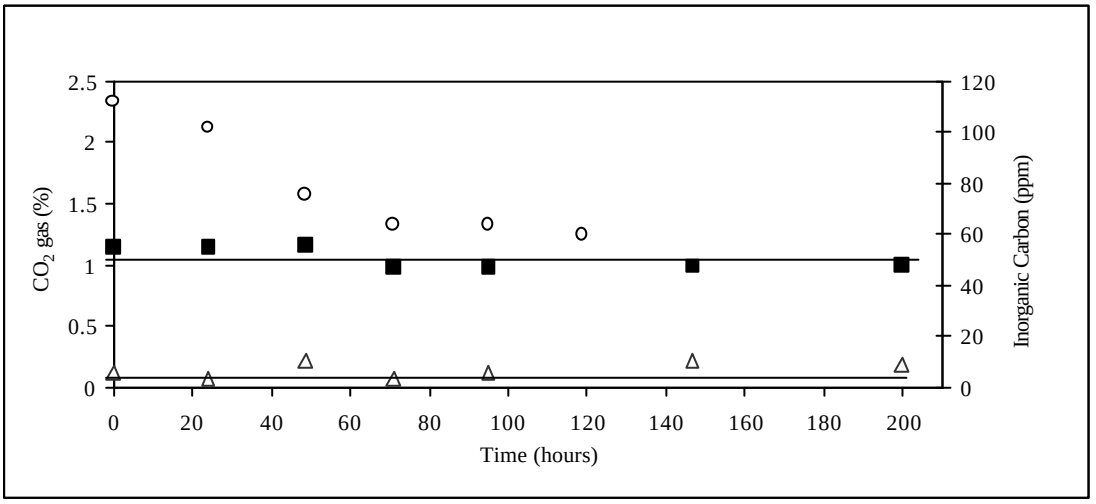

Figure 6 : Evolution total inorganic carbon concentration in liquid phase (O) , CO_2 concentration in the gas phase in the inlet (■) and the outlet (Δ) of the photobioreactor during experiment CO_2-2. D = 0.026 h^{-1}, Fr = 305 W/m^2.

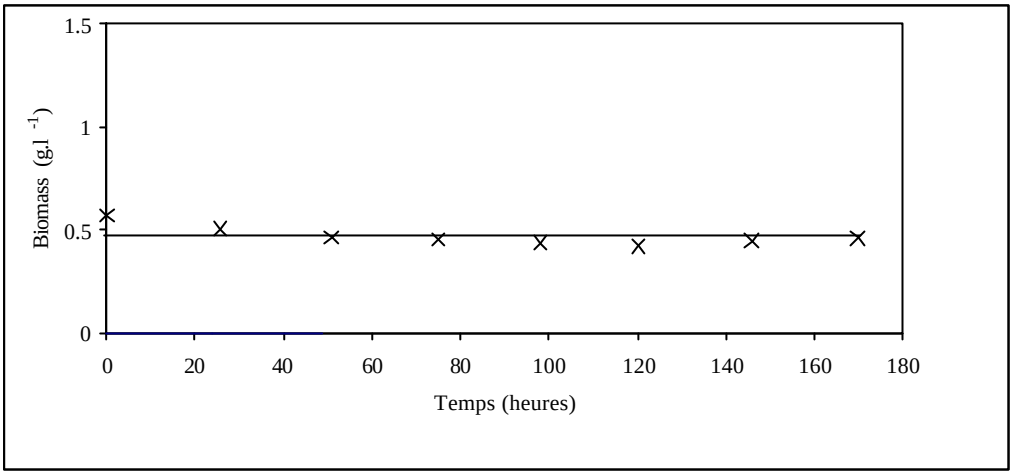

Figure 7 : Evolution of total biomass concentration during experiment CO_2-3. D = 0.026 h^{-1}, [CO_2]in = 0.5% ; Fr = 155 W/m^2. (x : biomass concentration determination by dry weight ; ● : biomass concentration determination by spectrophotometry).

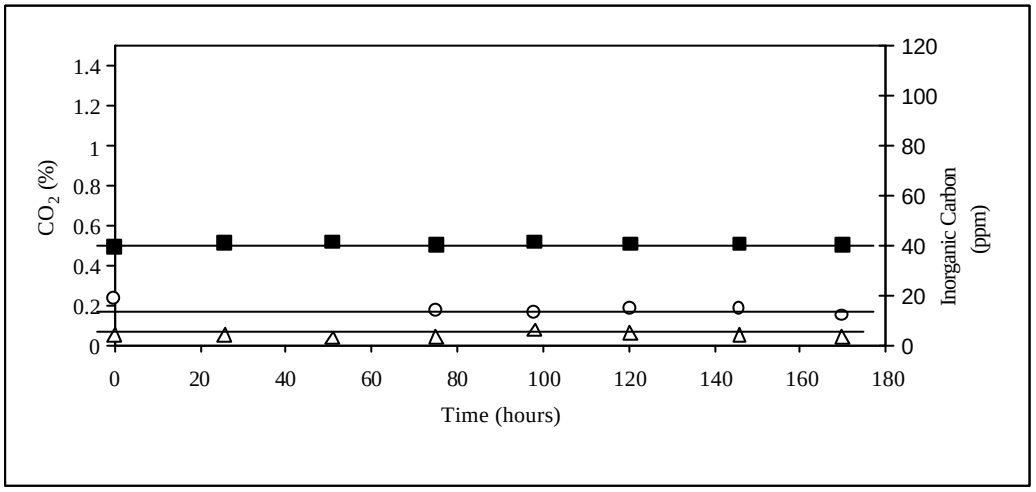

Figure 8 : Evolution total inorganic carbon concentration in liquid phase (O) , CO_2 concentration in the gas phase in the inlet (■) and the outlet (Δ) of the photobioreactor during experiment CO_2-3. D = 0.026 h^{-1}, Fr = 155 W/m^2.

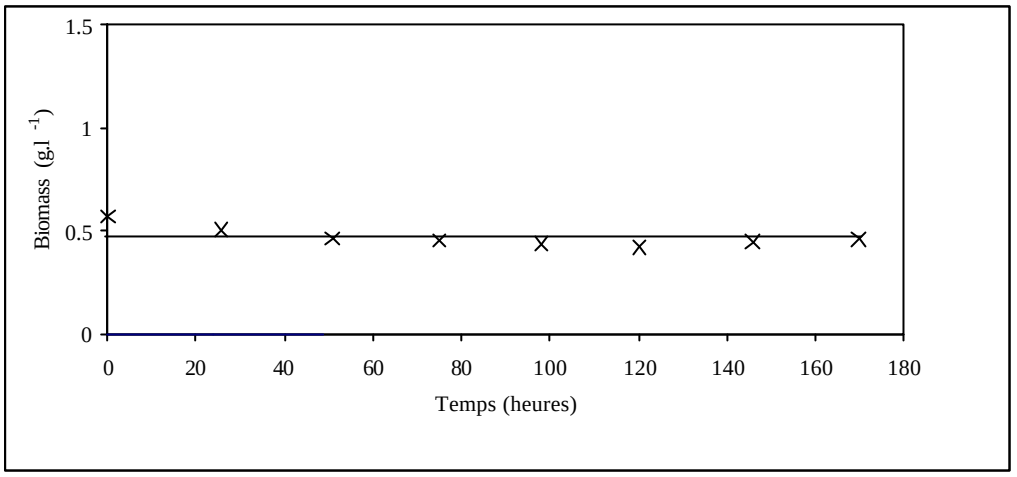

Figure 9 : Evolution of total biomass concentration during experiment CO_2-4. D = 0.026 h^{-1}, [CO_2]in = 0.5% ; Fr = 305 W/m^2. (x : biomass concentration determination by dry weight ; ● : biomass concentration determination by spectrophotometry).

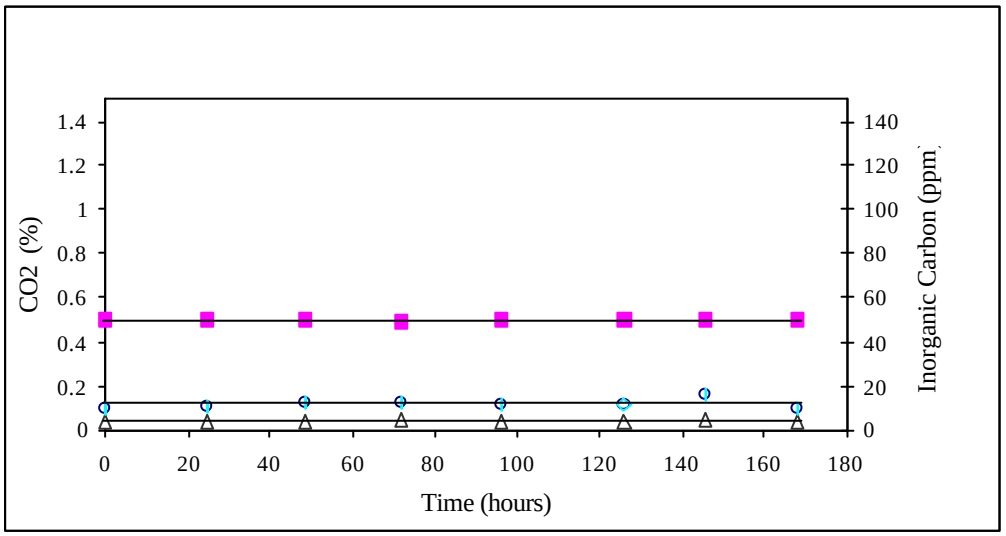

Figure 10 : Evolution total inorganic carbon concentration in liquid phase (O) , CO_2 concentration in the gas phase in the inlet (■) and the outlet (Δ) of the photobioreactor during experiment CO_2-4. D = 0.026 h^{-1}, Fr = 305 W/m^2.

Table 2 : Biomass concentration , growth rates and C concentration in the liquid and gas phases obtained during C-limited continuous cultures in the 77 l photobioreactor ($\%CO_{2inlet}$: CO_2 concentration in the gas phase at the inlet of the bioreactor ; r_{XT} biomass growth rate ; $\%CO_{2outlet}$: CO_2 concentration in the gas phase at the outlet of the bioreactor ; TIC : total inorganic carbon)

D (h^{-1})	0.026	0.026	0.026	0.026
Fr	155	223	155	223
% CO_{2Inlet}	0.5	0.5	1	1
[Biomass] ($g.l^{1}$)	0.47	0.46	0.6	0.73
r_{XT} (* 10^3 $g.l^{1}.h^{-1}$)	12.21	11.94	15.58	18.96
$\%CO_{2Outlet}$	0.05	0.05	0.15	0.13
TIC liquid (ppm C)	13	13	120	70

As shown in the different figures, in the case of a CO_2 concentration in the inlet of the reactor of 1 % (corresponding to steady states CO_2-1 and CO_2-2), an increase of the value of Fr from 155 to 223 $W.m^{-2}$ induces an increase in total biomass concentration, that demonstrates a clearly co-limitation between light and CO_2 in this situation. On the contrary, when the CO_2 concentration is lower (0.5%), no changes in biomass concentration are observed for the two values of Fr tested.

Moreover, for a CO_2 concentration of 1 % in the entrance of the bioreactor, limiting carbon concentration in the liquid medium was lower at higher radiant light intensities. This effect confirms that carbon concentration mechanisms in *Spirulina platensis* are strongly dependent on light intensity. This phenomenon was demonstrated by other authors that studied the mechanisms of carbon concentration in cyanobacteria (Kaplan et al, 1987; Miller et al, 1991). In fact, these mechanisms are energy consuming and can reduce the efficiency of photosynthesis under carbon limitation. In other words, when the energy provided to the cells was more important, the amount of CO_2 that *Spirulina platensis* was able to metabolize was higher.

The comparison of the biomass growth rate with the results obtained by Marty 1997, revealed that the results are of the same order of magnitude. As explained before, we choose experimental conditions as close as possible (but in continuous culture) to the ones used by Marty in her phD thesis. However, it is important to remark that the

experiments performed by Marty were all run in batch mode and in a stirred tank photobioreactor. On the contrary, we worked in continuous mode in an airlift reactor, and a direct comparaison of the data obtained is difficult.

Chemical composition of the samples obtained during the different steady-states is presented in table 3. The data show that protein content is not significantly affected by changes in the CO_2 concentration and in the light intensity, whereas total sugars concentration presents a little decrease when light is increased. In T.N 32.4, Cornet et al observed an inhibition of exopolysaccharide synthesis concomitant with a decrease in total proteins fraction. In our case, the deviations observed are not so strong (especially for protein content), but total sugars concentration is rather low when compared to previous experiments performed in no-limiting CO_2 concentration conditions.

Table 3 : Concentration and composition of the biomass obtained during the carbon limitation experiments in the 77 l pilot reactor (*Spirulina* compartment).

Experiment	CO_2-1	CO_2-2	CO_2-3	CO_2-4
Total Biomass ($g.l^{-1}$)	0.6	0.73	0.47	0.46
Proteins (%)	43.5	47.5	48.3	44
Total Sugars (%)	25.5	19.1	31.1	20.6

The analysis of elemental composition (table 4) and of the corresponding carbon molar formulae (table 5) demonstrates that, in the conditions tested, the application of different CO_2 concentration in the inlet gas flow and of different incident light fluxes (Fr) did not produce marked effect on the C, H, N, O, P fractions of the biomass. However, the brut formula of the biomass obtained is quite similar to the one described by Marty (1997) during batch experiments in carbon limitation conditions and corroborates the observation that, the H/O ratio is higher than in no-limiting conditions. This modification of the elemental composition was explained by Marty by the fact that the metabolism of carbon-limited *Spirulina* cells could be modificated and lead to an increased synthesis of unsaturated lipids.

Table 4 : Elemental composition of the biomass obtained during the carbon limitation experiments in the 77 l pilot reactor (*Spirulina* compartment).

Experiment	CO_2-1	CO_2-2	CO_2-3	CO_2-4
C	41.1	42.4	41.4	37.6
H	6.2	6.5	6.35	6.2
O	30.5	27.5	28.6	31.5
N	9.2	9.4	8.58	8.16
P	1.9	1.71	1.22	1.85

Table 5: Brut formula of the biomass obtained during the carbon limitation experiments in the 77 l pilot reactor (*Spirulina* compartment).

Experiment	Brut formula
CO_2-1	$CH_{1.81}O_{0.55}N_{0.19}$
CO_2-2	$CH_{1.84}O_{0.48}N_{0.19}$
CO_2-3	$CH_{1.84}O_{0.5}N_{0.18}$
CO_2-4	$CH_{1.97}O_{0.61}N_{0.19}$

5 Conclusions

The operation of the new pilot reactor was demostrated by carbon supply in the gas-phase. The operation of the reactor was stable during a period that was considered relevant.

When working with a low CO_2 concentration (0,5%) in the inlet of the reactor, total inorganic concentration in the liquid phase was of 13 ppm. In this case, the operation was limited by the carbon source, obtaining similar results for different illumination conditions.

At higher carbon concentration conditions at the inlet (1%), the results indicate an interaction between CO_2 and light limiting effects, probably as a consequence of the energy requirements for CO_2 uptake in these conditions.

Althought the obtained results allow us to perform preliminary calculations, it is proposed to perform new experiments in order to ascertain and better characterise the kinetics of these co-limitation processes.

6 References

Contreras A., Garcia F, Molina E. And Merchuk J.C., 1998. Interaction between CO2 mass-transfer, light availability and hydrodynamic stress in the growth of Phaeodactylum tricornutum in a concentric airlift photobioreactor. Biotechnol Bioeng, 60, 3, 317-325