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SET UP OF THE PHOTOSYNTHETIC PILOT REACTOR

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1 Introduction.

Compartment IV of MELISSA loop, consisting of a photobioreactor for the growth of the cyanobacteria *Spirulina platensis*, is the one that has been brought to a further degree of development. Indeed, it was the first compartment that was operated at the pilot scale level within the MELISSA Pilot Plant, in a 7 litres volume gas-lift photobioreactor.

Reliable, highly automated and controlled operation of this bioreactor has been fully achieved, and a complete set of data for the continuous operation of the bioreactor at different conditions has been generated in a period of three years of operation. As a step forward in the development of the Pilot Plant, and the demonstration of the MELISSA concept, it was decided to scale-up compartment IV to a new bioreactor about ten times in volume, and adapt the 7 litres reactor for compartment II, also a photosynthetic compartment, using the bacteria *Rhodospirillum rubrum*. Therefore, completing these actions, together with the start-up of the pilot reactor for compartment III, would bring the MELISSA Pilot Plant to a point of development where three compartments would be operating already at the pilot scale level, thus increasing the generation of data on the characterisation and operation of the bioreactors and the loop as such.

The design of the new photobioreactor for compartment IV was presented in TN 25.2 (Vernerey et al., 1996), and had as guidelines the following points:

- to scale-up by a factor of 10 the volume of the bioreactor to a size roughly enabling at least the oxygen production to sustain the life of three rats.
- to maintain as far as possible the current type of bioreactor, in order to use efficiently all the knowledge developed on the present unit (for example, the knowledge model establishing the relationship between cell growth and light intensity given to the reactor from the external illumination source)
- to improve the operation of the bioreactor with respect to some peripheral equipment and associated instrumentation. Some of the changes are associated to the availability of new equipment, as some others are due to the change in the reactor size.

The calculations made with respect to the first point, taking into account previous studies on oxygen and *Spirulina* consumption by rats, and the productivity attained in

the first air-lift reactor used, as detailed in TN 25.2, suggested a 50 litres total volume for the new bioreactor, considering that 20% would not be illuminated. With respect to the type of bioreactor, the air-lift concept was retained, as well as the cylindrical geometry, this allowing to use all the mathematical formulation already developed for the control of the reactor operation.

Different design possibilities where considered, and compared taking into account the ratio between the illuminated and total volume (that should be as high as possible) and the different constraints (as for example total height due to the room available in the pilot plant laboratory; reactor diameter, as a too wide diameter would cause dark zones in the inner part of the illuminated volume of the reactor, especially at high cell concentrations). Finally, the selected option for the design, as discussed in more details in TN 25.2, was an external loop air-lift reactor. This design was a good compromise, that fulfilled all the restrictions imposed to the system, reaching a high ratio between illuminated and total volume, of about 70%.

Under the process of construction of the reactor, that was carried out by Bioengineering AG (Wald, Switzerland), an important issue not considered in the design arose, with respect to the material of construction of the transparent illuminated walls. It was considered not safe to build these two cylindrical parts (1.5 m in length each) in glass, as being connected to stainless steel sections at both sides (top and bottom), the steam sterilisation process could easily brake them. Therefore, it was decided to apply the concept of "foil" reactor, developed by Bioengineering for photobioreactors, and these parts where made of a sterilisable flexible plastic material (in this case sterilisation is still done by steam, surrounding the plastic material with a metal jacket). Also, during the process of construction the sizes of different parts of the reactor where adapted to the standard sizes available in the company, for reasons of easy construction and lower cost.

Therefore, the final sizes that will be presented in this technical note do not coincide exactly with the final design presented in TN 25.2. As an example, the diameter of the cylindrical illuminated columns had to be changed from the designed 12 cm to 15 cm, as the last was the closest standard diameter available for the plastic foil, while their length was reduced from 1.65 m to 1.50, as this was the standard size available for the metallic jackets for sterilisation. All the changes introduced along the discussion with the manufacturer where done taking the standard sizes that did not introduce any reduction

in the volume of the reactor. As a consequence, the final volume of the reactor was increased to 77 litres.

With respect to the auxiliary equipment, the first aspects considered had been the illumination system and temperature control. For the illumination system, the same type of halogen lamps used previously has been kept, as these lamps have an spectra adequate for the absorption spectra of *Spirulina* cells. The total number of lamps of the new reactor is 350 (Sylvania 12V 20W), and the total power of the lamps 7000 W. It has been considered for the design that 5% of this power is converted into light energy, as the rest is dissipated as heat. Another important point is that the voltage of the lamps should be regulated for control purposes. The technical solution to this point is to regulate the voltage directly in the main power line to the complete set of lamps, at 380V AC. After this regulation the power is transformed to 12V AC for the 380V or proportionally for the regulated lower voltages. Later it is distributed to the individual lamps. This option was chosen as its cost is markedly lower than the option followed in the previous reactor, with an array of variable power supplies, thus first transforming and second regulating the voltage to the lamps.

With respect to the heat generated by the lamps, two actions have been incorporated in the reactor design: first, the hot air generated around the lamps is evacuated by means of a set of tubes distributed along the lamps supports, connected to a fan that pumps the hot air out of the laboratory; second, the heat absorbed by the liquid in the reactor is compensated by means of a refrigeration system, pumping a cold fluid (cooling fluid temperature can be decreased to –19 °C in the cooler, with a max. flow of 4.8 m^3/h, at 1.9 bar) through the external jackets in the stainless steal parts of the reactor.

Another aspect considered in the design of the new reactor is the management of liquid and gas flows. For the continuous operation of the 75 litres volume reactor, liquid medium is pumped in by means of gear pumps, and sterilised by filtration. Two different liquid tanks supply the medium.

The liquid tanks are mounted on electronic balances. By this way it is possible to detect when one bottle is empty, and allows to change to the other one. The balances have a 4-20 mA output for monitoring the weight, this information can be used for monitoring the decrease in weight of the bottle and therefore the liquid flow rate. Automatic flow control using this measurement is not yet implemented because it is necessary to take into account about 20 min delay in the signal. That is, a modification

of the flow at time zero will not be correctly identified as a modification in the speed of weight decrease, until enough time has passed to securely identify the change in the slope of weight decrease.

Liquid outlet is done by means of a peristaltic pump and the liquid flow is calculated as a function of the inlet flow, to get a constant level in the bioreactor. Based on a calibration curve, the output pump is set to a 10% higher flow rate than the input pumps. In this way the liquid level is maintained at the position of the medium extraction tube.

The gas flow control is a key parameter as it concerns liquid circulation, pressure regulation and O_2 and CO_2 concentration measurements. A gas pump and four mass flow-meter/controllers have been installed and a gas circuit has been implemented to improve the existing system and allow normal operation in closed gas loop.

The main flow controller regulates the flow of gas inlet in the reactor, that governs its hydrodynamic pattern. Two flow controllers regulate the external air input or output flow in order to maintain a constant pressure in the reactor and the fourth controller regulates the CO_2 entrance for pH regulation.

In this technical the following aspects of this new 75 l bioreactor are described:

- general design of the equipment
- auxiliary equipment
- control and measurement loops
- listing of variables and connections
- description of reactor set up and results obtained during the first batch experiment.

2- General charateristics of the equipment.

As explained in the introduction the final design selected was an airlift bioreactor having the riser tube and the down-comer section as separated units connected by a stainless steel pipe. This arrangement increases the illuminated area when it is compared with equivalent concentrical designs. The riser and down-comer constitute the illuminated parts of the bioreactor and are made of plastic foil for safety reasons. That is to avoid any glass wall breaking due to steam sterilisation.

The main characteristics of this reactor are:

Main loop tube diameter: 0.15 m. Length illuminated tube 1.5 m.

Foil : polyamid tripan DN-150 (thickness 80 μm).

Working volume : 77 l (up to 83 l after swelling).

Illuminated volume : 53. l. Illuminated area: 1.41 m^2.

Illuminated volume/working volume : 0.688, (up to 0.71 after swelling of plastic material due to hydrostatic pressure).

Illuminated area/ working volume : 18.3 m^{-1}.

In figure 1 a general drawing of the bioreactor and peripheral connections is presented. In appendix 1.1 a list of parts corresponding to that figure can be found. In appendix 1.2 the peripheral instrumentation is listed. One of the improvements added to the bioreactor has been the set up of a gas loop. A global overview of this gas loop is presented in Figure 2.

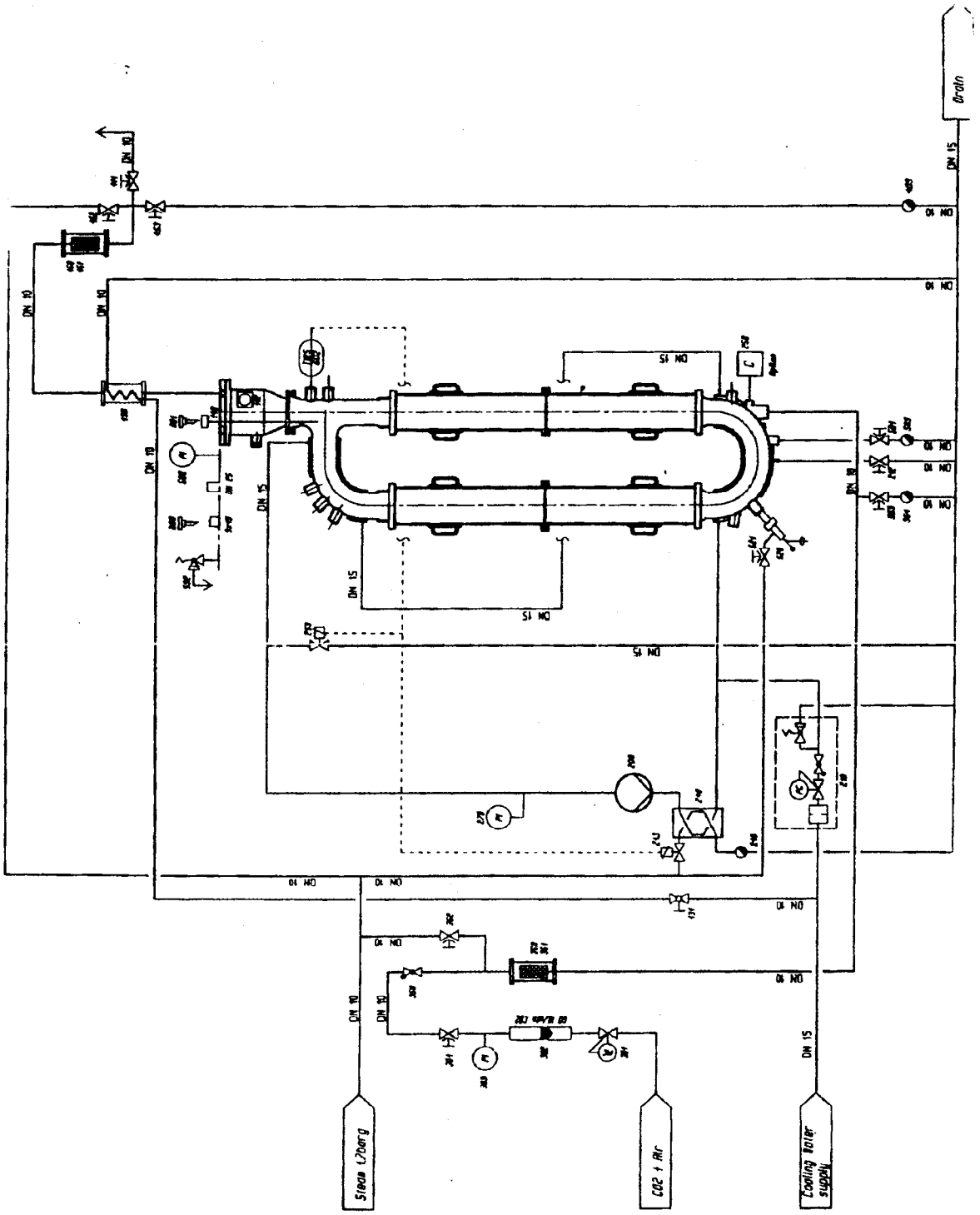


Figure 1: General overview of the bioreactor.

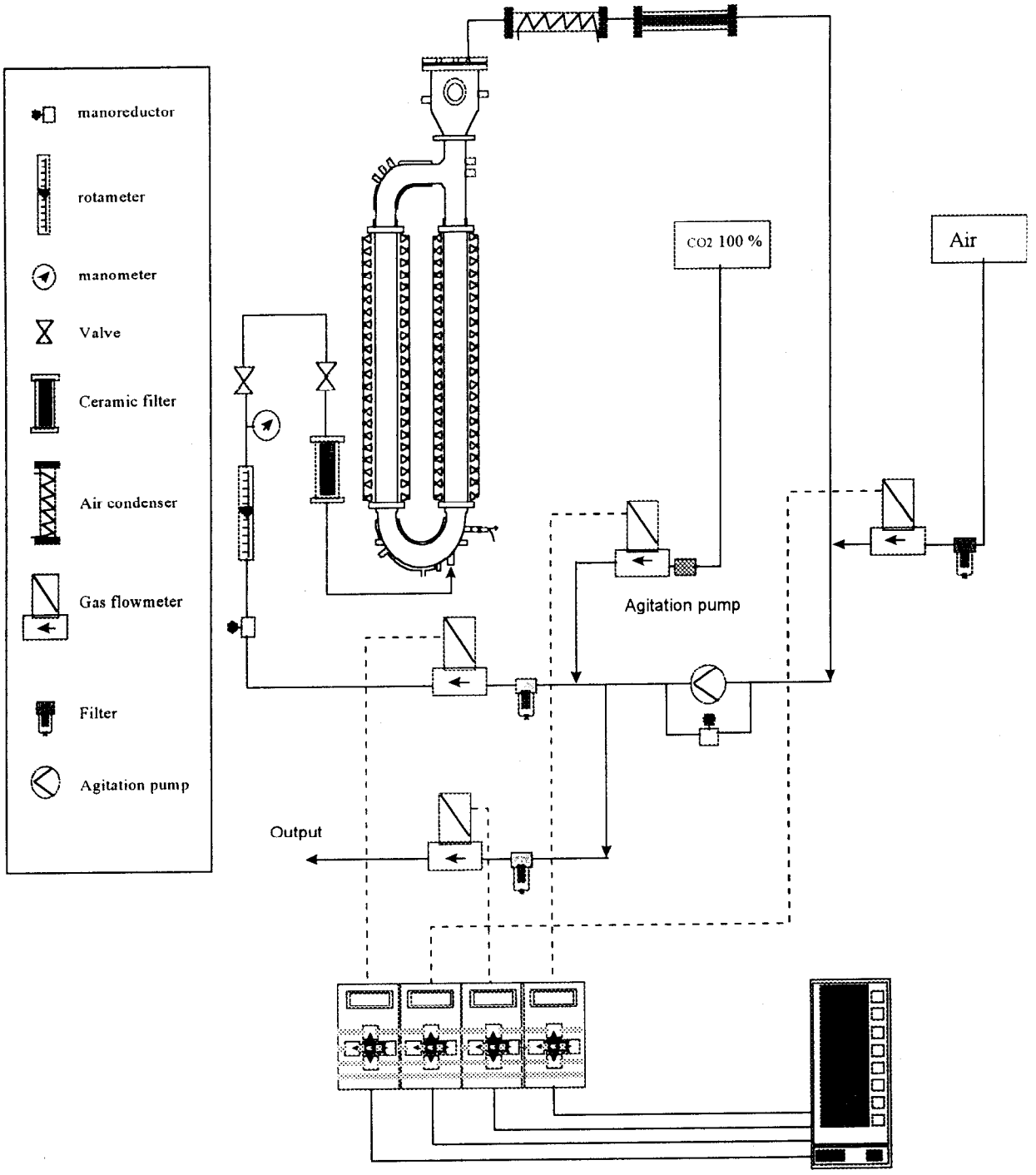


Figure 2: General scheme of the gas loop

3. Description of control and measurement loops

3.1 DESCRIPTION OF THE CONTROLLERS

ASCON 20 controllers are multifunctional, panel-mounted, controllers that use graphic technologies. Their main technical characteristics are the following :

I/O Capacity of the instrument

8 Analog Inputs (AI)

4 Analog Outputs (AO)

4 Digital Inputs (DI)

1 Frequency Input

8 Digital Outputs (DO)

2 RS 485 serial ports

1 RS 232 serial port

1 LAN on ARCNET standard

Analog Inputs AI	0-5 V or 1-5V cc selectable in the parameters, block AI 0-20 mA or 4-20 mA with external shunt resistance 250 Ω. 16 bits conversion resolution. Input impedance in cc ≥1000MΩ
Frequency Input	Connectors 13 (+)and 14 (-) can be connected in the AC 20 controller, for a frequency measurement. Measurement ranges selectable : 0.01-200 Hz, 0.1-2000 Hz. 1-20 kHz in the range 0.01-200 Hz a digital anti-bounce filter of 1.5 ms is automatically inserted (width limit 8-36V)
Analog Outputs	0-5 V, 1-5 V, 0-20 mA, 4-20 mA, selectable in the parameters, AO block load : under tension minimum 500 Ω maximum resolution ~13 bit.
Logical Inputs	24 V cc (min 8 Vcc, max 36 V cc) opto-isolated, passive, input resistance 4700 Ω, bi-directional, operable with positive or negative continuous voltages.
Logical Outputs	24 V cc/ca, max. 36 V cc/ca, 300 mA. NA Output protection against excess voltages and short circuits with self-restoring fuse.

Serial Communications	RS485 port (Main Com) for connection to a supervision system, multi-drop protocol MODBUS or JBUS (RTU), maximum length of the line 1200 meters with a capacity for up to 32 controllers by means of twisted pair, and terminated at the ends with a load of 120 Ω. Transmission velocity up to 19200 baud. RS 485 port (Aux Com) for expansion unit or backing up data of central unit. Galvanic isolation from inputs and outputs. RS232 service frontal port for connection to a programming computer by means of the program Prograph and saving of parameterisation by the program Ac_Edit.

Programming of the control loop was done with AC-PROGRAPH. This program allows to create a control strategy graphically, drawing it on the screen, and, later, downloading and running it on the AC 20 ASCON Controller. A control strategy is defined by functional modules, taking analog and digital signals via their inputs, processing them according to different algorithms and then passing the results to their outputs, interconnected together through wiring, like on a circuit diagram, defining the flow of signals between the modules.

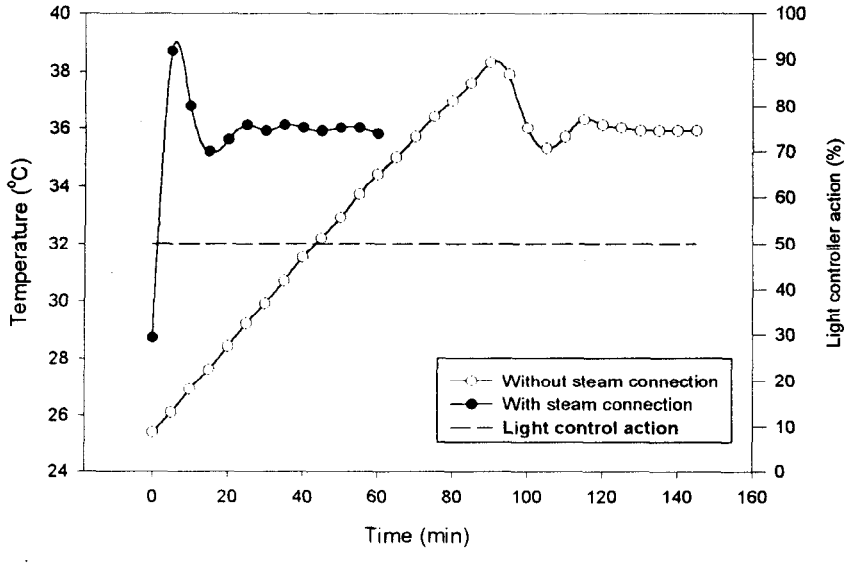
3.2 TEMPERATURE REGULATION

The temperature regulation subsystem was supplied by the bioreactor manufacturer and consists of a temperature controller which obtains the temperature data from the corresponding temperature probe located in the bioreactor. Depending on this measurement it acts on two different valves (Figure 4). The first one allows for a cool fluid to enter and replace the fluid in the heat exchanger jackets located in the metallic parts of the bioreactor. In case of the temperature being too low there exist a second heat exchanger foreseen to heat the fluid flowing in the metallic jackets by means of using steam. This method requires a source of steam continuously connected to the bioreactor.

As it can be seen in Figure 3, the effect of connecting the heat exchanger to the steam line is important during the start-up of the bioreactor were it accelerates the speed at which the working temperature is reached. Using the steam exchanger, the working temperature begins to be stabilised in about half an hour while without using it the working temperature is reached in about two hours.

To verify the operation of the temperature controller in front of a change in illumination conditions, several light step changes were done with and without using the heat exchanger. Bioreactor was filled with water and wood chips were added to increase heat uptake from the lamps. In the first series of tests the light intensity was increased in several steps. The controller operated using the steam generator (Figure 5 top) or without using the steam generator (Figure 5 bottom). The light step changes did not result in a significant variation of the temperature value. This reflects an appropriate operation of the cooling line, which is the part in charge of evacuating the excess of heat generated by the light energy increase. There was also not a relevant variation between using or not the steam generator, which may reflect the fact that the main part of the action is done on the cooling line.

Figure 3: Temperature test during start-up of the bioreactor with and without steam line connected to the heat exchanger.



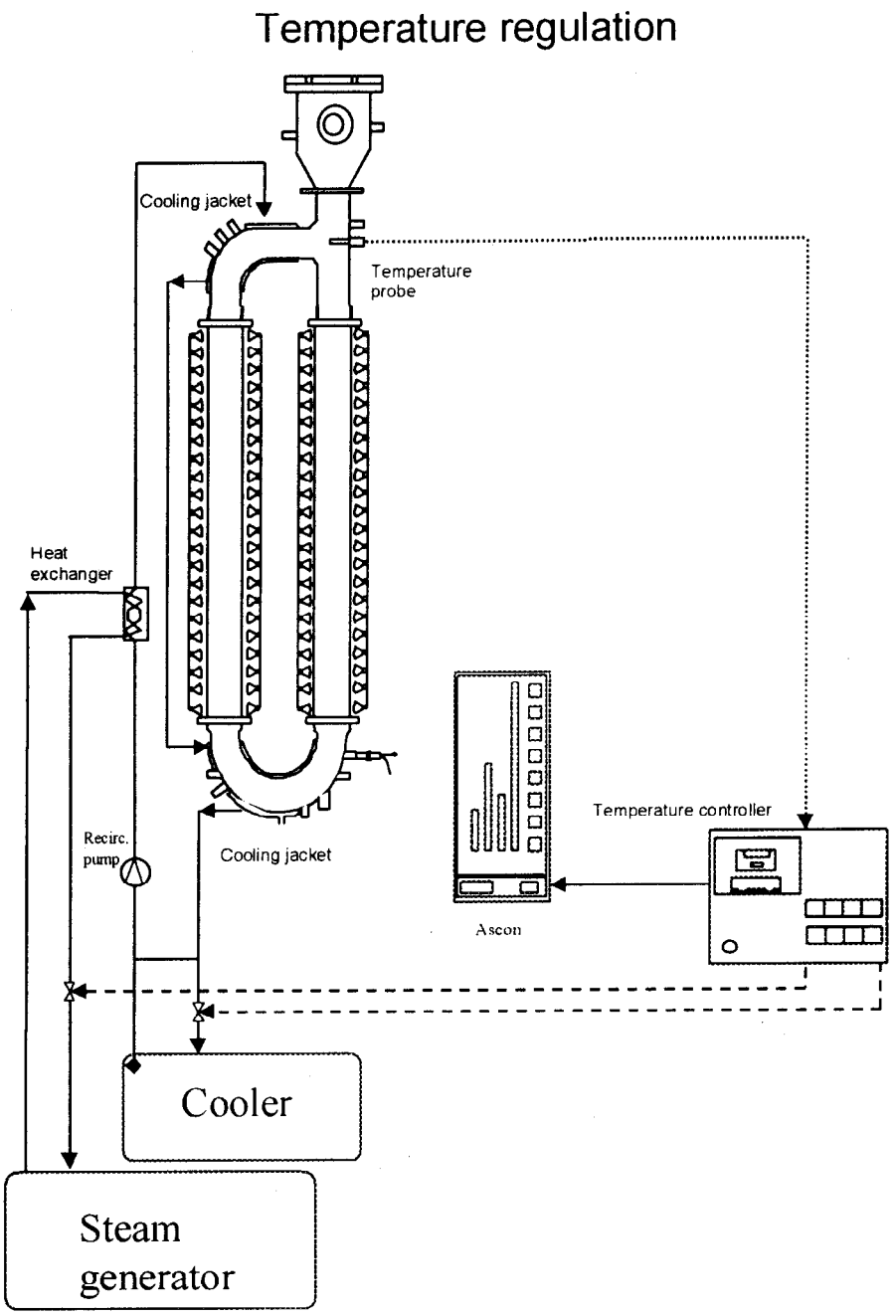


Figure 4: Temperature regulation loop

Goal	Maintain the temperature of the culture at the setpoint value (36 °C).
Measurements	A PT-100 probe measure the temperature of the culture. Range : 0-150 °C
Actions	The regulation action (opening of the valves for admission of refrigerating liquid in the cooling jacket or steam for heating) is performed by the temperature regulator.
Analog values	AI 0502 : temperature measurement

A second set of tests was done where light intensity was decreased, to verify the effect of the heating elements on the behaviour of the controller. The controller worked as before either using the steam generator (Figure 6 top) or not (Figure 6 bottom) the steam generator. In this case the stability of the set point appeared slightly less stable without using the steam generator than if the steam generator was on-line. Nevertheless the performance was considered very good in both cases.

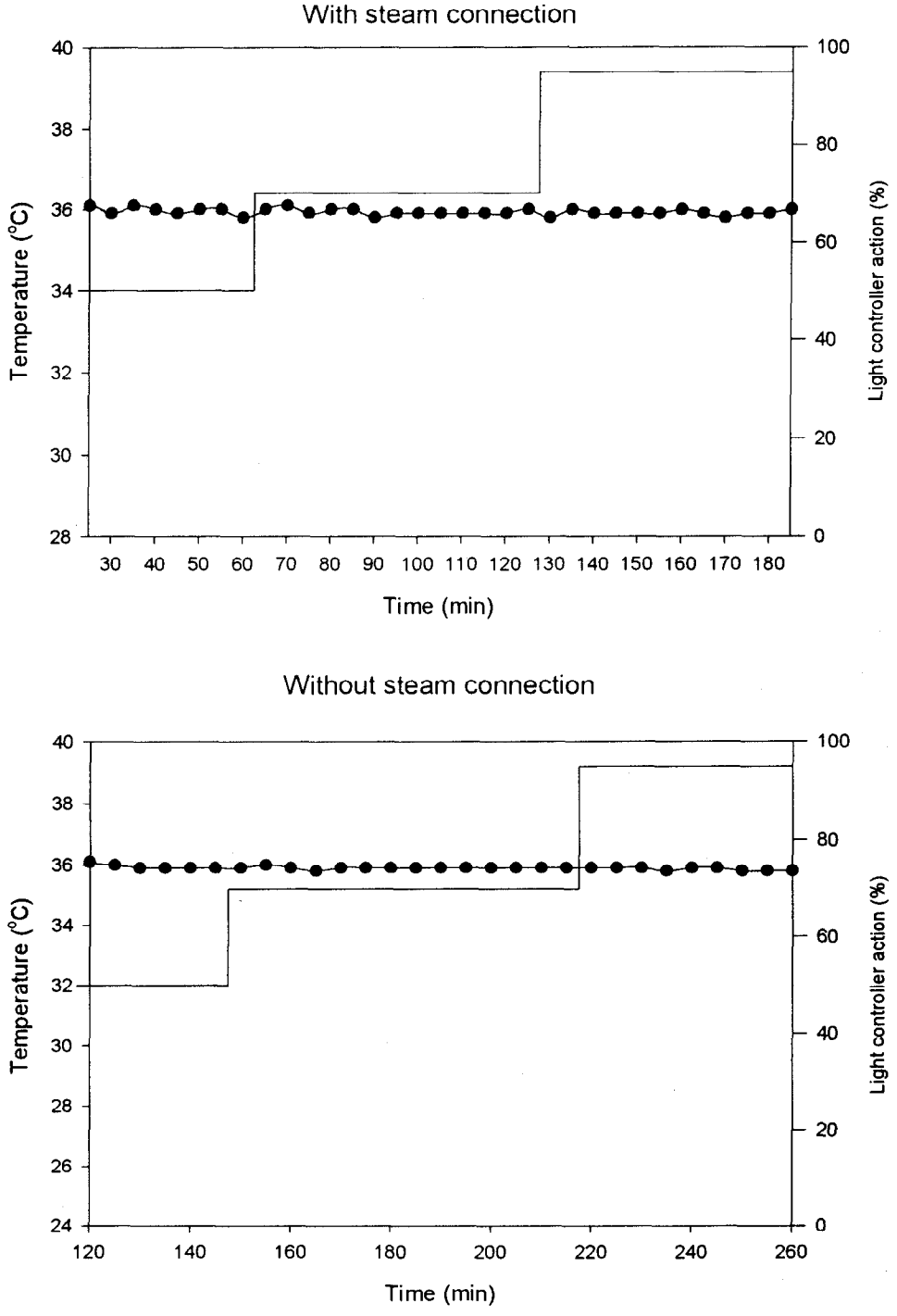


Figure 5: Increasing light steps at different light intensities with and without using the connection to the steam generator.

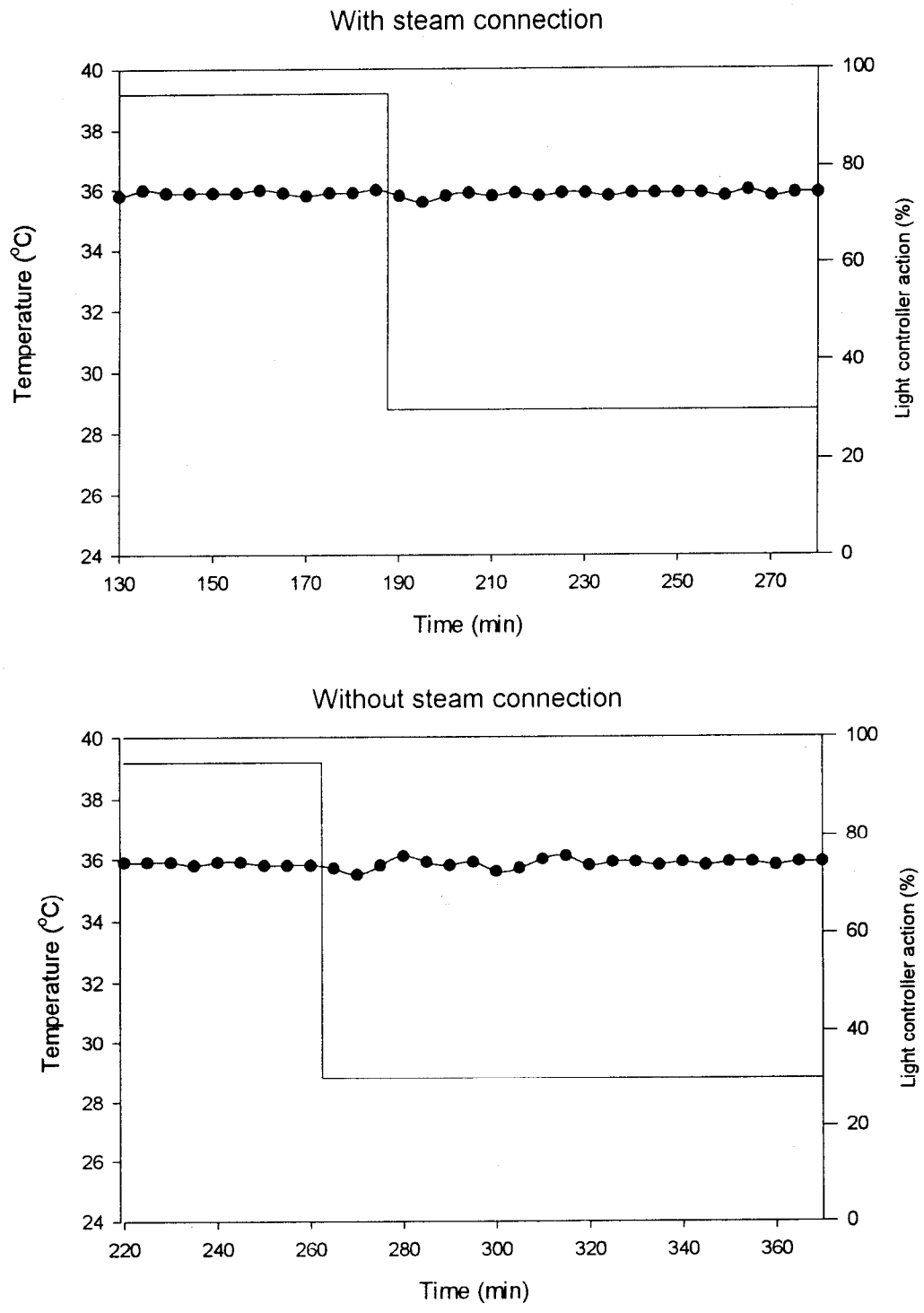


Figure 6: Decreasing light steps at different light intensities with and without using the connection to the steam generator

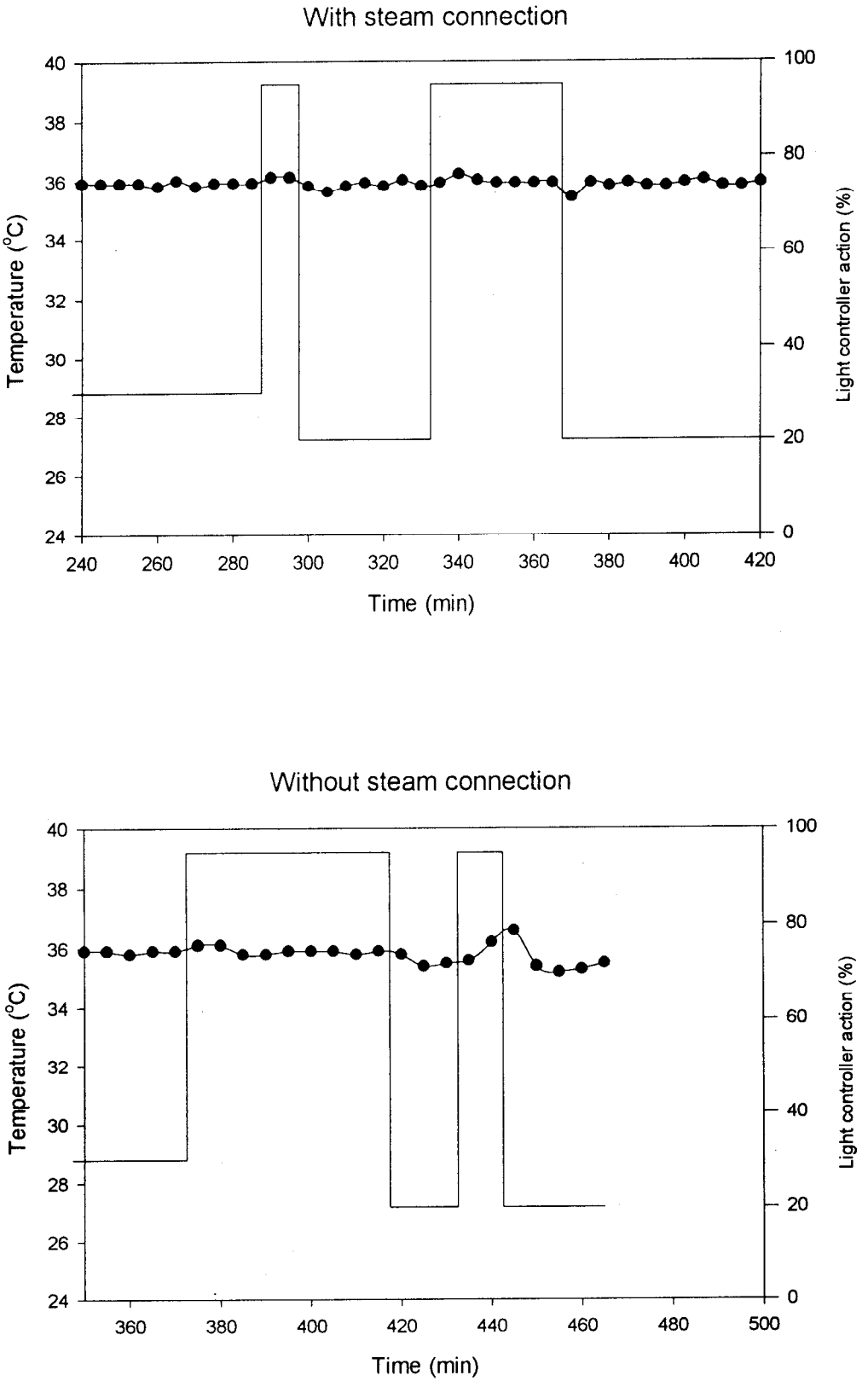


Figure 7: Increasing and decreasing light steps with a higher concentration of wood chips.

To further investigate the performance of the controller, the concentration of the wood chips was further increased so as to simulate a higher biomass concentration absorbing light energy. The controller worked as before either using the steam generator (Figure 7 top) or not (Figure 7 bottom) the steam generator. In this case it was observed that the control system showed a slower response in reaching the value of the temperature set-point, specially if the light intensity was changed before allowing enough time for the controller to correct for the previous disturbance. Nevertheless the variation showed in the worst case was only of about +/-1 °C and the period of time necessary to recover the stability around the set-point was short (~30 min).

3.3 pH REGULATION

Goal	**Maintain the pH in the culture medium around a fixed value (usually 9.5) to compensate pH increase due to *Spirulina* growth**
Measurements	A probe measures the pH of the medium. Range : 0-14 Calibration : before starting the culture The flow of $C0_2$ delivered is measured by HI-TECH flowmeter/controller Range : 0-5 l/min Calibrated with $C0_2$ at 1 bar
Actions	CO_2 is introduced to reduce pH using the chemical reaction : $CO_2 + OH^- \rightarrow HCO_3^-$ ASCON 20 fixes the values of the set-point of CO_2 flow rate.
Analog values	AI 0606 : pH measurement AI 0604 : CO_2 flow measurement AO 0604 : CO_2 flow set-point

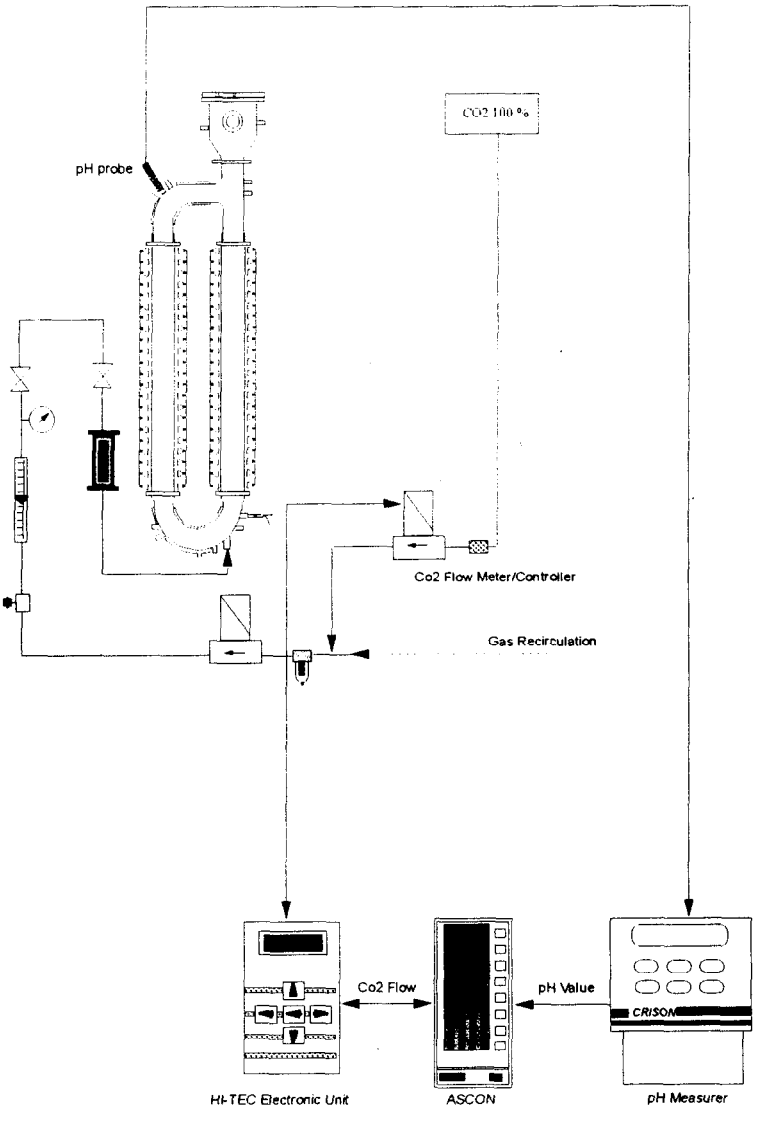


Figure 8: Scheme of pH regulation loop.

3.4 LIGHT REGULATION

Goal	**Maintain the light intensity at the surface of the bioreactor around a setpoint**
Actions	Either the light can be set to a fixed value by Ascon 20 or the setpoint can be fixed by the GPS. The signal is sent to the light supply system.
Analog values	AO 0504: light set-point (4-20 mA)

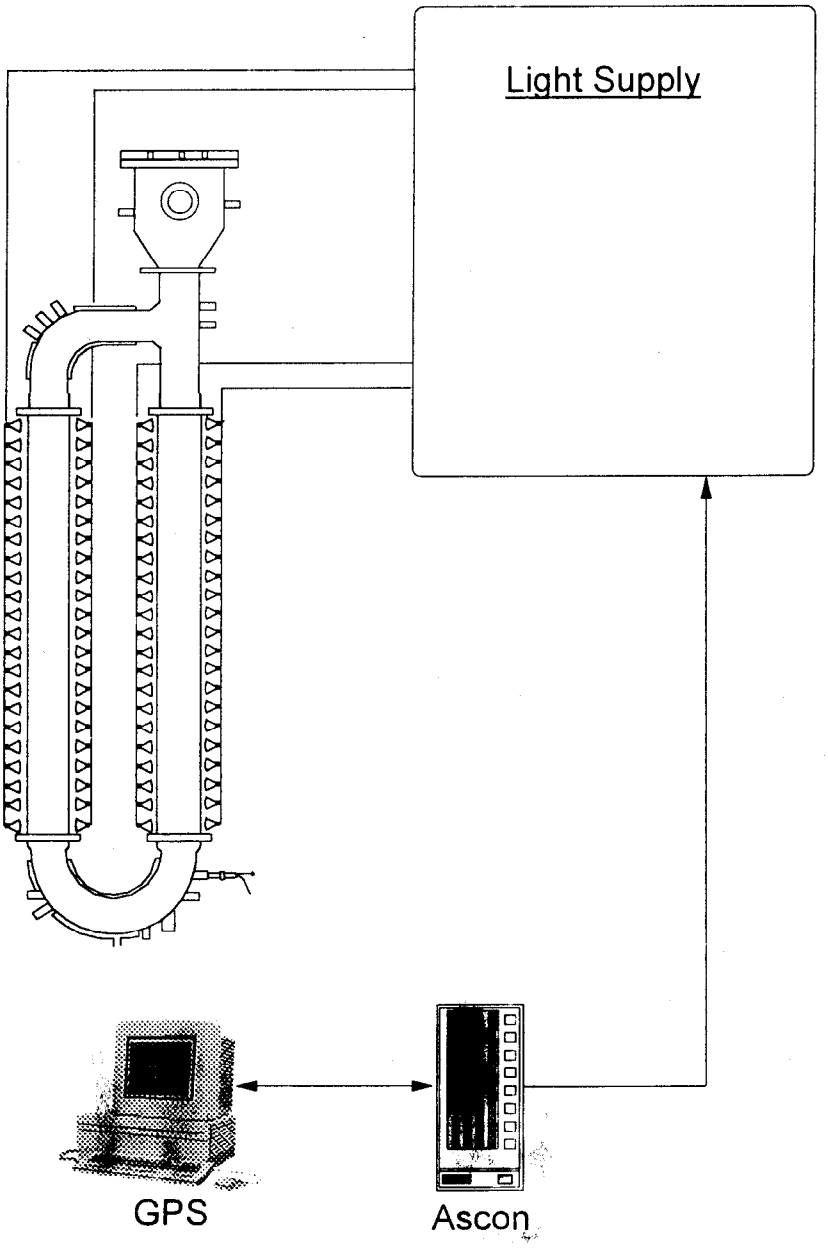


Figure 9: Scheme of the light regulation loop.

3.41 LIGHT INTENSITY CALIBRATION

As mentioned in the introduction, 350 lamps compose the illumination system, with a maximum power of 7000 W. These lamps are fixed on two metallic supports (one for each column of the bioreactor) that can be opened and removed during cleaning and sterilisation operations. Each support contains 7 straight bars of 25 lamps.

A calibration was done to establish a relationship between the percentage of controller action and illumination intensity at the surface of the bioreactor (Fr). Conversion of the light intensity measured by a spherical sensor located in the axis of the riser to the light intensity at the surface of the bioreactor can be done using the following equation:

$$Fr = 0.291 \times Eb \times \frac{rb}{\pi \times Rb}$$

Where Fr is the light flux at the bioreactor surface, Eb is the light intensity ($\mu mol/m^2 \cdot s^2$) measured by the sensor, rb is the radius of the sensor (0.03 m), Rb is the radius of the bioreactor (0.075 m).

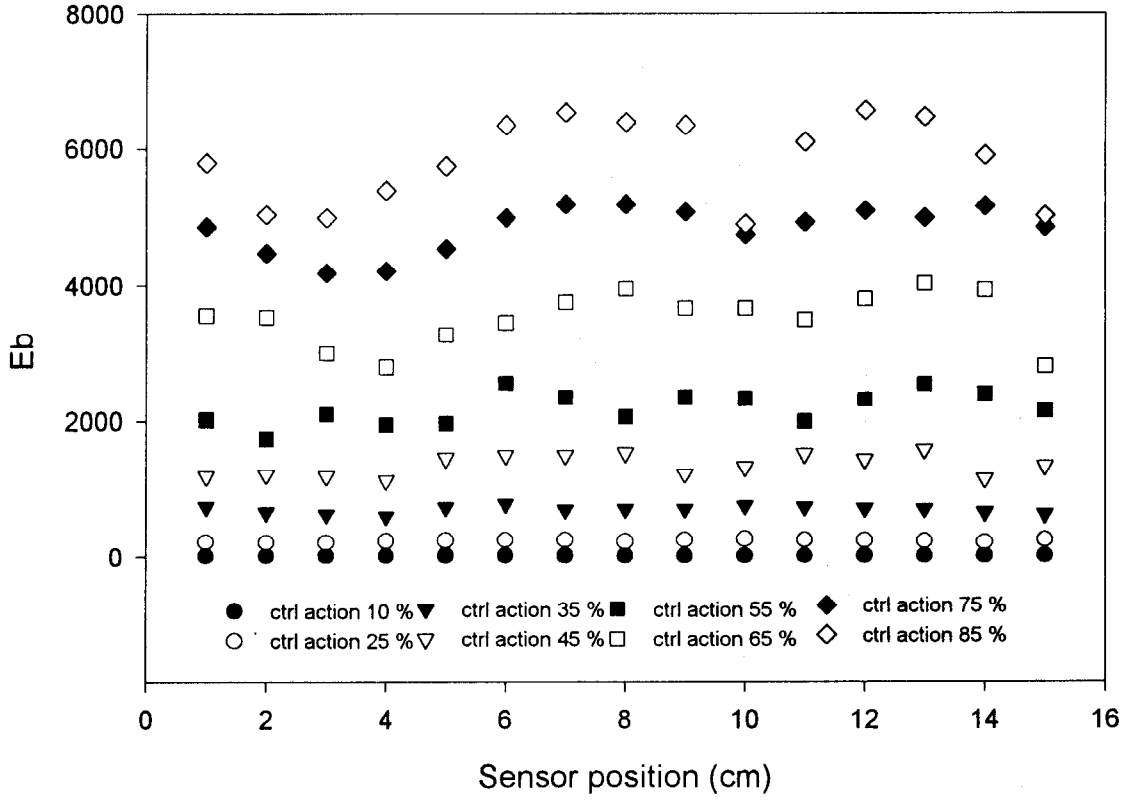


Figure 10: Light intensity obtained in the centre of the reactor for various controller actions and at different height levels of the illuminated volume.

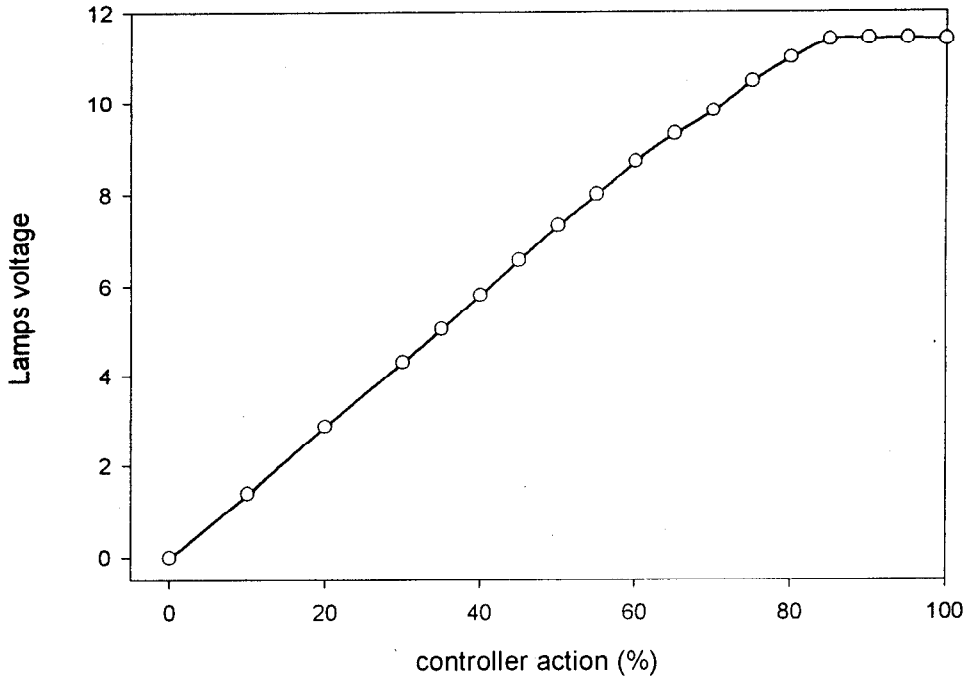
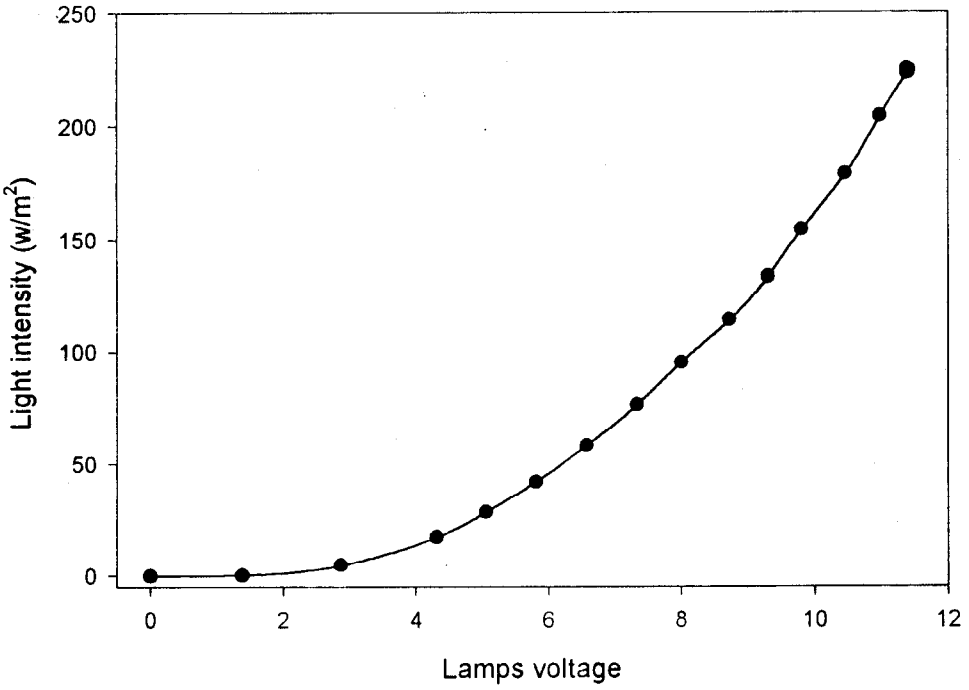


Figure 11 Lamps voltage as a function of controller action

Figure 12: Light intensity at the surface of the bioreactor as a function of voltage



Light intensity measurements were done at different vertical positions and for different voltages applied to the lamps. The results of these measurements are plotted in Figure 10.

The measurements obtained for each voltage at different vertical positions were averaged and the light intensity values measured by the sensor in μmol/(m^2 s^2) were converted to Fr values using the formula mentioned above.

As a result of these measurements, a relationship between the voltage applied to the lamps and the Fr of the airlift bioreactor was obtained (**Figure 12**).

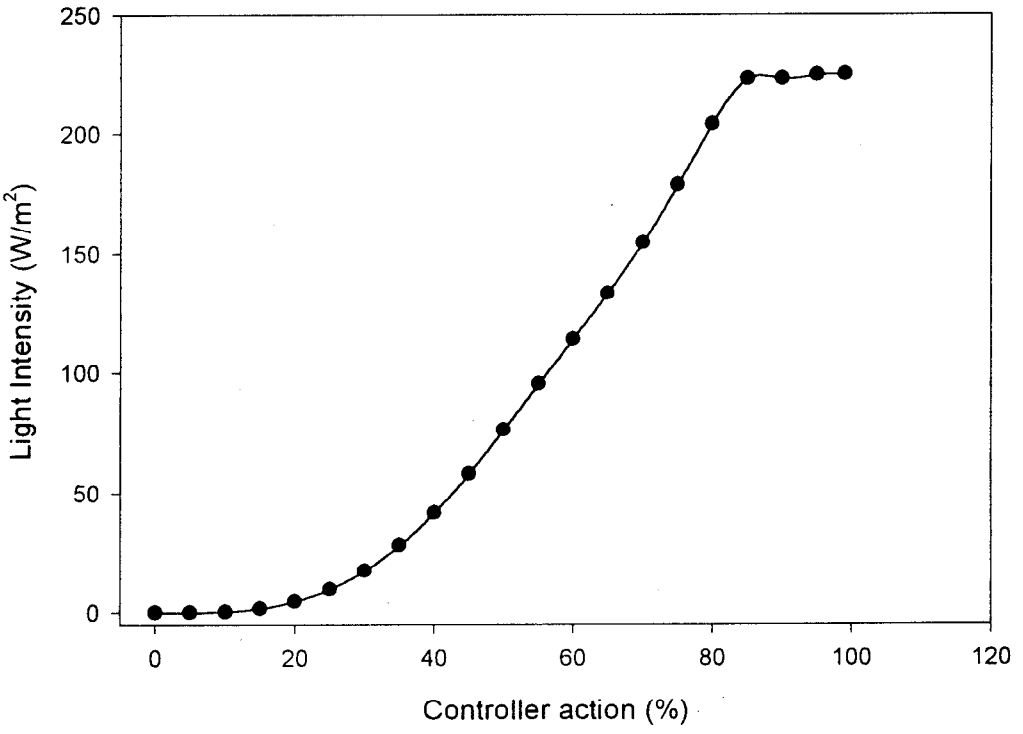


Figure 13: Light intensity at the surface of the bioreactor as a function of controller action

The voltage applied to the lamps is modified by the controllers by means of an electric signal (4-20 mA) sent to the illumination power supply system. In order to know the voltage applied to the lamps from the action of the controller it is necessary to obtain a relationship among the controller action and the lamp voltage. Different percentages of controller action were set and the voltage received by the lamp on its support was measured (Figure 11). The obtained data allows to calculate the Fr values from the known value of the controller action (Figure 13) using the following relationship:

$$Fr = -8*e^{-8}*(\%ctrl)^5 + 1*e^{-5}(\%ctrl)^4 - 0.0003(\%ctrl)^3 + 0.0399*(\%ctrl)^2 - 0.5389*(\%ctrl) + 0.9646$$

3.5 NITRATES MEASUREMENT

Goal	**Measure NO_3 concentration in the fermentation medium**
Measurements	Free-biomass medium is obtained by the Tech-Sep module. Then it is analysed by ultraviolet light absorption in the Dr Lange analyser. Characteristics : - range 0 to 25 mg/l $N\text{-}NO_3^-$ - continuous measure - calibration step during 20 min every day
Analog values	AI 0607 : Nitrate concentration in the reactor
Digital values	DI 0601 : Nitrate analyser calibration

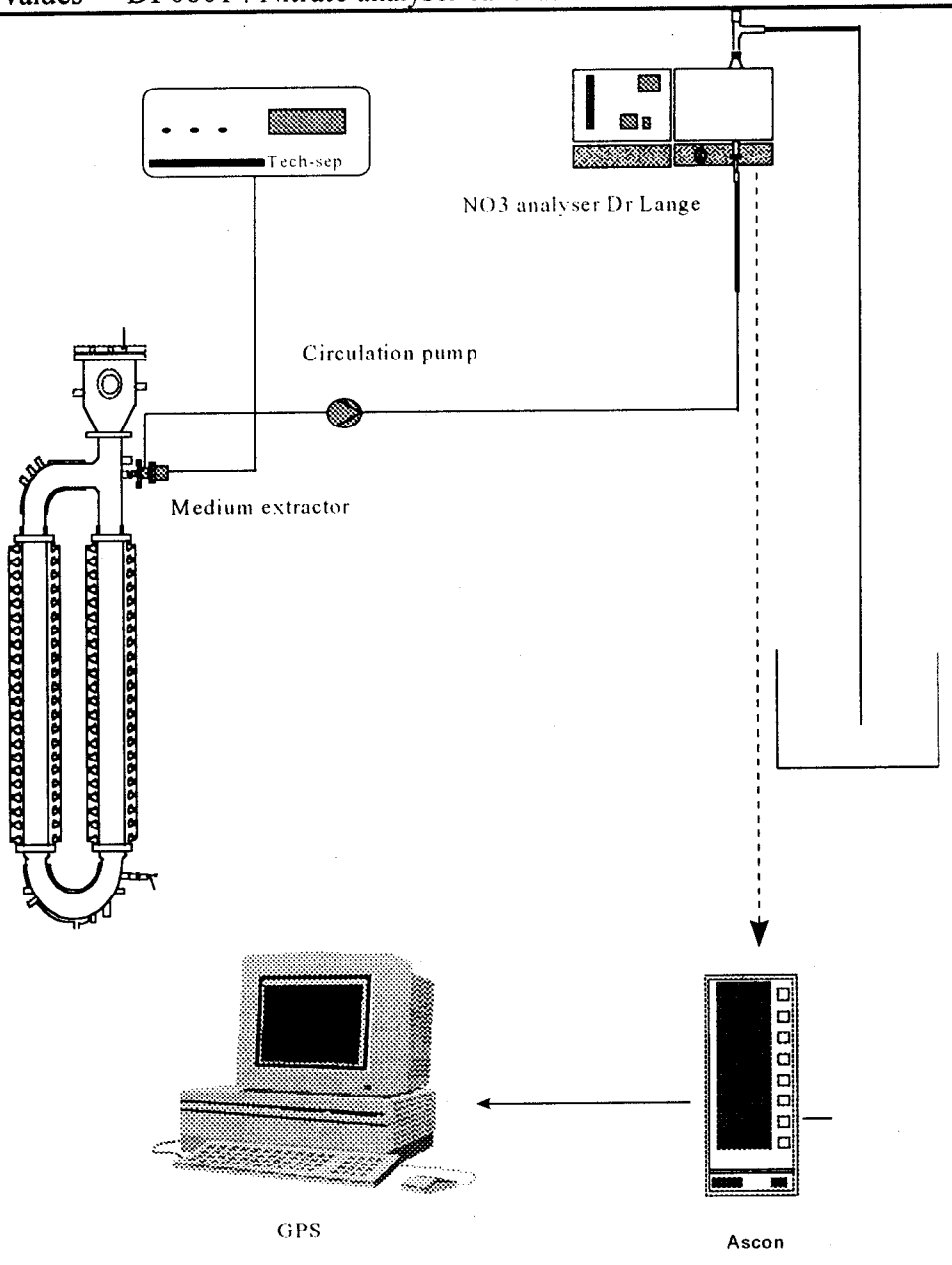


Figure 14: Scheme of the nitrates measurement loop.

3.6 BIOMASS REGULATION

The biomass regulation loop is a more complex loop than the ones previously described. It relies in the operation of the GPS computer which allows the use of more developed control routines. This evolved routines, make use of mathematical models and can predict the evolution of biomass growth and their composition. For its operation the GPS require to know at any time, the biomass concentration, light intensity and flow rate. The GPS can modify the productivity values by acting either on the light intensity, as the primary manipulated variable, or on the flow rate. Operation on the light intensity has already been described above. Operation on the flow rate, is done by acting on the analogic output connected to the input and output pumps.
As a new feature the controller has available the value of the weight of the culture medium input bottles. In the first set up, the weight value is used to allow the control system to change from one culture medium bottle to the next one as soon as one is empty or stop the flow if both are exhausted. The flow rate is calculated by the GPS and the control action sent to the controllers. In a future implementation the GPS could use the weight information to evaluate and correct, if necessary, the flow rate of the culture medium. This feature is proposed as a future improvement to be incorporated into the GPS.

Goal	**Maintain the biomass productivity in culture medium around a set-point.**
Measurements	A biomass probe (Monitek) measure the attenuation of a light beam through the culture. Then a correlation law is used by the controller to calculate the biomass concentration in the reactor. Characteristics : - range 0 to 2 attenuation - continuous measure - calibration before the culture for zero value. Two balances measure the diminution of weight in the input medium bottle. Flow rate calculated by the GPS. Characteristics : -range 0 to 150 kg -continuous measure of weight and flow -calculation of liquid flow by the GPS to accommodate the productivity desired.
Actions	The ASCON 20 calculate liquid input flow rate and decides which of the two liquid input pump has to run. GPS fixes the set-point of input pump velocity and ASCON 20 calculates the set-point of output pump. GPS regulates the flow and light intensity to maintain the set-point of productivity.

Analog values	AI 0501 : Monitek attenuation AI 0503 : Weight input medium tank 1 AI 0504 : Weight input medium tank 2 AO 0501 : Input pump 1 set-point AO 0502 : Input pump 2 set-point AO 0503 : Output pump set-point
Digital values	DO 0501 : Input pump 1 on DO 0502 : Input pump 2 on

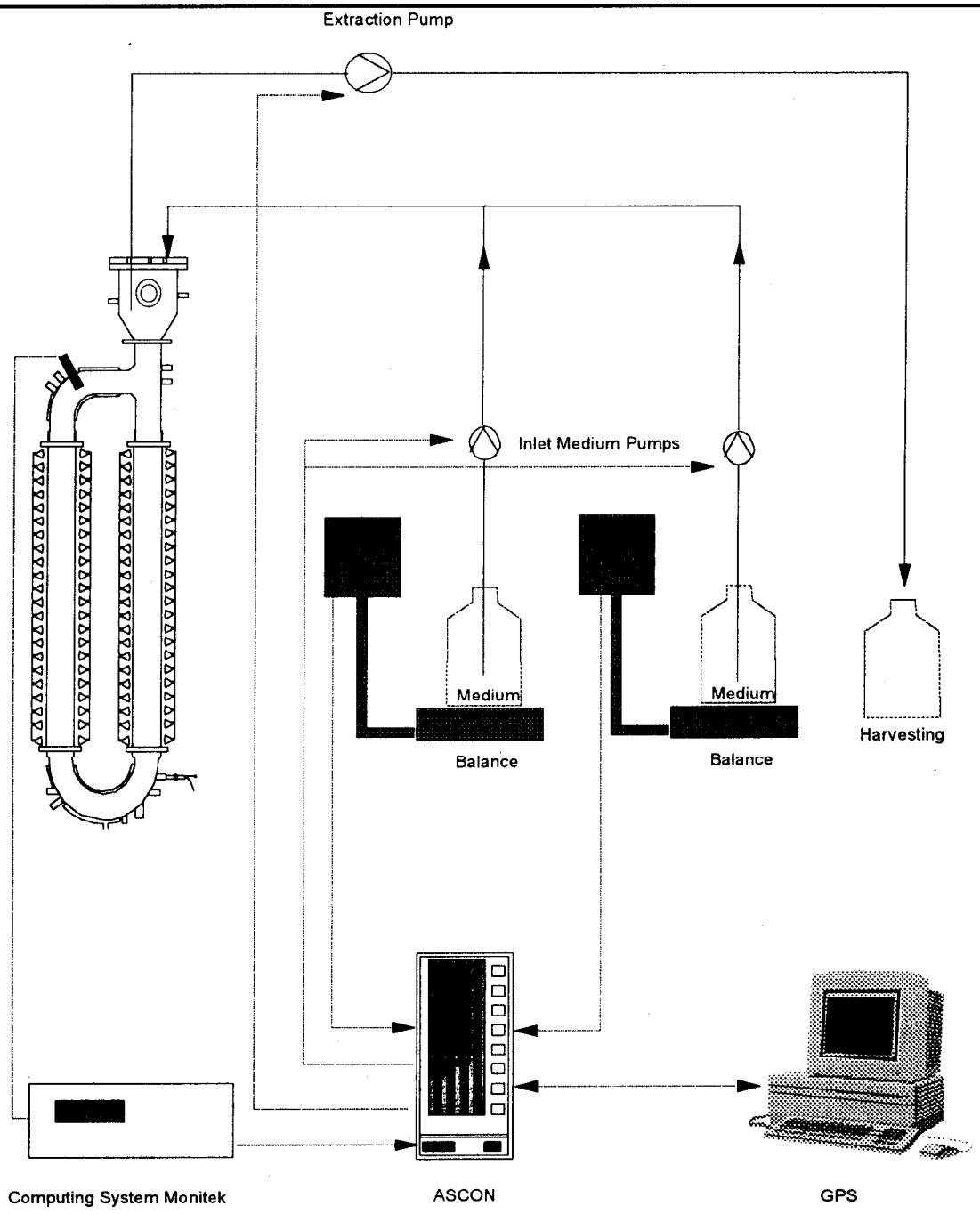
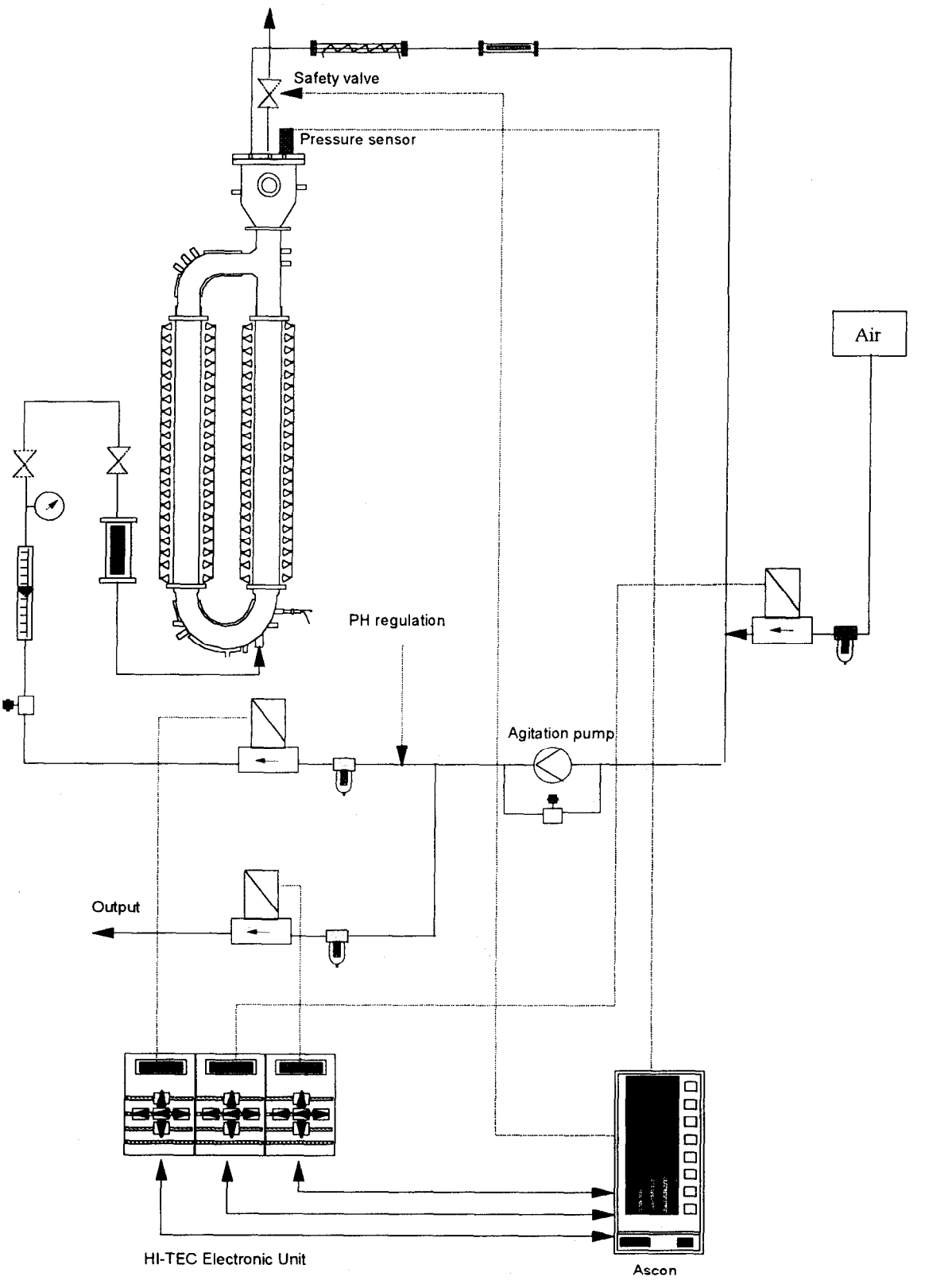


Figure 15: General view of the biomass and liquid flow control.

3.7 GAS FLOW AND PRESSURE REGULATION

Goal	**Maintain the head pressure in the reactor around a set-point (0.01bar). Regulate the gas flow rate and liquid agitation and aeration**
Measurements	The pressure sensor measure the pressure in the headspace of the reactor Characteristics - range 0 to 1.5 bars - continuous measure The mass flow meters/controllers measure the gas flow of gas recirculation, external input and output Characteristics : - range 0 to 30 l/min - continuous measure - calibration at 1.25 bars.
Actions	ASCON 20 control setpoint of hydrodynamic, input and output mass flow controllers If P>0.01 bar->increase output gas flow. If P<0.01 bar->increase input gas flow. When there is an overpressure of 0.02 bars, security valve opens
Analog values	AI 0601 :gas recirculation flow measurement AI 0602 : gas external input flow measurement AI 0603 : gas external output flow measurement AI 0605 : pressure measurement AO 0601 :gas recirculation flow setpoint AO0602 : gas external input flow setpoint AO 0603 : gas external output flow setpoint
Digital values	DO 0601 : safety valve


Safety valve
Pressure sensor
Air
PH regulation
Agitation pump
Output
HI-TEC Electronic Unit
Ascon

4 List of variables and connections

4.1 List of variables

ASCON 1

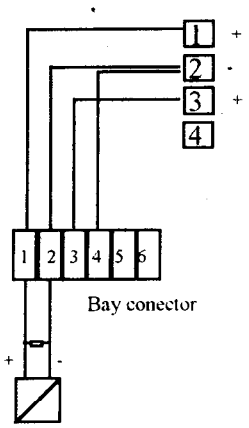
AI 0501	Biomass sensor
AI 0502	Temperature sensor
AI 0503	Balance 1
AI 0504	Balance 2
AI 0505	NO_3 concentration (0-25 mg/l)
AI 0506	NO_3 pressure sample
AI 0507	pO_2 liquid
AI 0508	Not connected
DI 0501	Calibration switch NO_3
DI 0502	Not connected
DI 0503	Not connected
DI 0504	Not connected
DI 0505	Not connected
DI 0506	Not connected
DI 0507	Not connected
DI 0508	Not connected
AO 0501	Input liquid pump 1 setpoint
AO 0502	Input liquid pump 2 setpoint
AO 0503	Output liquid pump setpoint
AO 0504	Light regulation
DO 050 1	Input liquid pump 1 On
DO 0502	Input liquid pump 2 On
DO 0503	Not connected
DO 0504	Not connected
DO 0505	Not connected
DO 0506	Not connected
DO 0507	Not connected
DO 0508	Not connected

ASCON 2

AI 060 1	Flow meter 1 (Hydrodinamic)
AI 0602	Flow meter 2 Inp. Flow
AI 0603	Flow meter 3 Outp. Flow
AI 0604	Flow meter 4 CO_2 Flow.
AI 0605	Head Pressure
AI 0606	pH
AI 0607	[CO_2] gas measurement
AI 0608	[O_2] gas measurement
DI 0601	Not connected
DI 0602	Not connected
DI 0603	Not connected
DI 0604	Not connected
DI 0605	Not connected
DI 0606	Not connected
DI 0607	Not connected
DI 0608	Not connected
AO 0601	Set point Flow meter 1
AO 0602	Set point Flow meter 2
AO 0603	Set point Flow meter 3
AO 0604	Set point Flow meter 4
DO 060 1	Pressure safety valve
DO 0602	Hydrodynamic flow pump.
DO 0603	Not connected
DO 0604	Not connected
DO 0605	Not connected
DO 0606	Not connected
DO 0607	Not connected
DO 0608	Not connected

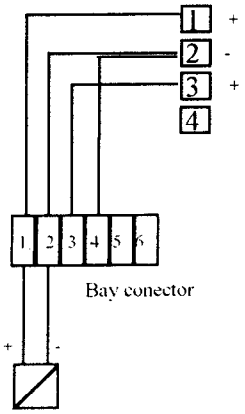
4.2 General scheme of Ascon connections

Analogical inputs



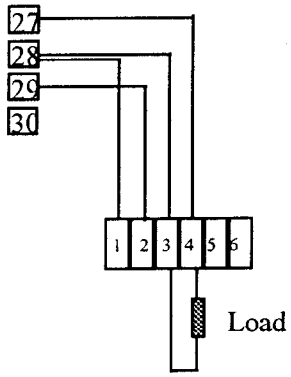
4-20 mA signal

Analogical inputs

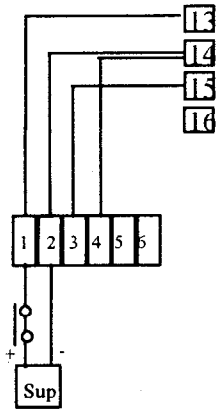


0-5 V signal

Anological outputs

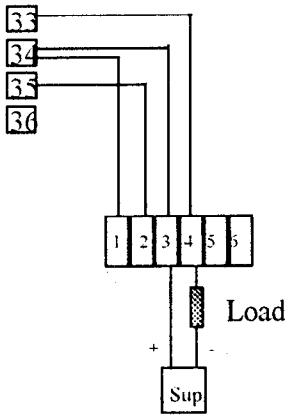


Digital inputs



External power supply

Digital outputs



External power supply

4.3 List of connections

Bay connector 1

No	Signal name	Hardware voltage/current	ASCON voltage/current	From	To
1 2	+ Biomass - Biomass	4-20 mA	1-5 V	Monitek	AI 0501
3 4	+ Temperature -Temperature	4-20 mA	1-5 V	Temperature controller	AI 0502
5 6	+ Balance 1 - Balance 1	4-20 mA	1-5 V	Balance 1	AI 0503
7 8	+ Balance 2 - Balance 2	4-20 mA	1-5 V	Balance 2	AI 0504
9 10					
11 12					
13 14					
15 16					
17 18	+ Set-point pump 1 - Set-point pump 1	0-5 V	0-5 V	AO 0501	Pump 1
19 20	+ Set-point pump 2 - Set-point pump 2	0-5 V	0-5 V	AO 0502	Pump 2
21 22	+ Set-point output pump - Set-point output pump	0-5 V	0-5 V	AO 0503	Output pump
23 24	+ Light regulation - Light regulation	4-20 mA	4-20 mA	AO 0504	Light supply
25 26					
27 28					

No	Signal name	Hardware voltage/current	ASCON voltage/current	From	To
29 30					
31 32					
33 34					
35 36					
37 38					
39 40					
41 42					
43 44					
45 46					
47 48					
49 50					
51 52					
53 54					
55 56	+ Liquid pump 1 on - Liquid pump 1 on		DO 0501		Liquid pump 1
57 58	+ Liquid pump 2 on - Liquid pump 2 on		DO 0502		Liquid pump 2
59 60					

Bay Connector 2

No	Signal name	voltage/current		From	To
1 2	+ Flowmeter 1 - Flowmeter 1	0-5 V	0-5 V	Flowmeter 1	AI 0601
3 4	+ Set-point Flowmeter 1 - Set-point Flowmeter 1	0-5 V	0-5 V	AO 0601	Flowmeter 1
5 6	+ Flowmeter 2 - Flowmeter 2	0-5 V	0-5 V	Flowmeter 2	AI 0602
7 8	+ Set-point Flowmeter 2 - Set-point Flowmeter 2	0-5 V	0-5 V	AO 0602	Flowmeter 2
9 10	+ Flowmeter 3 - Flowmeter 3	0-5 V	0-5 V	Flowmeter 3	AI 0603
11 12	+ Set-point Flowmeter 3 - Set-point Flowmeter 3	0-5 V	0-5 V	AO 0603	Flowmeter 3
13 14	+ Flowmeter 4 - Flowmeter 4	0-5 V	0-5 V	Flowmeter 4	AI 0604
15 16	+ Set-point Flowmeter 4 - Set-point Flowmeter 4	0-5 V	0-5 V	AO 0604	Flowmeter 4
17 18	+ Head pressure Sensor - Head pressure Sensor	4-20 mA	0105 V	Pressure Sensor	AI 0605
19 20	+ pH - pH	4-20 mA	0105 V	pH Meter Crison	AI 0606
21 22	+ NO3 - NO3	4-20 mA	0105 V	NO3 Dr Lange	AI 0607
23 24	+ pO2 - pO2	4-20 mA	0105 V	pO2meter Mettler	AI 0608
25 26					
27 28					
29 30					

No	Signal name	Hardware voltage/current	ASCON voltage/current	From	To
31 32					
33 34					
35 36					
37 38					
39 40					
41 42	Calibration Switch NO_3	Switch		Dr Lange	DI 0601
43 44					
45 46					
47 48					
49 50					
51 52					
53 54					
55 56					
57 58	Pressure Safety valve	220 V		DO 0601 via relay	Safety valve
59 60	Agitation pump	220 V		DO 0602 via relay	Pump power supply

4 Procedure of set-up of the photobioreactor

4.1 SET UP AND STERILISATION

The first step consists on mounting the two polyamide foils according to the manufacturer manual. After fixing tensing and positioning the foil in the reactor, the final length of each foil is 150 cm. This operation is a delicate operation due to the fact that the polyamide foil can get wrinkled, pierced or cut easily.

Once both plastic parts are mounted, pressure jackets have to be mounted and screwed. These pieces are necessary during the operation of sterilisation of the reactor. They allow the foil to be submitted to the sterilisation pressure without deformation.

Before sterilisation, the fermenter is prepared for culturing, i.e. all openings are closed, needle connections are equipped with new silicone septums, the biomass and pH sensors are calibrated . The steam is supplied by the laboratory steam line.

Sterilisation consists in two steps, respectively sterilisation of air inlet filter and sterilisation of fermenter and exhaust air filter. The operations of opening and closing of the valves are explained in the manual (page 8/9). The time of sterilisation is of 15 minutes for the inlet filter and at least 30 minutes for the fermenter. After the sterilisation and during the cooling, air has to be let flowing in to prevent the foil from colapsing. When the desired temperature is reached, the pressure jackets can be removed.

4.2 START UP OF THE REACTOR

First, the light supports have to be placed and its wires connected. The fermenter is filled with appropriate culture medium. For *Spirulina* cultures the modified Zarrouck medium is pumped into the reactor under aseptic conditions. The light level is fixed at the desired value, and the aeration and temperature control are switched on. Special attention must be paid to the fact that pressure control is working well in order to avoid swelling or shrinking of the plastic material. This material is very sensitive to pressure changes and is plastic but not elastic. Then its deformation is not reversible. Due to this

fact it is important that the gas loop is not closed until pressure regulation has been first verified.

After polarisatin and calibration of dissolved oxygen probe, the reactor can be inoculated.

4.3 FIRST BATCH EXPERIMENT

A first batch experiment has been realised with this reactor in order to test its performance. The volume of inoculum was 10 litres and three levels of light were tested during 15 days of operation. At the beginning of this test, light intensity was set at 55 % of the controller action which corresponded to a calculated value of the light intensity at the bioreactor surface (Fr) of 95.2 W/m^2. At this light level, the biomass concentration reached a top of 1.6 g/l. When light was increased to 65 % of controller action (Fr = 133 W/m^2), a maximal concentration of 3 g/l was obtained. Finally, during the last phase of the experiment, light was set at the maximum value, that is a Fr value of 225 W/m^2 . In these conditions, biomass concentration increased to a value of 4.2 g/l.

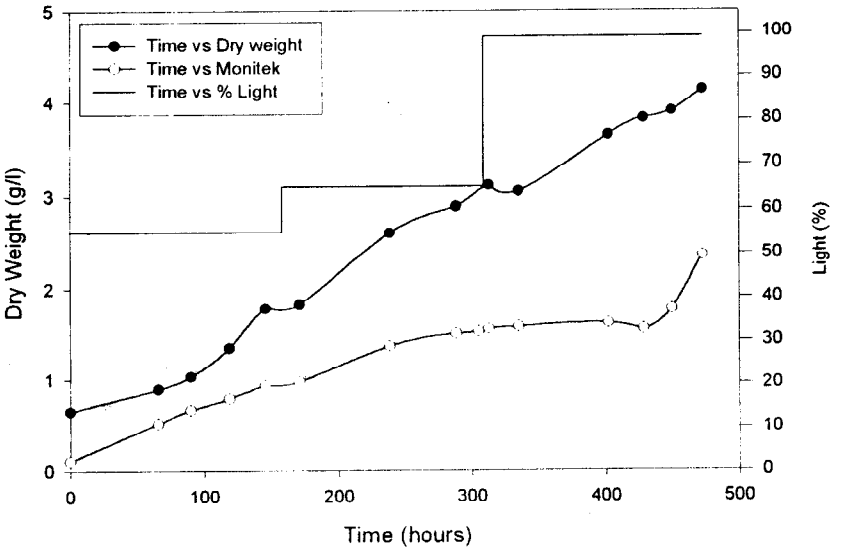


Figure 16: Biomass concentration evolution during the first batch experiment.

It is important to note that at the end of the experiment, the biomass sensor was out of range, due to the high biomass concentration in the reactor. The biomass sensor is not suitable of measurement at absorbances higher that 2 AU.

The bioreactor operation has been very satisfactory as biomass concentration increased rapidly after a step of light. Moreover, with the higher light level (Fr = 225 W/m^2)), the temperature was stable around 36 °C, and therefore corroborating the efficiency of the cooling system.

Appendix 1

A.1.1 LIST OF PARTS

Vessel

112	Round viewing glass

Temperature circuit

200	Inline pump for temperature control liquid circuit
210	Safety valve unit
212	Diaphragm valve with manual indicator
240	Heat exchanger
243	Solenoid valve
246	Thermostatic steam trap
253	Solenoid valve
270	Pressure gauge

Air inlet

301	Pressure reducing valve
302	Flow meter
303	Pressure gauge connection at rear
304	Diaphragm valve with manual actuator
350	Non return valve
360	Filter housing
361	Ceramic filter cartridge
362	Diaphragm valve with manual actuator
363	Diaphragm valve with manual actuator
364	Steam trap

Air outlet

409	Steam trap
414	Diaphragm valve with manual actuator

430	Reflux cooler
431	Ball valve with manual actuator
450	Filter housing
451	Ceramic filter cartridge
452	Diaphragm valve with manual actuator
453	Diaphragm valve with manual actuator

Vessel accessories

504	Diaphragm valve with manual actuator
508	Sterile pressure gauge
509	Steam trap
520	Diaphragm valve with manual actuator
521	Diaphragm valve with manual actuator
532	Safety valve sterile

A 1.2 PERIPHERIAL INSTRUMENTATION

Description		Type	Quantity
pH meter	Crison	PH Rocon 18	1
Temperature controller	Bioengineering		1
Pressure transmitter	STW	A05-5	1
PO_2 meter	Mettler	O2 transmitter 4500	1
Mass flowmeter/controller	HI TEC	F202D-FA-44-V	3
Mass flowmeter/controller	HI TEC	F202D-FA-33-Z	1
Electronic unit	HI TEC	E 7200-AAA	1
Inlet liquid pump	ISMATEC	BP Z Head : P186	2
Outlet liquid pump	Watson-Marlow	505 U	1
Balance (liquid feed medium)	Avery BerKel	L115	2
Air filter	Headline filters	360-50 C	3
Liquid filter	Millipore	Millipack 200	3
Medium extractor	Tech-Sep	PERSEP	1
NO_3 meter	Dr Lange	Nitrate/process spectrophotometer	1
Safety valve	ASCO		1
Hydrodynamic pump			1