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

MELISSA

Memorandum of Understanding
ECT/FG/MMM/97.012

Contract Number: ESTEC/CONTRACT13292/98/NL/MV

TECHNICAL NOTE: 47.6

Connection of compartments III and IV at pilot scale

Version: 1
Issue: 0

CREUS N.; PÉREZ J.; MENGUAL J.; MONTESINOS J.L.; ALBIOL, J.; GÒDIA, F.

JULY 2001

Document Change Log

Version	Issue	Date	Observations
Draft	0	20/3/01	Preliminary Version
1	0	26/07/01	Final Version

TABLE OF CONTENTS

1.- INTRODUCTION

The MELISSA project (Microbiological Ecological Life Support System Alternative) of the European Space Agency (ESA) is a tool for 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.

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 behavior of any of them.

Once the connection between compartments II, III and IV at laboratory scale (Creus *et al.*, 1999 and 2001) has been successfully tested, the connection between compartments III and IV at pilot plant scale has been studied.

In this work, a long term connection (more than 3 months), including different deviations from the optimal operational conditions, of compartments III and IV at pilot plant scale has been studied. The three principal aims of this study have been:

1.- Studying the feasibility of a long term connection.

2.- Studying the effect of the income of nitrite and ammonium in compartment IV due to a change of the dilution rate or due to the decrease of oxygen in compartment III.

3.- Verification of the performance of the control system of compartment IV while compartments III and IV are interconnected.

2.- SET-UP AND MATERIALS AND METHODS

2.1.- SET-UP

2.1.1.-Compartment III

A 3.8L fixed bed reactor with immobilized cells on a Biostyr support has been used. A more detailed description of this column and its instrumentation is found in Pérez (1997). A co-culture of *Nitrosomonas europaea* and *Nitrobacter winogradskyi* is grown in this compartment. This reactor operates at the optimal conditions required for the nitrification process. The pH is set at 8.2, the temperature at 30ºC, an aeration of 3L/min is used. The whole reactor is covered with aluminium foil to avoid the presence of light inside the reactor. In order to have a better homogeneization between the inlet and the recirculation flows a magnetic stirring at 400rpm is used. The recirculation flow rate is set at 30L/day. A picture of this compartment is found in figure 1.

2.1.2.-Compartment IV

A 77L air-lift with an external loop is used as compartment IV. A more detailed description of this reactor and its instrumentation is given by Vernerey (Vernerey, 2000). This reactor works at the optimal conditions required by *Spirulina platensis*. The pH is set at 9.5 and the temperature at 36.5ºC. The calibration of the incident light intensity as a percentage of the controller signal is found in section 2.2.4 of this document. A picture of this compartment is found in figure 2.

2.1.3.-Connection between compartments III and IV

The connection between compartment III and IV is done by means of two filtration steps and a 10L buffer tank. The two filtration steps (liquid filters MILLIPORE OPTICAP ^{TM} 4") eliminate the biomass outcoming from compartment III and, taking into account that one of the filters is placed in the outlet of compartment III and the other one in the inlet of compartment IV, these filters maintain both reactors isolated allowing their disconnection without having any contamination problems. The buffer tank provides a safety margin in order to allow some fluctuations or small changes in one of the compartments.

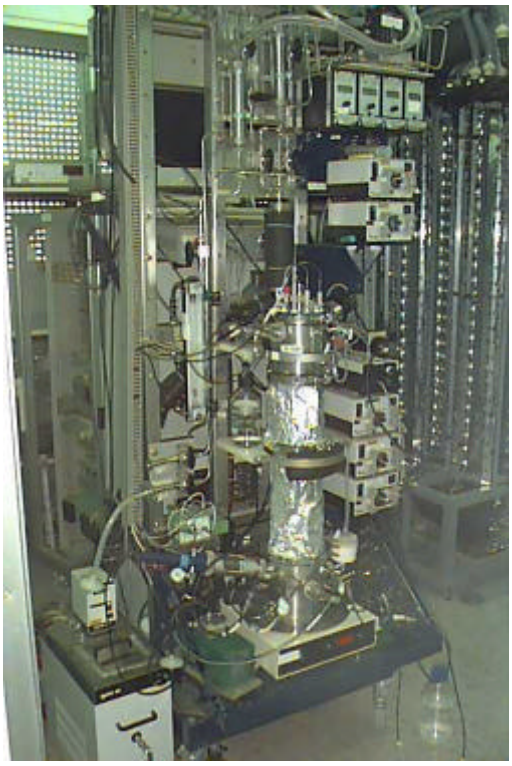

Figure 1.- View of compartment III at pilot scale

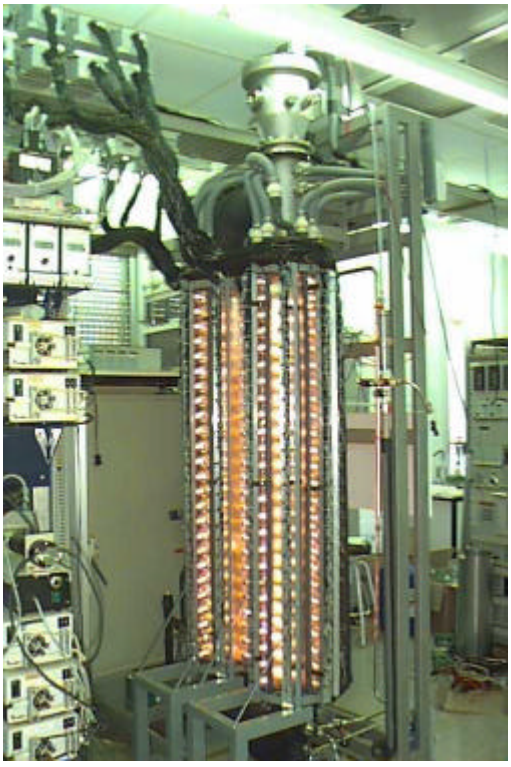

Figure 2.- View of compartment IV at pilot scale

2.2.- MATERIALS AND METHODS

2.2.1.- Strains and inoculum

The strains of *Nitrosomonas europaea* and *Nitrobacter winogradsky*i used were 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, J. *et al*., 1997 a) is used to inoculate the reactor.

The *Spirulina platensis* strain was obtained from the Pasteur Institute*: Arthospira platensis* (PCC 8005). *S. platensis* is revived and the subcultures are done using their recommended medium (provided in section 2.2.2). The inoculum's volume is fixed as the 10% of our working volume (77L).

2.2.2.- Culture media

Compartment III

The culture medium used in compartment III when disconnected from compartment IV is based in a mixture of an adapted medium for *Nitrosomonas europaea* (Wijffels, 1994) and an adapted medium for *Nitrobacter winogradskyi* (Hendrikus et col., 1992) and (Wijffels, 1994). This medium is described in (Pérez J., 1997) and is given in table 1.

***N. europaea and N.winogradskyi* medium**	
Compound	*g/L*
$FeSO_4 \cdot 7\ H_2O$	2.5E-3
KH_2PO_4	0.68
$NaHCO_3$	0.8
$MgSO_4 \cdot 7\ H_2O$	0.052
$CaCl_2 \cdot 2\ H_2O$	7.4E-4
$(NH_4)_2SO_4$	1.32
$CuSO_4 \cdot 5\ H_2O$	4.0E-6
Na_2HPO_4	0.71
$ZnSO_4 \cdot 7\ H_2O$	4.3E-6
$(NH_4)_6Mo_7O_{27} \cdot 4H_2O$	0.177

Table 1.- Composition of the *N. europaea* and *N. winogradskyi* medium

Compartment IV

The culture medium used in compartment IV when disconnected from compartment III is the Zarrouk salt mixture medium (Zarrouk, 1966). This medium is presented in table 2.

***S. platensis* medium**	
Compound	*g/L*
$NaNO_3$	2.5
EDTA-Na_2	0.08
NaCl	1.0
$FeSO_4 \cdot 7\ H_2O$	0.01
Na_2CO_3	7.6
$NaHCO_3$	10.8
$MgSO_4 \cdot 7\ H_2O$	0.2
$CaCl_2$	0.04
K_2HPO_4	0.5
K_2SO_4	1.0
Dissolution	*mL/L*
A5	1.0
B6	1.0

Table 2.- Composition of the *S. platensis* medium where the composition of the dissolutions A5 and B6 are presented in table 2.1 and 2.2

A5	
Compounds	*g/L*
H_3BO_3	2.86
$MnCl_2 \cdot 4\ H_2O$	1.81
$ZnSO_4 \cdot 7\ H_2O$	0.222
$CuSO_4 \cdot 5\ H_2O$	0.079
MoO_3	0.015

Table 2.1.- Composition of A5 solution

B6	
Compounds	*g/L*
NH_4VO_3	0.023
$KCr(SO_4)_2 \cdot 12\ H_2O$	0.096
$NiSO_4 \cdot 7\ H_2O$	0.048
$(NO_3)_2Co \cdot 6\ H_2O$	0.049
$Na_2WO_4 \cdot 2\ H_2O$	0.018
$Ti(SO_4)_2 + TiOSO_4$	0.048

Table 2.2.- Composition of B6 solution

Connection between compartments III and IV

To be able to connect compartments III and IV, a media containing all the necessary compounds for the growth of *Nitrosomonas europaea, Nitrobacter winogradsky*i and *Spirulina platensis*, has to be defined. A medium based on the combination of the above-described media for each compartment has been designed and it is presented in table 3.

Connection medium	
Compound	*g/L medium*
EDTA-Na$\cdot 2\ H_2O$	0.08
$FeSO_4 \cdot 7\ H_2O$	0.01
KH_2PO_4	0.68
$NaHCO_3$	0.80
$MgSO_4 \cdot 7\ H_2O$	0.20
$CaCl_2 \cdot 2\ H_2O$	0.04
$(NH_4)_2SO_4$	1.32
Na_2HPO_4	0.71
$(NH_4)_6Mo_7O_{27} \cdot 4\ H_2O$	0.18
Dissolution	*mL/L medium*
A5	1.00
B6	1.00

Table 3.- Composition of the medium used for the connection of compartments III and IV (A5 and B6 compositions are presented in tables 2.1 and 2.2)

2.2.3.- Analytic procedures

Cell concentration

- Dry weight

S. platensis dry weight was determined by filtering through a 0.45μm pre-weighted filter, dried until constant weight in a 100ºC oven and cooled down in a desiccator.

- Optical density

The optical density measured at 750nm is a direct measurement of the *S. platensis* concentration. Polysaccharides do not absorb at this wavelength. Thus, this measurement reflects only the diffusion of the light produced by the presence of the microorganisms, fact that is directly related with the biomass concentration. The spectrophotometer used is a Kontron Instrument, Uvikon 941, Italy.

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

- Nitrate

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

BASIS: The 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 was measured using UV measurement determinations by means of LCK 341 nitrite analysis kits (Dr. Lange Nitrax).

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

Inorganic carbon measurements

The total inorganic carbon (TIC) is measured with an O.I Corporation 700 TOC analyser (Texas, EEUU). It is an automatic system that can either analyse solid and liquid samples. The total inorganic carbon is defined as the carbon that is converted to CO_2 when acidifying the sample. That includes the CO_2, carbonates and bicarbonates that are present in solution.

BASIS: The TIC is determined by measuring the CO_2 liberated when acidifying the sample. The pH decrease converts the carbonate and bicarbonate ions present in solution to CO_2. This CO_2 is trapped and concentrated and is analysed by a non disperse IR analyser.

Axenicity control

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

2.2.4.- Light calibration of compartment IV

For the operation of photobioreactors the light availability determination is of key importance. Determination of light availability at any point of the bioreactor can be done provided the light intensity at the bioreactor surface is known.

Determination of the light intensity at the bioreactor's surface is done by measuring the light intensity at the axis of the empty 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 can be done using the following equation:

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

Where: Fr is the light flux at the bioreactor surface, Eb is the light intensity measured by the sensor, rb is the sensor's radius (30mm) and Rb is the bioreactor radius (75mm).

As the available light measured by the sensor is given in units of $\mu mols/m^2s^2$ 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 has been 0.291, which was previously calculated by J.F.Cornet (Cornet *et al.*, 1992 a,b

and 1993) by integration of the used lamps spectra in the range 350–750nm used by *S. platensis* .The Eb values were measured in the empty bioreactor.

Light intensity measurements were done at different at different positions along the vertical axis and for different controller signals.

The measurements obtained for each controller position at different vertical positions were averaged to obtain a light intensity value for each controller position. The light intensity values measured by the sensor in μmols/m^2s^2, were converted to Fr values using the above mentioned formula and conversion factor. As a result of this measurements a relationship between the controller output and the Fr of the bioreactor is obtained as can be seen in figure 3.

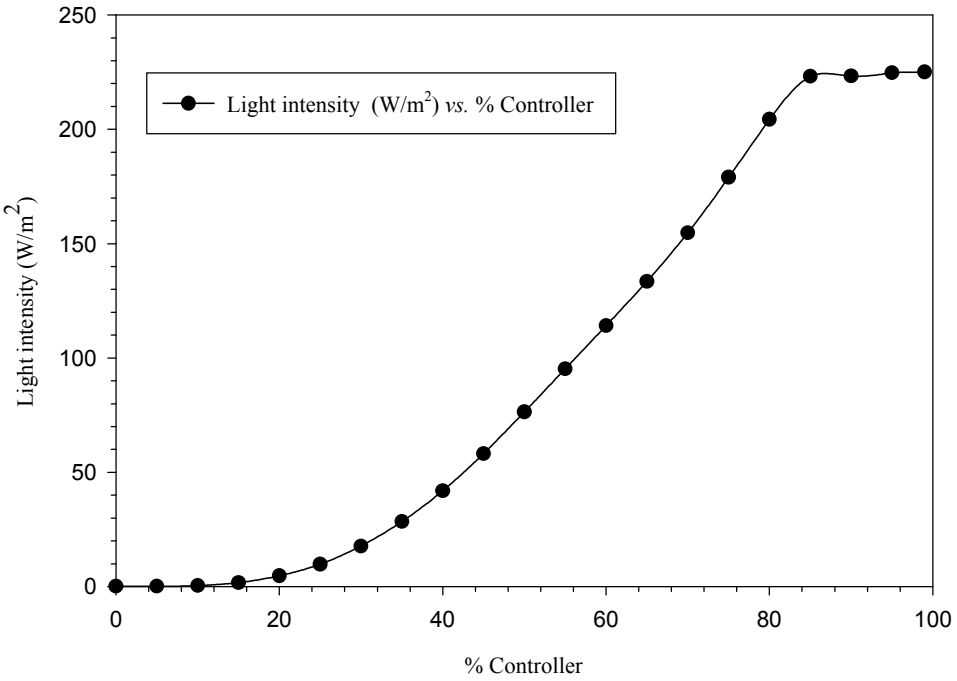

Figure 3.- Light intensity *vs.* % Controller

3.- RESULTS AND DISCUSSION

In this work the long term connection between compartments III and IV at pilot scale and the effect of an ammonium and nitrite inlet in compartment IV due to changes in the dilution rate and the oxygen concentration in compartment III have been studied. The operation of the MCS has been tested in compartment IV when connected to compartment III.

3.1.- EFFECTS IN COMPARTMENT IV DUE TO CHANGES IN THE DILUTION RATE OF COMPARTMENT III

In order to perform this experiment the media described in section 2.2.2 with an ammonium concentration of 300 N-ppm and a $NaHCO_3$ concentration of 5.0 g/L is used. This higher $NaHCO_3$ concentration is needed because in this experiment the carbon source of compartment IV is fully supplied by the $NaHCO_3$ coming from the third compartment.

	Conditions I		**Conditions II**		**Conditions III**	
	Comp. III	*Comp.IV*	*Comp. III*	*Comp.IV*	*Comp. III*	*Comp.IV*
T(ºC)	30	36.5	30	36.5	30	36.5
pH	8.2	9.5	8.2	9.5	8.2	9.5
V_{liquid} *(L)*	3.8	77	3.8	77	3.8	77
Light (W/m^2)	0	20	0	20	0	20
Q_L *(L/day)*	4	4	8	8	15	15
D (h^{-1})	0.044	0.002	0.088	0.004	0.164	0.008
i (days)	0.95	19.25	0.475	9.625	0.25	5.13

Table 5.- Summary of the different conditions of compartments III and IV during this experiment

The evolution of compartments III and IV when performing these changes in the dilution rate is presented in figures 4, 5 and 6.

As can be observed in figure 4, when the dilution rate in the third compartment is increased, the ammonium to nitrate conversion decreases, remaining thus, nitrite and ammonium in the outlet of this compartment. This transient state after the perturbation, with non complete conversion lasts, approximately, 72 h. By that time the required biofilm to convert the new apport of ammonium to nitrate is formed. Then, the normal operation of the bioreactor is recovered converting all the entering ammonium to nitrate.

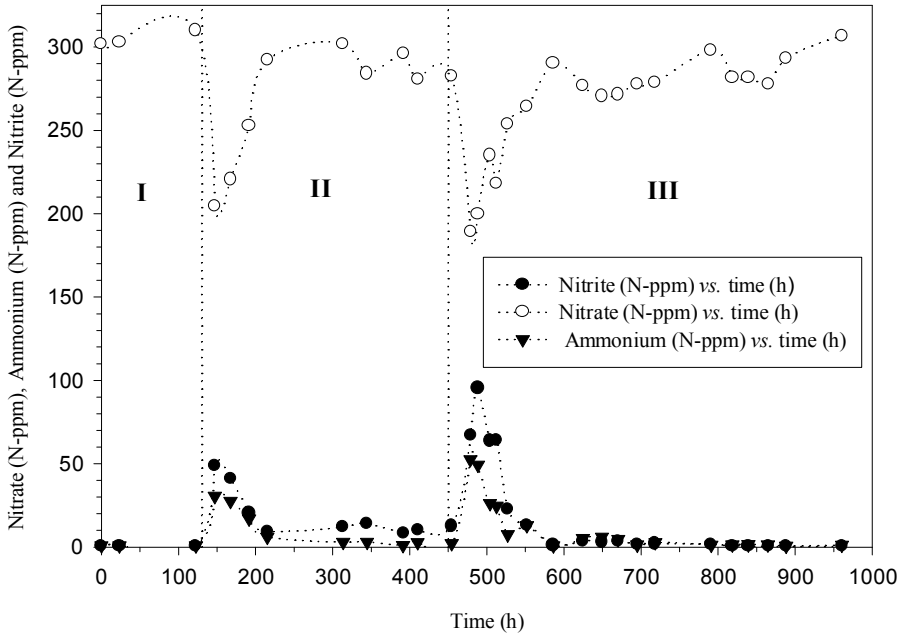

Figure 4.- Evolution of compartment III operation in the connection experiment between compartments III and IV. I, II and III are the different operational conditions presented in table 5

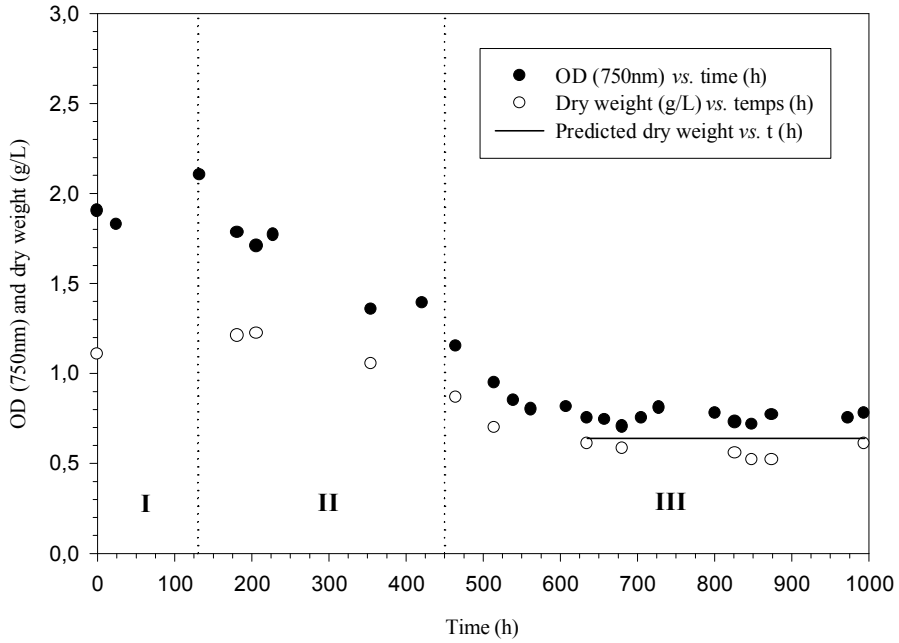

Figure 5.- Evolution of the biomass concentration in compartment IV in the connection experiment between compartments III and IV. I, II and III are the different operational conditions presented in table 5

In relation with the evolution of compartment IV, a biomass concentration decrease is observed when increasing the dilution rate. For the 0.002 h^{-1} and 0.004 h^{-1} dilution rates of compartment IV a new change in the dilution rate was forced before obtaining the steady state. Waiting until the steady state in these two dilution rates would require a long period of time taking into account that with these two dilution rates the residence time in compartment IV is very high as can be checked in table 5. Thus, the dilution rate was increased, when compartment III had reached its normal operation; neither nitrite nor ammonium were present at the output of this compartment.

In order to evaluate the global behaviour of the connection, the steady state in compartment IV was reached for the 0.008 h^{-1} dilution rate. As can be observed in figure 4 the experimental dry weight is in agreement with the predicted dry weight using these conditions by PHOTOSIM 2 (Cornet, 1993). To be able to evaluate the effect of nitrite and ammonium in the fourth compartment and in order to be able to carry out the carbon balances; the ammonium, nitrate, nitrite concentrations in the outlet and the carbon concentration in the outlet and in the inlet were measured and are presented in figure 6. The inlet ammonium, nitrite and nitrate concentrations in this compartment are the nitrate, nitrite and ammonium concentrations in the inlet of compartment III. These concentrations are presented in figure 6.

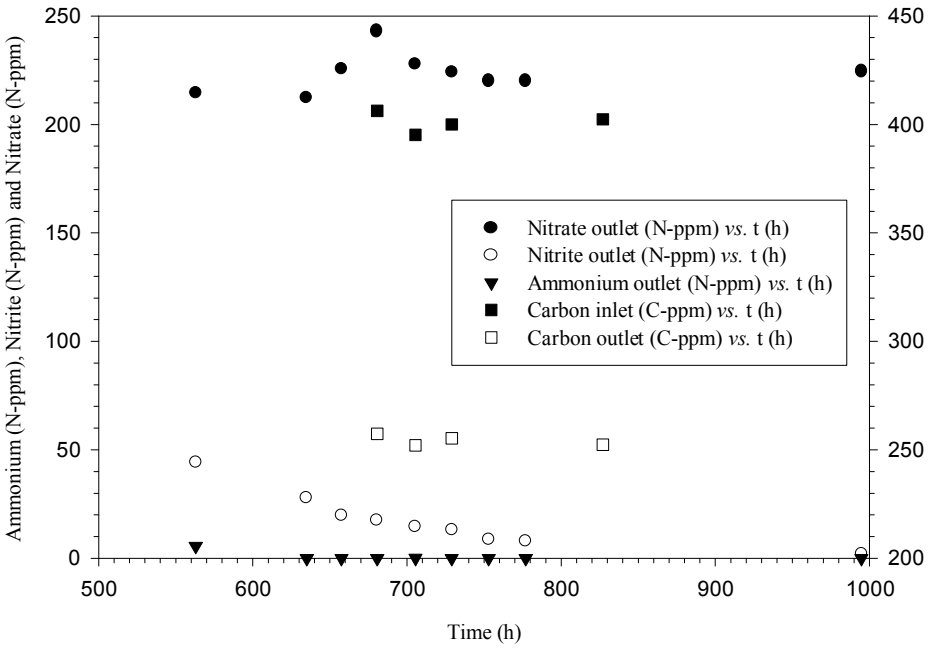

Figure 6.- Evolution of Ammonium, Nitrite and Nitrate concentration in the outlet of compartment IV and evolution of the carbon concentration in the inlet and outlet of compartment IV, for the set of operational conditions III

From the data presented in figures 5 and 6 the nitrogen and carbon balances in compartment IV have been done. In order to do these balances the S. *platensis*

composition of $CH_{1.650}O_{0.531}N_{0.170}S_{0.007}P_{0.006}$ (Cornet, 1992a) is assumed. In table 6 and 7 the carbon and nitrogen balances in compartment IV are presented.

	Inlet (gC/L)	**Outlet (gC/L)**
Carbon (C-ppm)	0.4	0.25
Cells	0	0.241
Total	*0.4*	*0.491*

Table 6.- Carbon balance in compartment IV

	Inlet (N-ppm)	**Outlet (N-ppm)**
Nitrate	280	225
Cells	0	48
Total	*280*	*273*

Table 7.- Nitrogen balance in compartment IV

From these tables it can be said that meanwhile the nitrogen balance is closed the carbon balance is not. This fact can be explained by the addition of CO_2 in compartment IV to regulate the pH. Thus, carbonates are added in solution. This fact can explain why the outlet carbon concentration is higher than the inlet carbon concentration.

The effect of the presence of ammonium and nitrite in compartment IV has to be analysed. Previously published experimental data indicates that even though nitrates are the main nitrogen source for *S. Platensis* ammonium and nitrite can be also assimilated. When growing with nitrate the *S. Platensis* growth rate is not significantly decreased until having a really high nitrate concentration, of about 16800 N-ppm (Ciferri, 1983). Ammonium can also be assimilated if its concentration does not exceed 100N-ppm (Richmond, 1986). It has been observed that when nitrate and ammonium are simultaneously used, ammonium is first consumed (Guerrero and Lara, 1987). Nitrite can be also assimilated even though it is toxic at high concentrations (Becker, 1994). The limit concentration above which nitrite begins to be toxic is not clearly defined in the literature. In a culture using both nitrate and nitrite as nitrogen sources, a toxic effect when the nitrite concentration was over 18 N-ppm was observed (Soyer, 1992). Nevertheless, in another work where the *S. platensis* growth rate was measured using different nitrogen sources, similar growth rates were obtained using concentration values of 28 N-ppm of nitrate or nitrite (Filiali and Dubertret, 1996).

The fact that ammonium is first uptaken when having ammonium, nitrate and nitrite as nitrogen sources is also observed in figures 4 and 5. In those figures, an immediate ammonium assimilation in compartment IV is observed. The inlet ammonium peak is similar as the nitrite peak, see figure 4, and almost no ammonium is left in the outlet of compartment IV. Thus, ammonium is partially consumed in this compartment. Ammonium is also partially washed out from compartment IV through the gas phase due to its conversion to ammonia caused by the high pH of the culture. An increase in the nitrate consumption in compartment IV is also observed when a decrease in the ammonium inlet is produced. This fact is explained by the lower ammonium availability. Therefore, the preference of *S. platensis* in consuming ammonium in front of nitrate is demonstrated.

In order to see whether nitrite was partially consumed in compartment IV, the experimental nitrite concentrations in compartment IV were compared to the theoretical wash out curve of nitrite in this compartment. These results are presented in figure 7.

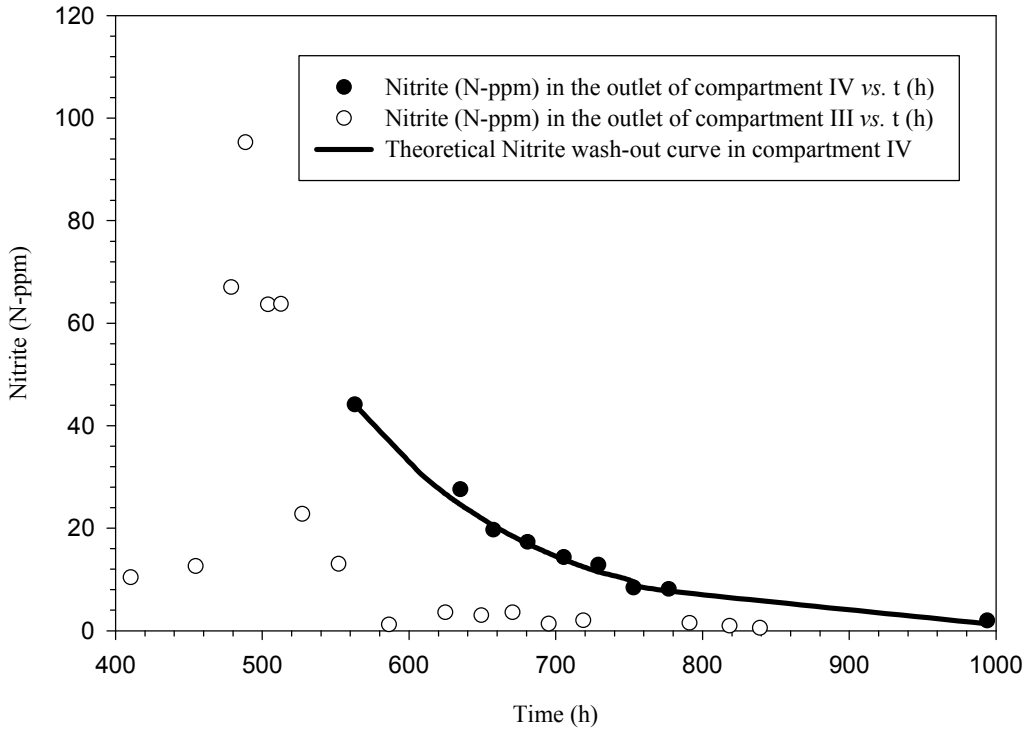

Figure 7.- Nitrite evolution in the outlet and inlet of compartments III and IV respectively and theoretical nitrite wash-out curve in compartment IV

To simulate the nitrite wash-out curve, the nitrite inlet concentration in compartment IV was not considered due to the fact that if compared with the existent nitrite concentration in the bioreactor, it is negligible. Making this assumption and considering the air-lift reactor perfectly mixed, the nitrite wash-out curve is described by equation 1.

$$[nitrite] = C_0 \exp^{-(t-t_0)/t} \qquad (1)$$

where:

[nitrite] is the nitrite concentration (N-ppm)
t_0 is the initial time (h)
t is the time (h)
ι is the residential time (h)
C_0 is the initial concentration (N-ppm)

When substituting the operational conditions of compartment IV in this equation, equation 2 is obtained.

$$[nitrite] = 4257.34 \exp^{-0.0082\ t} \quad (2)$$

where the nitrite concentration is given in (N-ppm) and the time in (h).

As can be seen in figure 7 the evolution of the nitrite concentration in compartment IV is fully in agreement with the nitrite wash-out curve. Therefore, it can be concluded that when having in the fermentor an excess of ammonium or nitrate, nitrite is not consumed.

3.2- LONG TERM CONNECTION

In order to evaluate the long term operation of the connection between compartments III and IV at pilot scale, once the two compartments were operating in a continuous mode, they were connected using the medium presented in section 2.2.2. This connection has been carried out continuously during three months. During these three months the reactors have been working together operating under different conditions. These different conditions are presented in table 8.

	Compartment III					*Compartment IV*			
	NH_4^+	Q_L (L/day)	D (h^{-1})	ι (days)	O_2 $\%DO_{mean}$	Q_L (L/day)	D (h^{-1})	ι (days)	Light (W/m^2)
Conditions I	900	4.5	0.05	0.84	80	4.75	2.6e-7	16.21	35
Conditions II	300	14.5	0.16	0.26	80	15.5	8.4e-3	4.96	35
Conditions III	300	14.5	0.16	0.26	40	15.5	8.4e-3	4.96	35
Conditions IV	600	14.5	0.16	0.26	40	15.5	8.4e-3	4.96	35
Conditions V	600	14.5	0.16	0.26	20	15.5	8.4e-3	4.96	35
Conditions VI	600	14.5	0.16	0.26	15	15.5	8.4e-3	4.96	35
Conditions VII	600	14.5	0.16	0.26	10	15.5	8.4e-3	4.96	35
Conditions VIII	600	14.5	0.16	0.26	5	15.5	8.4e-3	4.96	35
Conditions IX	600	14.5	0.16	0.26	40	15.5	8.4e-3	4.96	35

Table 8.- Different operational conditions of compartments III and IV during a 3 months interconnected run.

The evolution of compartments III and IV during this period is presented in figures 8, 9 and 10.

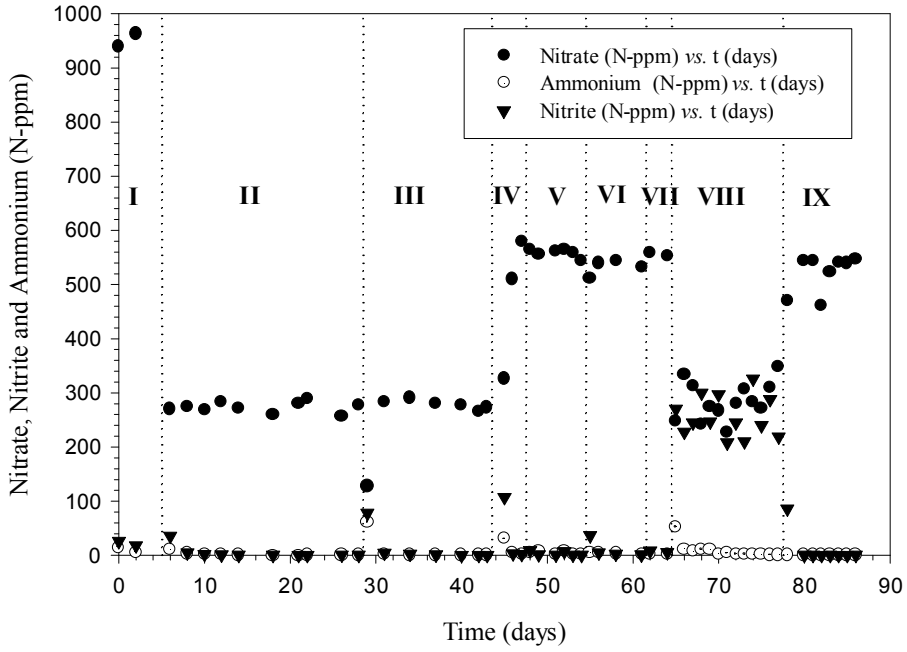

Figure 8.- Evolution of compartment III during the long term connection experiment. I, II, III IV, V, VI, VII, VIII and IX are the different operational conditions used in this experiment.

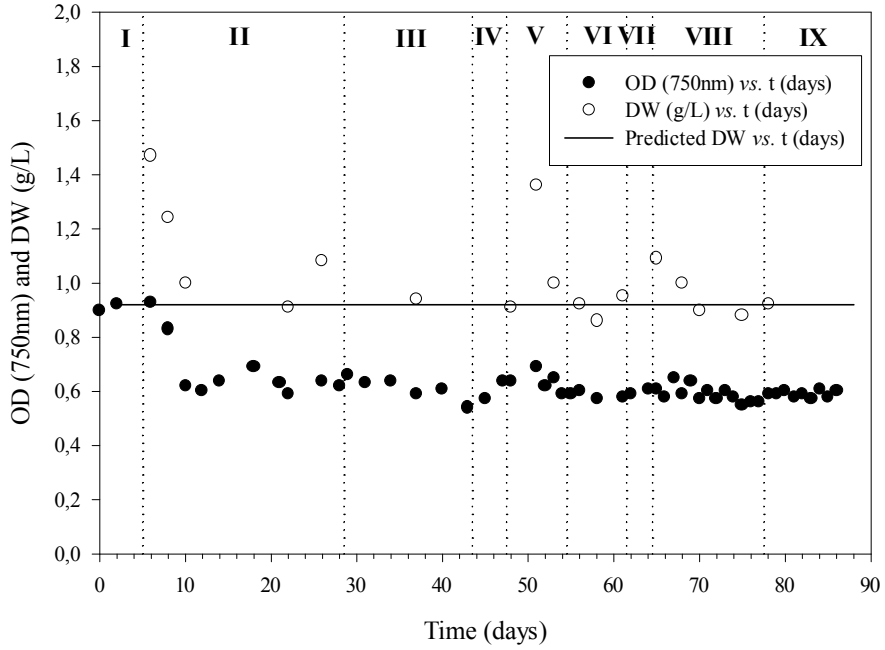

Figure 9.- Evolution of the biomass concentration of compartment IV during the long term connection experiment. I, II, III IV, V, VI, VII, VIII and IX are the different operational conditions used in this experiment.

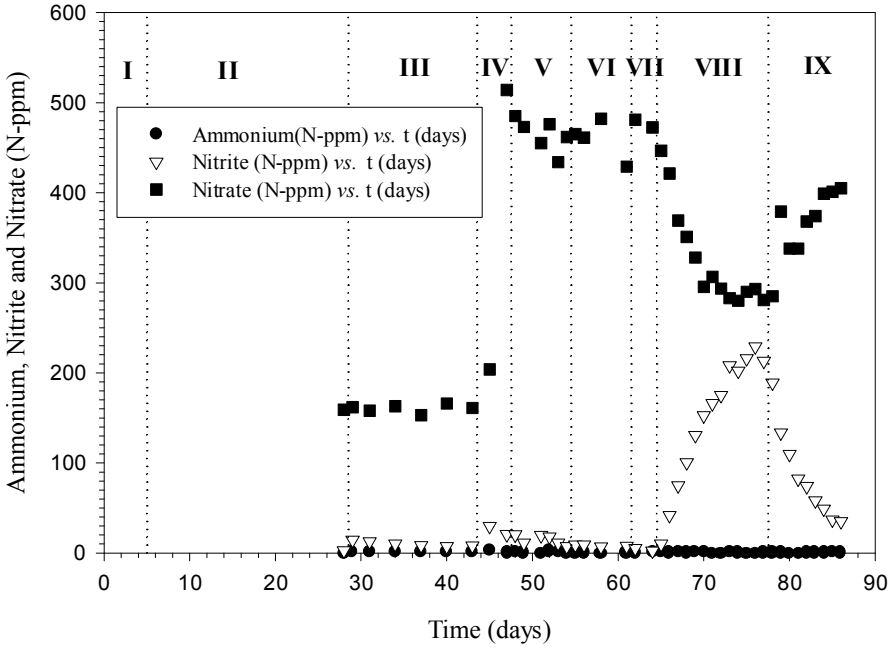

Figure 10.- Evolution of the ammonium, nitrate and nitrite concentrations in compartment IV during the long term connection experiment. I, II, III IV, V, VI, VII, VIII and IX are the different operational conditions used in this experiment.

As can be seen in figures 8, 9 and 10 during the long term connection of compartments III and IV no relevant toxic effects are presented. The experimental dry weight in compartment IV matches with the predicted by PHOTOSIM 2 (Cornet, 1993).Thus, it can be said that the connection of these two compartments can be carried out without any major difficulty.

Carbon balances have not been calculated in compartment IV because neither the inlet nor outlet CO_2 nor the inlet carbonate concentrations were measured in compartment IV.

The nitrogen balance in compartment III can be considered almost closed; as during conditions II and III, when 300 ammonium N-ppm are entering in this compartment, an average of 270 N-ppm are found in the outlet , and during conditions III, IV, V, VI, VII, VIII and IV, when 600 ammonium N-ppm are entering in this compartment, an average of 550 N-ppm are found in the outlet.

Taking into account that the biomass concentration is maintained during the whole experiment at 0.9-1 g/L the nitrogen balance in compartment IV can be calculated assuming the *S. platensis* composition as $CH_{1.650}O_{0.531}N_{0.170}S_{0.007}P_{0.006}$ (Cornet, 1992a). Then ~90 N-ppm are needed. As observed in figures 8 and 9 the nitrogen concentration gap between the outlet of compartment III and the outlet of compartment IV is in between 80-100 N-ppm. Thus, the nitrogen balance can be considered closed.

3.3- EFFECTS IN COMPARTMENT IV DUE TO CHANGES IN THE OXYGEN CONCENTRATION OF COMPARTMENT III

This experiment was carried out during the long term connection presented in section 3.2. As can be observed from table 8 and figures 8 and 9 when changing the dissolved oxygen in compartment III ammonium and nitrite were found in the outlet of this compartment and afterwards they were introduced in compartment IV. As it is also observed in section 3.1 ammonium is immediately consumed in compartment IV, showing the preference of *S. platensis* in consuming ammonium when, ammonium, nitrate and nitrite are available. Ammonium is also partially washed out from the fermentor through the gas phase due to its conversion to ammonia caused by the high pH of the culture. In figures 11 and 12 the changes when decreasing the oxygen concentration in compartment III for conditions IV, V, VI, VII, VIII and IX are presented.

When a high nitrite inlet concentration in compartment IV is produced no decrease in the biomass concentration is observed in figure 12, and the experimental biomass concentration is in agreement with the predicted by PHOTOSIM 2 (Cornet, 1993). Thus, no irreversible toxicity effect is observed when having high nitrite concentrations in compartment IV. Ammonia is immediately consumed when entering in compartment IV. In order to see whether nitrite is consumed in this compartment the theoretical nitrite concentration curve, for condition VIII, and the nitrite washing out curve, for conditions IX, have been compared with the experimental nitrite concentration in compartment IV as can be seen in figure 13.

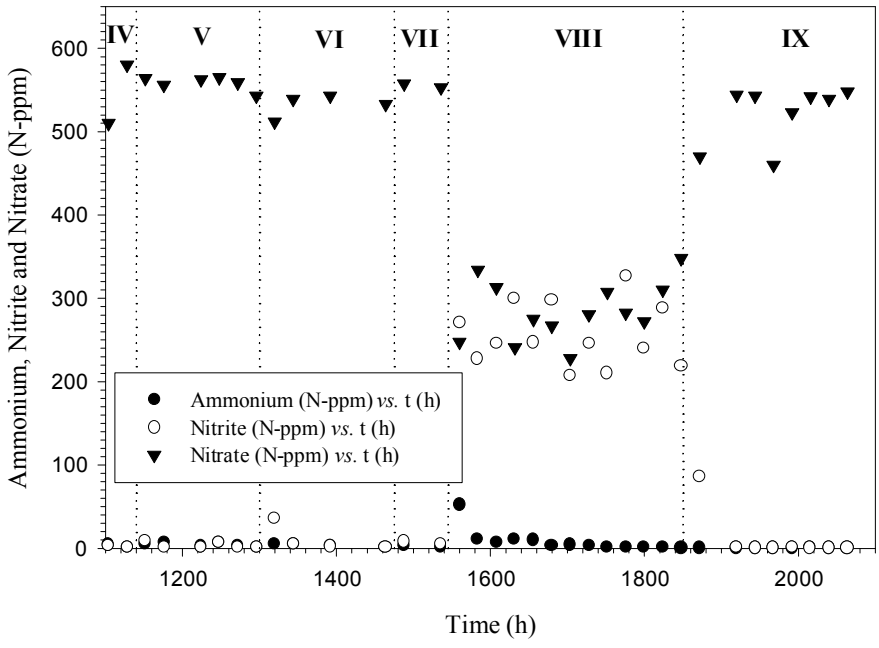

Figure 11.- Evolution of compartment III when decreasing the oxygen concentration

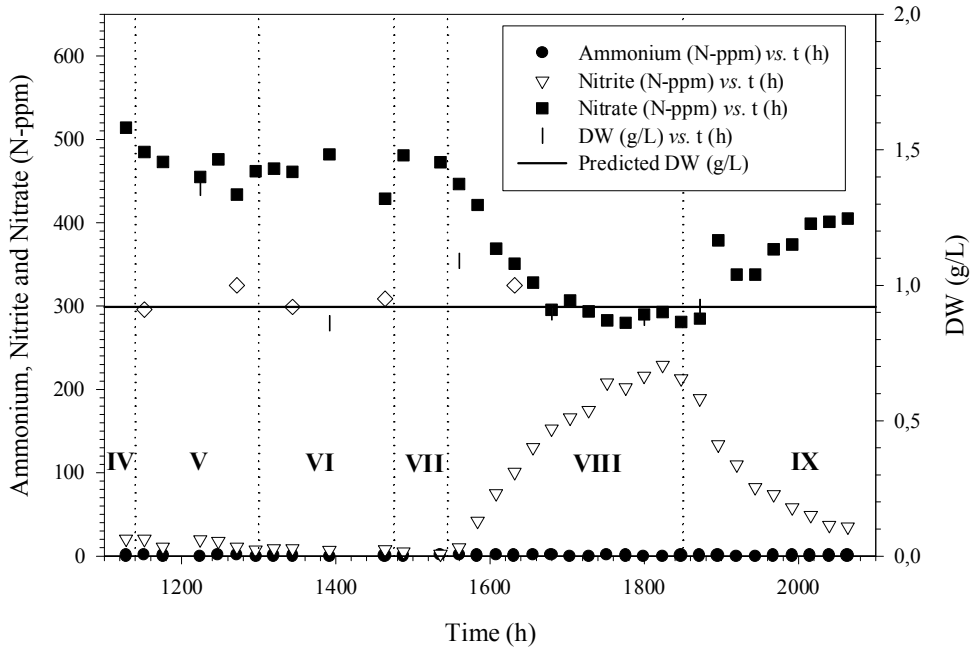

Figure 12.- Evolution of compartment IV when decreasing the oxygen concentration

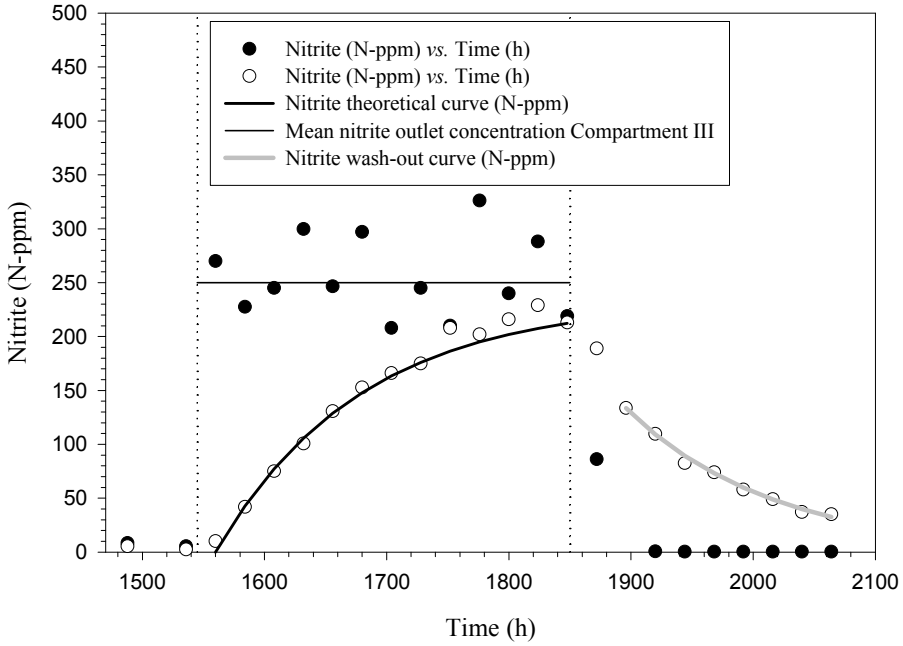

Figure 13.- Nitrite outlet concentration of compartment III and nitrite experimental evolution, theoretical nitrite concentration curve and washing out curve in compartment IV

The theoretical nitrite concentration curve is obtained by means of equation (3) when considering a perfect mix in the air-lift.

$$[nitrite] = C_E - C_E \exp^{-(t-t_0)/t} \quad (3)$$

where:

[nitrite] is the nitrite concentration (N-ppm)
ι is the residence time (h)
t_0 is the initial time (h)
t is the time (h)
C_E is the inlet nitrite concentration (N-ppm)

Substituting the residence time and considering the inlet nitrite concentration as 233 N-ppm (from figure 13 the average outlet nitrite concentration of compartment III is 250, but if the dilution due to the 1L/day NaOH used to adjust the pH is taken into account, the inlet concentration to compartment IV is decreased to 233 N-ppm) equation 3 is converted to equation 4.

$$[nitrite] = 233 - 233\exp^{-(t-1559.96)/119.23} \quad (4)$$

The washing out curve, equation 5, is obtained when substituting in equation 1 the correspondent parameters.

$$[nitrite] = 133.6 \exp^{-(t-1895.96)/119.23} \quad (5)$$

As concluded in section 3.1, figure 13 allows to conclude that nitrite is not consumed in compartment IV.

3.4.- MCS TESTS

Once the connection between compartments III and IV has been successfully tested the verification of the performance of the control system of compartment IV while compartments III and IV are interconnected has to be tested. In order to do these tests, the MCS controlled the input pump of the third compartment. Thus, the flow rate of compartment IV is also controlled as all the exiting media from compartment III is entered in compartment IV.

3.4.1.- Biomass sensor's cleaning device

Problems of cell attachment on the biomass sensor's surface appeared during the MCS tests. In order to solve these problems an air cleaning device was developed. Every 5 minutes high pressured air was blowed on the biomass sensor during 10 seconds in order to clean its surface. A scheme of the cleaning device is shown in figure 14.

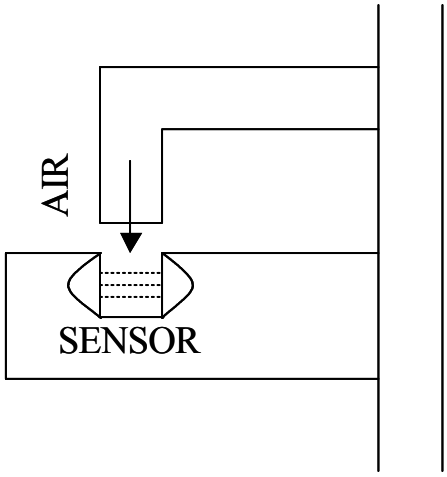

Figure 14.- Scheme of the biomass sensor's surface cleaning device.

3.4.2.- MCS tests results

Different steps up in productivity rate were done. The productivity was increased from 0.55 to 0.9, from 0.7 to 0.9 and from 0.9 to 1.2 g/h. In order to achieve these changes the MCS accepted the inlet flow rate at 0.6 L/h and varied the light intensity depending on the biomass requirements. In figures 15, 16 and 17 the evolution of the productivity rate along these tests is shown.

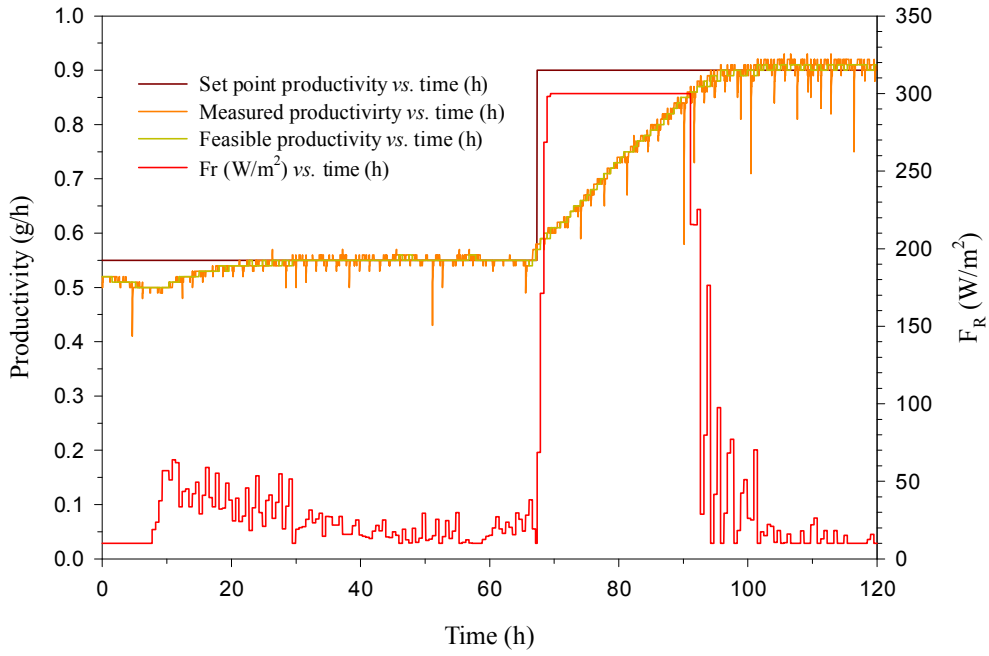

Figure 15.- Evolution of the productivity rate and light intensity when a step up from 0.55 till 0.9 is required in the MCS.

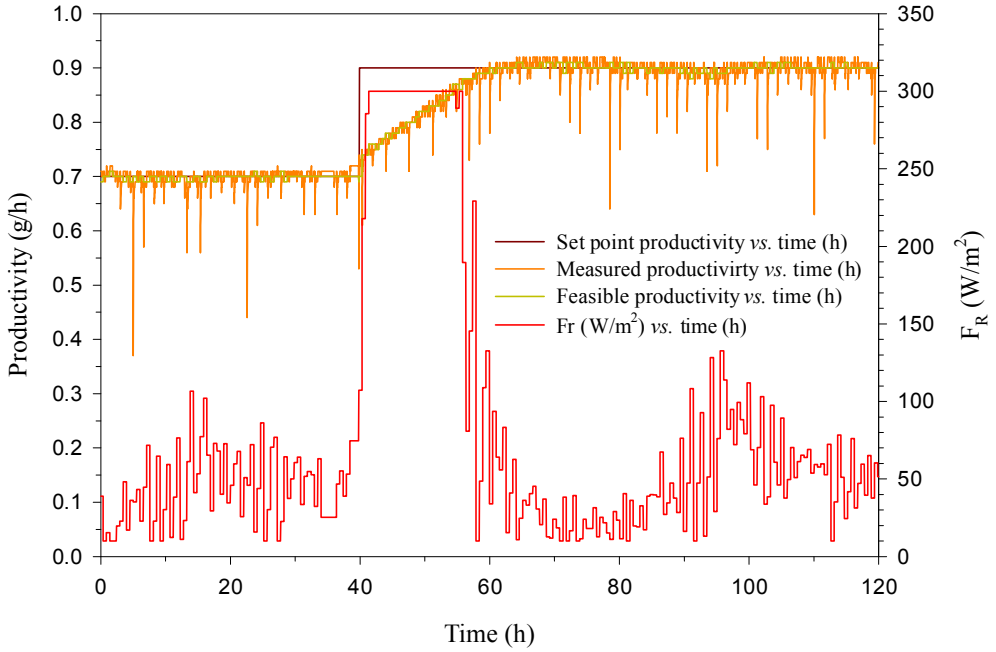

Figure 16.- Evolution of the productivity rate and light intensity when a step up from 0.7 till 0.9 is required in the MCS.

As it is shown in figure 15, 16 and 17 when the productivity rate step up is done in the MCS, the measured productivity increases due to the increase of the light intensity until the new set point is reached. Then, the light intensity fluctuates in order to maintain the required productivity. Thus, the MCS has been able to control compartment IV, as it was desired.

Figure 17 shows a test were the MCS controlled light intensity is suddenly decreased. Fact caused by a stop and restart of the MCS program. The stop was identified as being caused by an invalid writing operation in the control system. Thus, it can be seen that the MCS has no memory of the near past operation and restarts the control from the beginning increasing the light intensity step by step and it does not recover immediately the light intensity used before the restart of the computer.

In order to achieve these productivities the biomass maximum concentration limit of the control law has been increased as the reactor reached a higher biomass concentration than the maximum limit previously set. Up to this point this maximum is located at 2.5 g/L.

The measured productivity graph, as can be seen in the above figures, has a constant tendency but presents sudden drops. These drops are due to the cleaning device. When the air is blowed on the sensor surface its absorbance measure is affected by the presence of the air. Stable measurement is recovered about 20 seconds after the air blowing is stopped. After discussion with ADERSA it was agreed to maintain the last measurement before the air blow during the air blow period in order to avoid the drops.

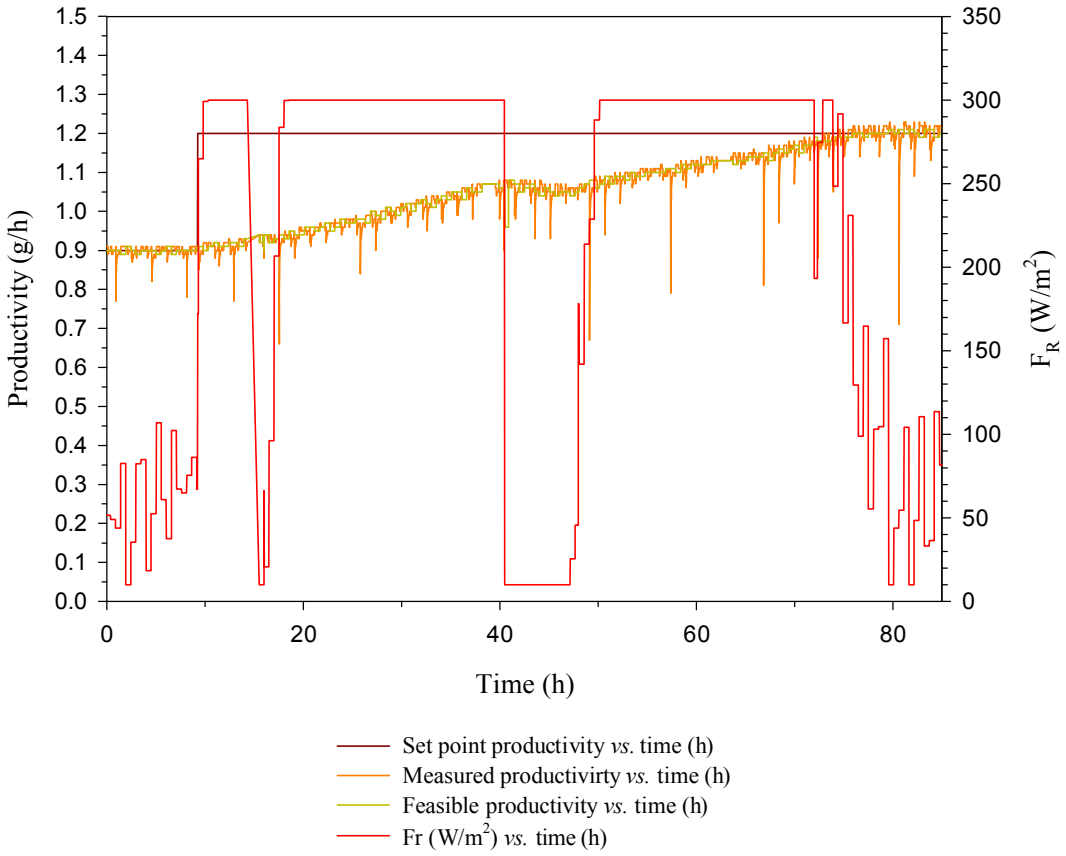

Figure 17.- Evolution of the productivity rate and light intensity when a step up from 0.9 till 1.2 is required in the MCS.

4.- CONCLUSIONS

In this work:

- The long term connection of compartments III and IV at pilot scale has been tested without detecting neither any malfunction nor any toxic effects.

- The nitrite inlet in compartment IV has been studied by:
  1.- Increasing the dilution rate in compartment III
  2.- Decreasing the oxygen concentration in compartment III.
  Concluding that:
  1.- When ammonium, nitrate and nitrite are present at the same time in the culture medium, *S. platensis* preferably consumes first ammonium and then nitrate.
  2.- Thus, Nitrite is found in the outlet of compartment IV. This fact has to be considered in case the outcoming water has to be used as, for example, potable water.

- The MCS is not affected by the connection of compartment III and IV and can control compartment IV as desired, provided the input flow of compartment III is increased in connection with the increase in compartment IV. Thus, further corroborationn of the accuracy of compartment IV operation control through MCS has been obtained.

REFERENCES

Becker, E. W. (1994) Microalgae: biotechnology and microbiology. *Ed. Cambridge University Press*. Cambridge.

Creus, N., Albiol, J. and Gòdia, F. (1999) Preliminary connection between 3 compartments. Technical Note 43.8. ESTEC/CONTRACT11549/95/NL/FG.

Creus, N., Albiol, J. and Gòdia, F. (2001) Tests with the 3 linked bench compartments. Technical Note 47.5. ESTEC/CONTRACT11549/95/NL/FG.

Ciferri, O. (1983) *Spirulina*, the Edible Microorganism. *Microbiological Rev.* **47**, 551-578.

Cornet, J. F., Dussap C. G. and Dubertret, G. (1992 a) A strucrured model for simulations of cultures of the cyanobacterium *Spirulina platensis* in photobioreactors: I. Coupling between light transfer and growth kinetics. *Biotech. Bioeng.* **40**, 817-825.

Cornet, J. F., Dussap C. G., Cluzel, P. and Dubertret, G. (1992 b) A strucrured model for simulations of cultures of the cyanobacterium *Spirulina platensis* in photobioreactors: II. Identification of kinetic parameters under light and mineral limitations. *Biotech. Bioeng.* **40**, 826-834.

Cornet, J. F., Dussap C. G. and Gros J. B. (1993) Modelling of physical limitations in photobioreactors. Application to stimulation and control of the spirulina compartment of the MELISSA artificial ecosystem. Technical Note 19.3. MELISSA CONTRACT PRF 130820.

Filali, R. and Dubertret, G. (1996) Growth capability of *Spirulina* on several nitrogen sources in the photosynthetic compartment of MELISSA. Technical Note 33.2. MELISSA. ESA-ESTEC/UNIVERSITE PARIS-SUD. *Res.* **9**, 865-871.

Guerrero, M. G. and Lara, C. (1987) Assimilation of inorganic nitrogen. In: The cyanobacteria. *Ed. Elsevier Science Publisher*. Amsterdam.

Hendrikus, J., Laambroek, H. J. and Gerards, S. (1992) Competition for limiting amounts of oxygen between *Nitrosomonas europaea* and *Nitrobacter winogradskyi* grown in mixed cultures. *Microbiology* **159**, 453-459.

Pérez, J. (1997) Caracterización y puesta a punto de reactores de lecho fijo para su aplicación en procesos de nitrificación. Treball de Màster. Universitat Autònoma de Barcelona.

Pérez, J., Montesinos, J.L. and Gòdia F. (1997a) Operation of the bench nitrifying reactors. Technical Note 37.510. ESTEC CONTRACT 11549/95/NL/FG.

Soyer, C. (1992) Étude d'une culture de *Spirulina platensis* sur un effluent du compartiment nitrifiant. Mémoire de DESS de microbiologie appliqueé et génie biologique. Université Paris VI.

Vernerey, A. (2000) Conception, contrôle et fonctionnement d'un photobioréacteur pour la culture en continu de la cyanobactérie *Spirulina platensis*. phD Tesis. Universitat Autònoma de Barcelona.

Wijffels, R. H. (1994) Nitrification by immobilized cells. Landbouwuniversitet Wageningen. PhD thesis.

Zarrouk, C. (1966) Contribution a l'étude d'une cyanophyceé: influence de divers facteurs physiques et chimiques sur la croissance de *Spirulina maxima*. Université de Paris. PhD thesis.