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PRELIMINARY DESIGN OF THE COMPARTMENT II PILOT PHOTOBIOREACTOR

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TABLE OF CONTENTS

1 INTRODUCTION

The MELISSA loop is a research tool to develop a biological life support system based on the growth of microorganisms and higher plants. Its main goal is to progress towards the definition and demonstration of regenerative life support systems of application in future long term and manned space missions.

The Pilot Plant, located at the UAB, has the specific goal to demonstrate the feasibility and controllability of the MELISSA loop concept while progressing towards complete loop closure. During the past years significant milestones were achieved. As examples compartments II, III and IVa have been successfully connected at bench scale (TN 43.8, Creus *et al.* 2000) and compartments III and IVa at pilot scale (TN 47.6, Creus *et al.* 2001) can be mentioned.

Moreover, the design and construction of several pilot chambers devoted to grow higher plants (HPC) (TN 65.5, Masot. *et al.* 2004) are in progress, to incorporate the higher plant compartment (IVb) into the Pilot Plant loop.

The next milestone towards increasing the level of closure of the loop requires to interconnect compartments II, III, IVa and IVb at pilot scale, having as a target the daily oxygen production equivalent to the one consumed by one man. Consequently, the scale-up of compartment II is a necessary step.

In the present Technical Note the preliminary design of the pilot Compartment II (photobioreactor, control system and the auxiliary equipment) is discussed.

The size of the pilot photobioreactor (PBR) for compartment II is a key point to address wile considering its interconnection to the other compartments. It depends on several factors that at present time have not yet been completely determined, such as the size of the higher plant compartment and the crops to cultivate on it or the operational conditions, volume and efficiency of the compartment I pilot reactor. On the other hand, previous data empirically obtained on the performance of compartment II at different illumination conditions, allow to

presume that its efficiency can be increased depending on the available light intensity, although not enough data is yet available to precisely calculate the improvement.

Therefore, in this Technical Note is proposed the construction of a 25 litres photobioreactor, which will allow to study satisfactory this complex system in a larger volume, as an intermediate step to the final C-II pilot photobioreactor for the Melissa PP. Depending on the variables previously mentioned, this size will be sufficient to achieve the productivity required for this compartment while interconnected to the other compartments in the PP. Nevertheless the data obtained with its operation will allow not only to advance in the development of other related areas such as control software design and test but also to further investigate the conditions for optimum performance and if necessary allow a proper design of a higher volume unit.

2 REQUIRED FACILITIES

As a global description and based on the preliminary design of the expanded Pilot Plant laboratory it is assumed that the laboratory where to install this pilot photobioreactor and its auxiliary equipment will have the following available services:

- Cool water line at 4 ºC for bioreactor temperature control.
- Low pressure steam line for steam sterilization: 4 kg/cm^2
- Air conditioned
- Electrical supply: 220/380 V, 5 kW (4 kW for 200 lamps, and 1 kW for the rest of the equipment).
- Technical gas lines: air, CO_2 and argon

3 PILOT PHOTOBIOREACTOR DESIGN

To achieve a high level of closure of the MELISSA loop in its pilot plant implementation the performance of the C-II photobioreactor has to be optimized. Also the size of the pilot photobioreactor has to be determined depending on the main requirements of the Melissa loop for this compartment, namely to consume all the volatile fatty acids (VFAs) produced in Compartment I.

The efficiency of the C-II photobioreactor relies on obtaining the best light transfer efficiency possible. In practical terms, and besides the transparent material selection and operating conditions (pe. applied light intensity and cell concentration) this implies that the length of the light path has to be as short as possible. The intention is to avoid the creation of internal dark volumes due to the light attenuation by the cells.

From the biological point of view, the physiology of the *R. rubrum* cells introduces some additional requirements in the design, such as to maintain anaerobic conditions for certain metabolic responses or the need of mechanical stirring to avoid the cell attachment in the walls of the photobioreactor.

Finally some other constraints arise from its final physical location (mainly the height of the lab) or possible manufacturer restrictions such as availability of materials and pieces of certain sizes or safety requirements. All of those facts impose limitations on the number of possible geometries to select from to a few possibilities. Taking into account all those facts the present document will make an initial proposal for the design of the C-II unit and its ancillary equipment including storage tanks.

Once the size and general characteristics of the pilot photobioreactor will be decided, the most appropriate materials for the construction will be chosen. Finally, the auxiliary equipment will be designed and sized. The resulting preliminary design will be submitted to ESA for approval as a previous step before final design decisions with the selected manufacturer. The

preliminary design is necessary to allow the manufacturer to provide an approximation of the cost of construction.

3.1 Basic design criteria. Characteristics and geometry.

As mentioned before, the performance of Compartment II photobioreactor (PBR), as also happens in Compartment IVa, depends heavily on the light energy availability in the culture volume. In Compartment II, a higher light energy availability for the photosynthetic cells, results in a higher volatile fatty acid (VFA) consumption rate.

Therefore, the available light inside the PBR is a parameter to maximize. This can be achieved taken into account two main factors:

a) The illuminated volume/total volume ratio (IVF): The rationale arises from the assumption that in the volume not illuminated (the dark volume) there is no cell growth and consequently it decreases the volumetric VFA consumption rate. Therefore, the illuminated volume has to be maximum in relation to the total volume.

b) Light intensity attenuation: As light energy radiation is transmitted through a participating medium such as the cell culture, three kinds of phenomena take place:

    a. Absorption: which causes a decrease of the light intensity,

    b. Emission: which usually increases intensity at different wavelengths

    c. Scattering: which causes a redirection of energy distribution.

    The global result of those facts imply that the longer the light path, the lower the light intensity available for the cells at increasing distances from the light sources and therefore in the bioreactor. Consequently, the diameter of the illuminated part of the PBR has to be as short as possible. Figure 1 graphically illustrates this effect.

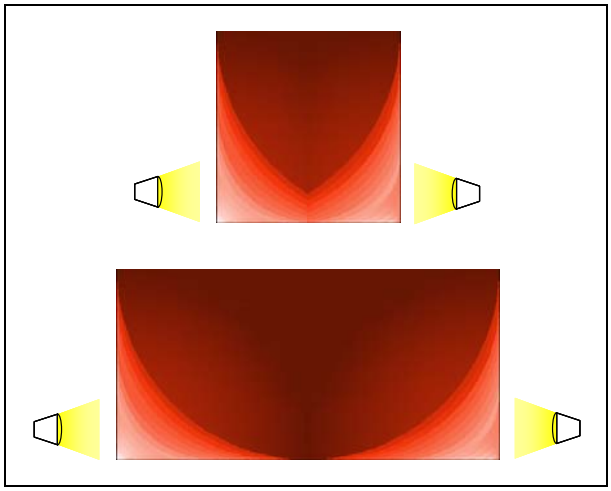


Figure 1: Effect of the light attenuation in relation to the PBR diameter. When the diameter is narrow the available light intensity in the centre of the PBR is higher.

The above mentioned effect indicates that the optimal design for the PBR is a narrow cylindrical column. Nevertheless, since the total height of the laboratory is limited, and the total volume of the reactor is also fixed by the total amount of VFA to consume, either a multicolumn bioreactor has to be chosen or a compromise among the column diameter, the bioreactor volume and PBR efficiency on the VFA consumption rate has to be reached.

In order to maximize the illuminated volume/total volume ratio (IVF), the most important part of the photo-bioreactor will be made using a rigid transparent material. This way only the part of the bioreactor were the sensor probes and bioreactor support will be located, will be built using a strong material such as stainless steel. It is required the transparent material to be rigid in order to sustain a gas overpressure during operation (to guarantee maintenance of anaerobiosis and axenicity) and to allow *in situ* sterilization.

3.2 Sizing

The total volume of the compartment II pilot photobioreactor mainly depends on the amount of volatile fatty acids (VFA) to be consumed and the rate at which this process take place.

Considering the demonstration loop to implement at the Melissa Pilot Plant, the VFA concentration in the inlet flow of compartment II (output of compartment I) depends on what is fed into Compartment I and its operational conditions. Also, the degradation efficiency of Compartment I is currently in the optimization process and the volume of this reactor is under design, although a 100 litres bioreactor can be expected for the Pilot Plant.

Assuming the operational conditions of compartment I are fixed, the VFA composition at its outlet will be variable depending on the amount of human faeces, edible and non edible amounts of crop biomass and percentages of *Spirulina* and *R. rubrum* biomass introduced into Compartment I. This fact opens the possibility to consider different scenarios. Depending on which assumptions are made, the VFA production of Compartment I will vary significantly.

Until all of these parameters will be precisely defined, the volume of the Compartment II PBR cannot be accurately determined, since the required VFA consumption rate can fluctuate along a wide range.

Therefore, in this Technical Note an assumption is made on C-I output compositions based on the reference operational conditions decided at different yearly meetings and taking into account previous results on its performance.

In TN 65.5 (Masot. *et al.* 2004), the design and construction of Compartment IVb HPC is studied and as a result it is proposed to operate 3 higher plant chambers (HPC) with 5 m^2 of growing area each, according to the future Pilot Plant oxygen productivity requirements equivalent to those consumed daily by one man. In addition, a crop culture consisting on 50% beet and 50% lettuce is also assumed.

The Crew Compartment for which the oxygen is generated consist of a number of rats as the human respiratory equivalent. Human crew is assumed to consume all the edible crop biomass and 40% of the *Spirulina* biomass produced and as a result provide the corresponding human faeces. Thus, the feed of the Compartment I will be the non edible crop biomass, the human faeces produced daily by one man, all the *R. rubrum* produced and the 60% of the *Spirulina* generated by the MELISSA loop.

Using the worksheet used for generating productivity data for the ICES 2004 (Waters *et al* 2004) congress and assuming the parameters detailed in table 1, it can be determined the required biomass production of Compartment II, as being of 33.0 g DW/day of *R.rubrum* biomass.

Crop total productivity:	6 g/(m^2·day)
Harvest index:	0.855
Protein degradation efficiency in Compartment I:	60%
Spirulina production:	36 g/day

Table 1: Parameters used for the determination of the required *R. rubrum* production in Compartment II (Masot, A. *et. al.*, 2004).

In TN 37.7 (Cabello *et al.*, 2002) the dry weight obtained in several steady states corresponding to different operational conditions for compartment II are reported. Taking those recorded data as a reference, and assuming a conservative criteria, the average productivity value reported is used. Therefore for an average continuous culture test (D=0.08 h^{-1}) using acetic acid as carbon source with an incident light at 58 W/m^2 a biomass productivity of 1.4 g DW·(L·day)$^{-1}$ was achieved. According to this biomass productivity and taking into account the required biomass production of Compartment II calculated previously, the volume of the pilot PBR required is of 23.6 L.

Considering, as an alternative the most unfavourable scenario, *i.e.* the entire fractions of edible and not edible crop biomass, *Spirulina* and *R. rubrum* and human faeces are fed into Compartment I, the required *R. rubrum* production in Compartment II rises to 66 g DW/day.

Following the same calculation approach as in the previous scenario a volume of 47.4 L PBR is required for Compartment II to consume all the produced VFA.

Taking a conservative approach will require to build a 50 litres volume photobioreactor for compartment II. However, different manufacturers have expressed their reluctance to build an *in situ* sterilizable bioreactor with glass walls of a volume higher than 25 litres in size. They also claim that European safety regulations would not allow its operation. Besides this point, and as mentioned before, previous operational data obtained at the Pilot Plant (Creus *et al.* 2001) using a 400 mL bioreactor, indicate that higher productivities can be obtained in this compartment if higher average light intensities can be reached. Due to the limited experimental data presently available on this particular issue it is advisable to scale the C-II bioreactor to an intermediate size and continue the experimental work so as to determine the optimum operational conditions of C-II PBR.

Consequently, in the present Technical Note it is proposed to construct a 25L PBR for Compartment II, which will be appropriate for the scope of different scenarios envisaged for the Pilot Plant integration tests.

3.3 Materials

As it has been explained, to obtain a suitable performance it is very important to achieve the maximum illuminated volume fraction and this fact drives the building issue towards using a transparent material for the main part of the PBR.

In the Melissa Pilot Plant there is working a pilot PBR (an external loop airlift) which its illuminated columns are made of polyamid foil. The drawback with this material is that it is very sensitive to small pressure changes and its volume varies slightly depending on the internal pressure. What is more the foil is extremely sensitive, and easily leaks.

As the new PBR has to sustain a gas overpressure (to guarantee the axenicity of the culture), this is certainly not a suitable material. Among the several rigid transparent materials available

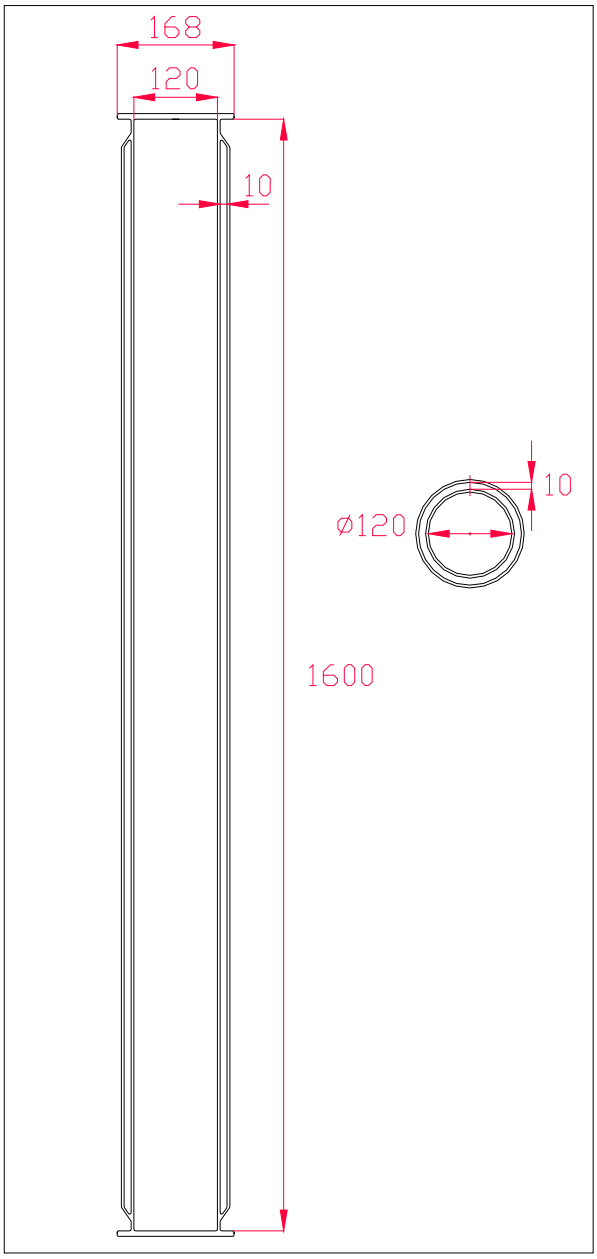
for this application, tempered glass has been chosen for its good behave among mechanical and light transmission properties.

However, metallic parts are also required to be able to fit probe ports in the PBR. Thus, two metallic parts will be needed, namely the head and the bottom sections. Stainless steel AISI-316 could be a suitable material for these sections due to its characteristics.

Liquid and gas lines have to be metallic in order to have welded fittings and then avoid leaks. The acid line, needed to control the culture pH, will be made of glass or plastic, since low pH in combination with moist heat during the sterilization process is a very aggressive atmosphere for metallic materials. The valves located in this line will have the part in contact with the liquid made of Teflon.

3.4 Pilot photobioreactor hardware

3.4.1 ILLUMINATED COLUMN



The illuminated column is the only part of the PBR where light energy can be supplied to the *R. Rubrum* cells, so this volume has to be maximum in relation to the total volume.

It is made in tempered glass and has a double jacket where cool water (4ºC) flow through it.

Dimensions:

- Internal diameter: 120 mm
- Glass thickness: 4.5 mm
- Double jacket Width: 10 mm
- External diameter: 156 mm
- Diameter ending: 168 mm
- Length: 1.6 m
- Internal volume: 18.1 L
- Illuminated surface: 0.60 m^2

Figure 2: Sizing of the illuminated column

3.4.2 HEAD AND BASE SECTIONS

The head and base sections connect the illuminated column with the other parts of the PBR (liquid and gas inlet and output). These parts are made of stainless steal (AISI-316) since they contain all the probes and ports.

Between the stainless steel part and the glass end of the illuminated column there is located an expansion joint in order to compensate the different expansion coefficients of the both materials during the sterilization process (121 ºC).

Head section

The main characteristic of the head section is the increase in the diameter in relation to the illuminated column, because it helps the gas-liquid separation. In its base the diameter is equal to the illuminated column diameter (0.12 m), and then it gradually increases to reach the final diameter of the head section (0.16 m). In the front part of the head section, there is a glass window in order to visually check the liquid level. In figure 3 the dimensions of this part are detailed.

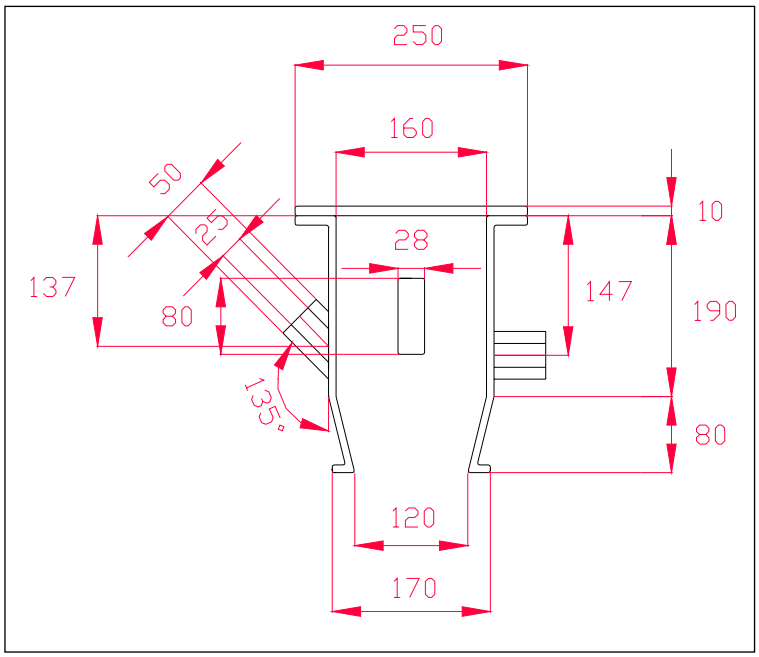


Figure 3: Cross section of the head part

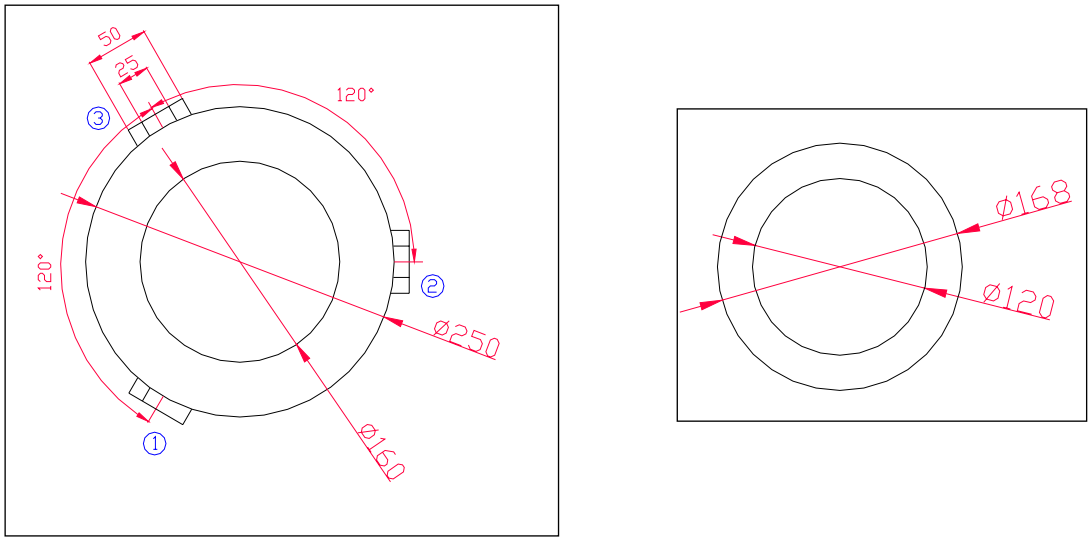


Figure 4: Plant view of the head section (top and base)

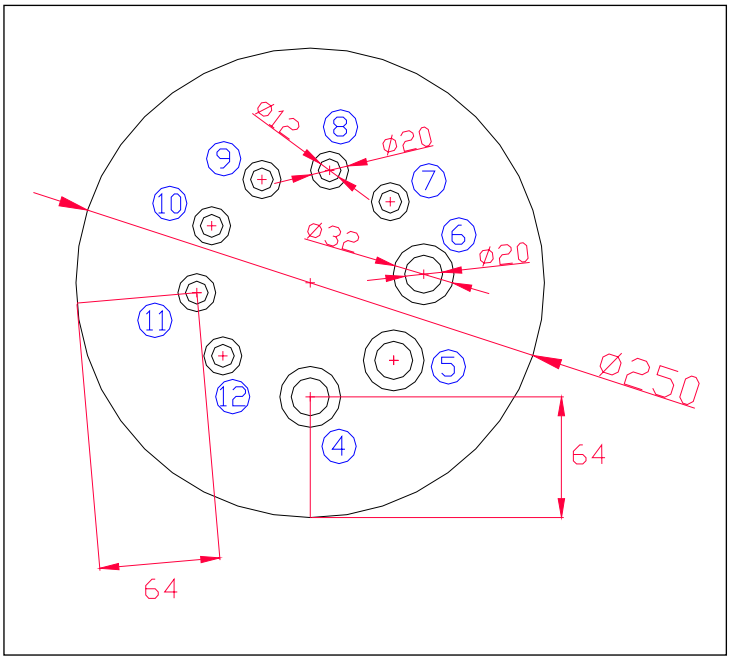


Figure 5: Scheme of the lid of the head section

In the head section there are 12 ports for probes and tubing; ports 1, 2 and 3 (internal diameter: 25 mm) are located in the wall of the head sector, as some probes have to be positioned horizontally or with a certain inclination. Nine ports are placed in the top lid (ports 4, 5 and 6 with an internal diameter of 20 mm and ports 7-12 with 12 mm). In figure 4 the position and size of the several ports is detailed.

Base section

The base section consists on a stainless steel vessel that is joined to the illuminated column and allows to locate some probes and tubing in this part of the PBR. There are seven ports, all of them with 12 mm as internal diameter. In figures 6 and 7 (cross-section and plant views respectively) its dimensions are specified.

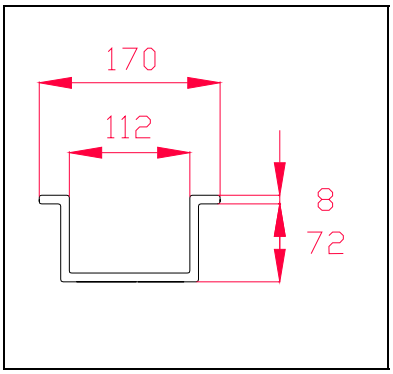


Figure 6: Cross section of the base part

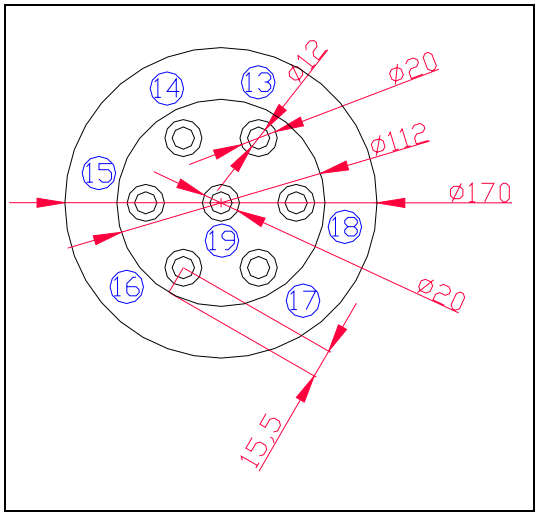


Figure 7: Plant view of the base of the PBR

In table 2 the function of all the ports of the head and base sections and its characteristics are detailed.

Head section			
	Port Num.	∅ Int. (Ext.) mm	Function
Wall	1	25 (50)	Biomass probe
	2	25 (50)	Biomass probe
	3	25 (50)	pH probe. Number 1
Lid	4	20 (32)	pH probe. Number 2
	5	20 (32)	Pressure sensor
	6	20 (32)	pO_2 probe
	7	12 (20)	pH control. Acid addition.
	8	12 (20)	pH control. Base addition.
	9	12 (20)	Gas output
	10	12 (20)	Overpressure valve
	11	12 (20)	Liquid output
	12	12 (20)	Sample port
Base section			
Lid	13	12 (20)	Temperature probe (Pt-100)
	14	12 (20)	Liquid input
	15	12 (20)	Inoculum port
	16	12 (20)	Redox probe
	17	12 (20)	Steam input/purge
	18	12 (20)	Gas input (Sparger)
	19	12 (20)	Extra port

Table 2: Function of all the ports of the head and base section and its internal and external diameter.

3.4.3 ILLUMINATION SYSTEM

To supply the necessary light energy for the cell growth, halogen lamps are used, since they have been used in the Melissa Pilot Plant for long time with an adequate performance for compartment II.

The halogen lamps have 12 V , 20 W and BAB 38º. Since is necessary to be able to vary the light intensity, and consequently, the voltage applied to the lamps.

Along the illuminated column there are 20 rows of lamps and each one have 10 lamps surrounding the illuminated column. Therefore in the PBR there are 200 lamps which need 4000 W of electric power supply. This is the maximum number of lamps, of this type, that can be located around the bioreactor.

To obtain 4000 W of continuous current, it is required an electrical transformer, to convert 220 V AC to 12 V AC which will receive the control action by means of a 4-20 mA signal).

The lights are set up on a mechanical support, made of stainless steel AISI-316, which allow the lamps to be in a fixed position. This support can be removed from the PBR, when the sterilization process takes place.

The light supply has a low efficiency and the main part of the energy is lost as heat. The air surrounding the illuminated column is heated up by the lamps and the

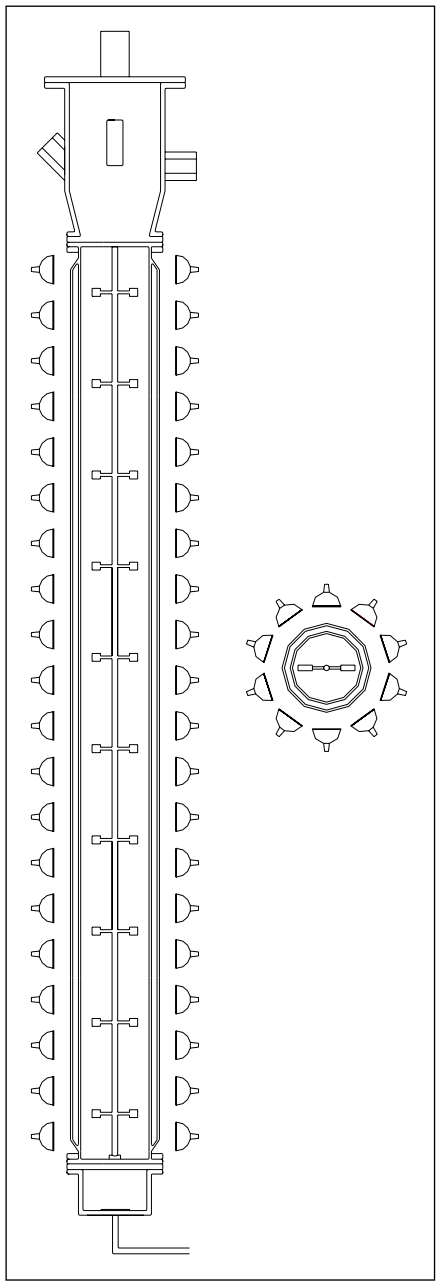


Figure 8: View of the lamp distribution around the illuminated column.

PBR refrigeration system has to work in excess. To avoid this extra load for the cooling system, a hot air extraction system has to be set up.

The hot air extraction system consists on an electric fan that propels air in the base of the PBR, forcing the air to rise up, and an air extractor located above the PBR, that removes the hot air driving it out of the laboratory facilities.

3.4.4 GAS LOOP LINE

The operation of this compartment consume VFA as carbon source and use the light as energy source in order to obtain the required resources for the *R. rubrum* cells. It also requires to maintain an anaerobic atmosphere for the cells. Otherwise, in presence of oxygen, the cells metabolism changes towards consuming the organic substrate as carbon and energy source, which at present time is not the role foreseen for this compartment in the Melissa loop.

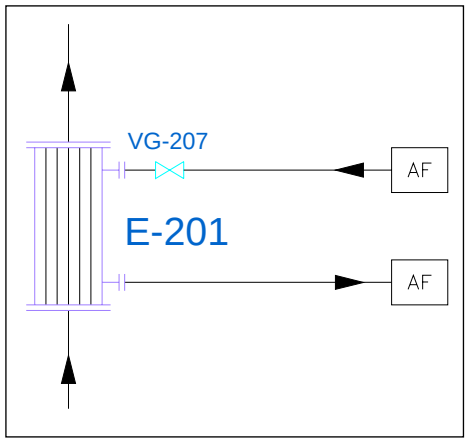


Figure 9: Scheme of the condenser E-201 in the PID.

This PBR has been conceived to operate with a closed gas loop, where the gas is continually recirculated through the illuminated column. There is also gas exchange with the other compartments because the gas exiting C-I it is fed into the PBR closed loop and also the gas in excess is driven to C-III.

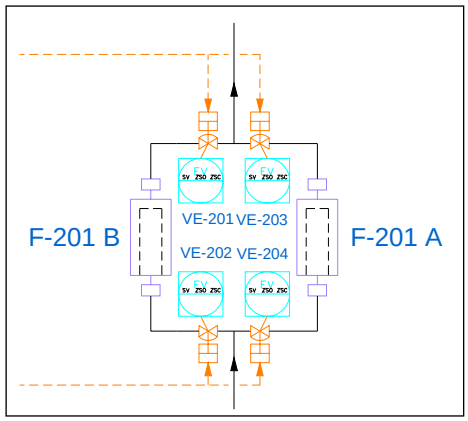


Figure 10: Scheme of the filters F-201 (A and B) in the PID.

To allow this kind of operation, a gas circuit has been designed and is depicted in the PID diagram in Appendix 1. The gas exits from the head section of the PBR and passes through the condenser E-201 (figure 9), where part of the humidity and VFA of the gas

condense and return to the culture.

After this step, the gas crosses the filter F-201 (figure 10) which it is duplicated (A and B) in order to allow to change the working filtration unit when clogging appears. As the pressure is measured before the filter (in the headspace of the PBR) and also after the filter (in the T-204 vessel), the clogging problems can be detected when the pressure in the PBR increases considerably without affecting the pressure after the filter. As soon as the controller detects this situation, it changes the working filter from F-201A to F-201B by means of the set of electrovalves VE-201, VE-202, VE-203, and VE-204. The filter F-201 acts a sterility barrier and after point the non-sterile part of the gas loop begins.

After filter F-201, valves VD-201 and VD-202 allow to open the gas loop and discharge the gases to the atmosphere if required, for example in case of an open loop mode of operation is necessary. The usual position of these valves, VD-201 closed and VD-202 opened, allow for the closed gas loop operation.

In the buffer vessel T-204 (figure 11), the gas pressure at the outlet of the PBR is stabilized, measured and, in case of overpressure, relieved out of C-II. Depending on the pressure inside the vessel, two actions may be triggered. If the pressure is slightly higher than the usual pressure set point, the VC-207 valve is opened and some gas is driven to C-III.

This situation will happen always when gas is introduced in the loop and also when the T-101 vessel will be filled up with more liquid medium. However, if the pressure in T-204 is considerably higher than the pressure set point, it indicates that some problem is occurring and the VC-208 will be opened to discharge the gas to the atmosphere. At design time the pressure set point value in T-204 is considered to be around 0.1 kg/cm^2. Nevertheless this value and the overpressure value set points will be more accurately determined after testing the performance of the system.

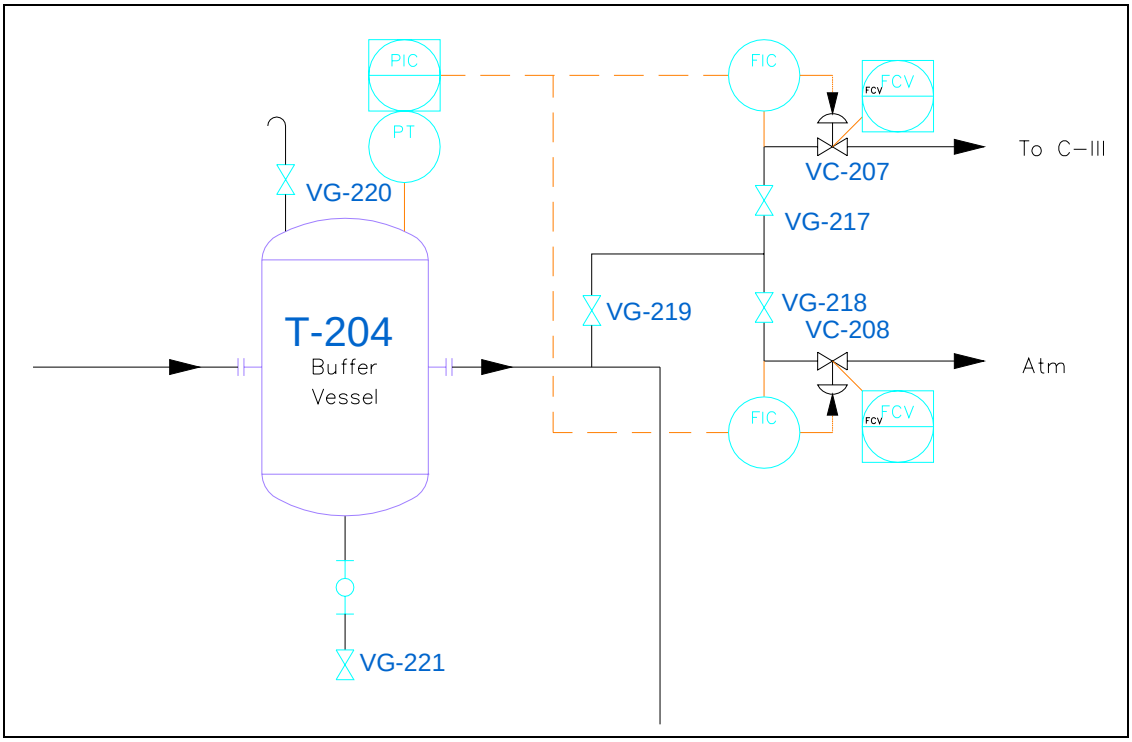


Figure 11: Pressure measurement and control at the outlet of the PBR.

External gas as CO_2, Ar or gas incoming from C-I can be fed into C-II gas loop. The connection points will be located close to the compressor input tube (figure 12). C-I connection is necessary if both compartments have to operate inerconnected; Ar gas is needed to maintain the gas pressure in the loop as well as the anaerobic conditions, and the CO_2 can be necessary to increase the CO_2 partial pressure depending on the type of test being carried out. Each gas connection has a mass flow regulation system that allows to know and adjust the flow rates independently.

The external gases will be added to the gas exiting from T-204 and the outlet gas from the gas analyzer loop (figure 12). The mixture will pass through the compressor K-201 increasing its pressure sufficiently to be able to overcome the liquid column pressure of the PBR. The

compressor has a bypass in order to be able to control the gas pressure. In case of overpressure in the compressor output line, a relief valve would be opened (figure 13).

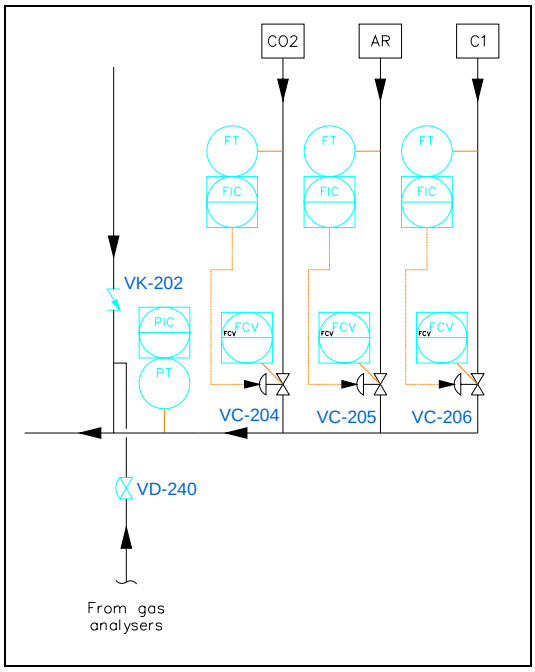


Figure 12: External gas inlet (Ar, CO_2 and gas proceeding from C-I) is added to the gas exiting from T-204 and the gas from the analyzer loop.

The compressed gas by K-201 goes into vessel T-203, which acts as a buffer tank to control the pressure and the flow rate of the gas inlet in the PBR. In addition, this vessel has the function of supplying gas to the vessels T-101 (feeding vessel) and T-301 (discharge vessel) when the their gas pressure decreases. As a result, the gas phase of both vessels and the PBR are connected, avoiding thus the external gas inlet when vessels T-101 and T-301 are being emptied. The pressure set points at the different tanks will be selected so as to allow the liquid movement from one tank to the other as described later. On the contrary, when these vessels are being filled up, the gas that is displaced is driven to the vessel T-204, avoiding the loss of a gas that may be rich in VFA. In this way, the gas closure in C-II is assured, since the only gas incoming in C-II is the one outcoming from C-I and the only gas outlet is going to C-III.

The gas outlet from vessel T-203 to the PBR will have the flow rate controlled by VC-202.

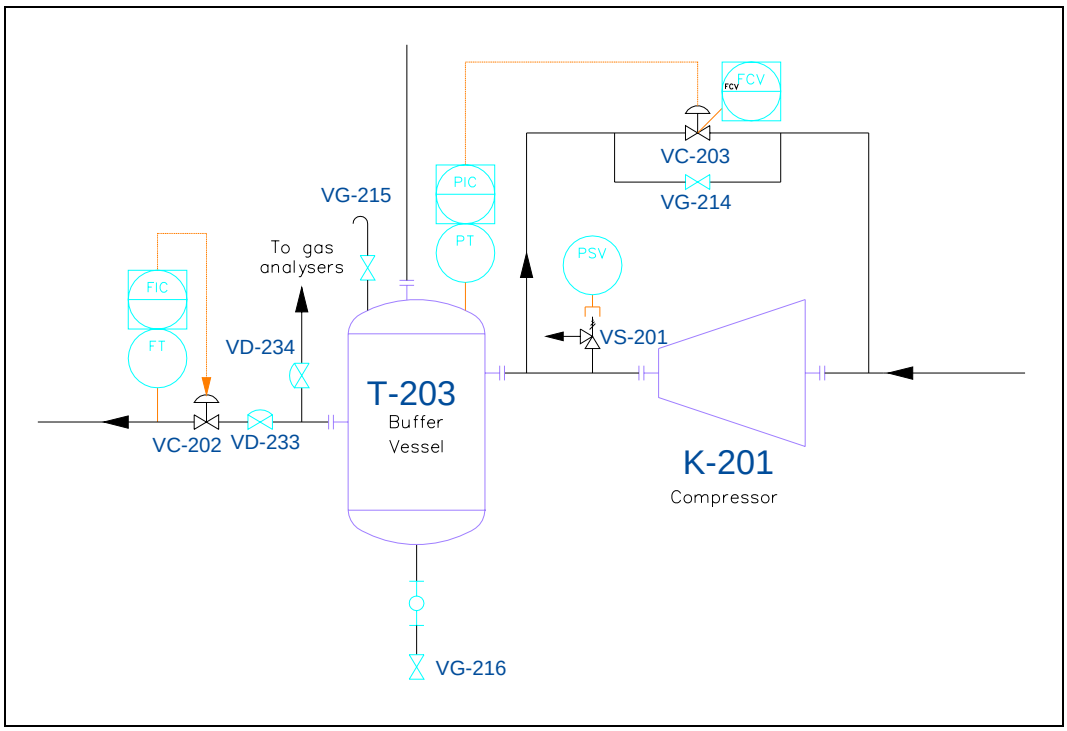


Figure 13: Gas compressor K-201 and the pressure and flow rate control loops of the gas inlet in the PBR. In the vessel T-203 there is a gas outlet to the vessels T-101 and T-301.

A fraction of this gas is driven to the closed analysis circuit, which will be composed by a CO_2 analyzer and a gas chromatograph, once measured the gas will be returned to the PBR gas loop, specifically at the compressor K-1 suction point. The gas flow rate needed to perform the analysis in this circuit is constant and negligible related to the gas flow rate inside the PBR gas loop.

The gas of the PBR loop is filtered before arriving to the PBR inlet. This filtration step consists on a prefilter, F-202, made of polypropylene with a cut-off of 0.65 μm, and a filter, F-203, made of Teflon with a cut-off of 0.22 μm. After the filtration, the gas is fed into the PBR and distributed by means of the sparger.

3.4.5 LIQUID SAMPLING SYSTEM

There will be two systems to take a liquid sample. The basic one would allow to take a sample manually from the bottom part of the PBR. It will be connected to a filtered steam circuit in order to sterilize the pipes before taking the culture sample while preserving the axenicity of the culture (figure 14).

In addition, there will be an automatic system to take a liquid sample free of cells, in order to be automatically analyzed by gas chromatography. This sample will be taken directly from the PBR or as close as possible to the liquid outlet pipe. This system is currently under study by specialized companies to select the best option.

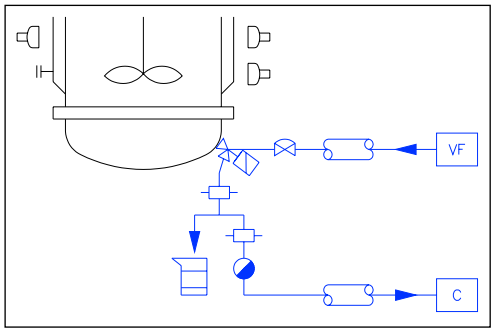


Figure 14: Manually culture sampling system

3.5 Auxiliary equipment design

From a global point of view, C-II is composed mainly by the PBR, and several auxiliary equipment such as the feeding vessel, the discharge vessel and the pipes that connect the liquid and gas lines of the three components.

3.5.1 FEEDING VESSEL

The inlet fresh medium, T-101, has been conceived to allow at least 48 hours of continuous operation of the PBR. Since the maximum inlet fresh medium flow rate is 4.5 L/h (when the PBR is running at 0.18 h^{-1}), the capacity of T-101 has been set in 250 L. It is made using stainless steel (AISI-316) and its dimensions are 0.70 m of diameter and 0.70 m of height.

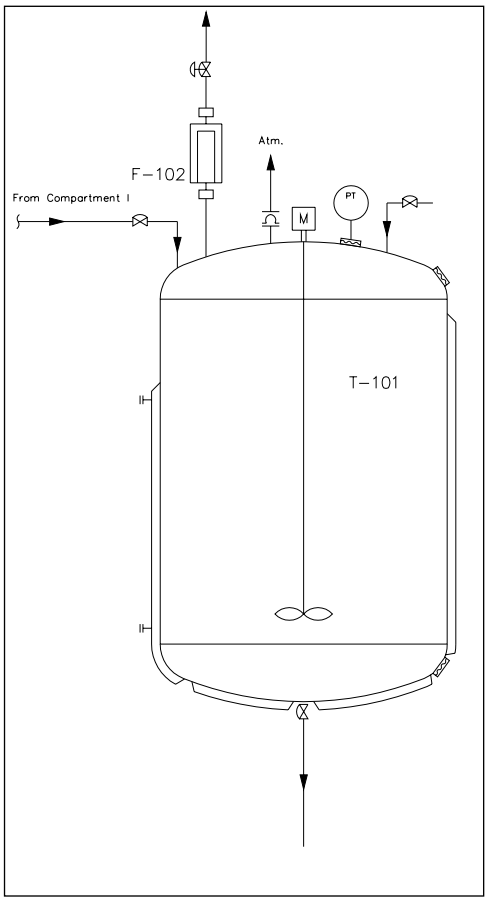


Figure 15: Scheme of the vessel T-101 in the PID.

The vessel has a double jacket in order to control the fresh medium temperature at 4 ºC, has a stirring system in order to avoid salt precipitation and has the possibility to maintain the pressure with inert atmosphere.

In this tank, it is a key factor to maintain the pressure controlled at a constant value. The reason being that pressure in this vessel is the driving force to make the liquid flow from this tank to the PBR.

The liquid flow rate between vessel T-101 and the PBR is regulated by means of a flow rate control valve, as described later.

3.5.2 DISCHARGE VESSEL

The discharge vessel, T-301, is similar to the feeding vessel T-101. T-301, as T-101, it has mixing and temperature control capabilities. Both have the same volume and dimensions.

This vessel has the possibility to be drained by means of a drainpipe located at its base and without compromising the sterility of the vessel by means of a steam sterilising loop.

As it has been described previously, the gas phase of this vessel is connected to the gas loop of the PBR by means of vessels T-203 and T-204.

When T-301 is being filled, the displaced gas is driven to T-204, where the gas is mixed with the PBR gas loop. On the other hand, when this vessel is drained, gas incoming from the vessel T-203 will be added to compensate the pressure decrease.

Since the gas phase of the PBR and the other vessels are connected, the VFA present in the gas phase (as a consequence of the dynamic equilibrium with the VFA of the liquid), will be retained.

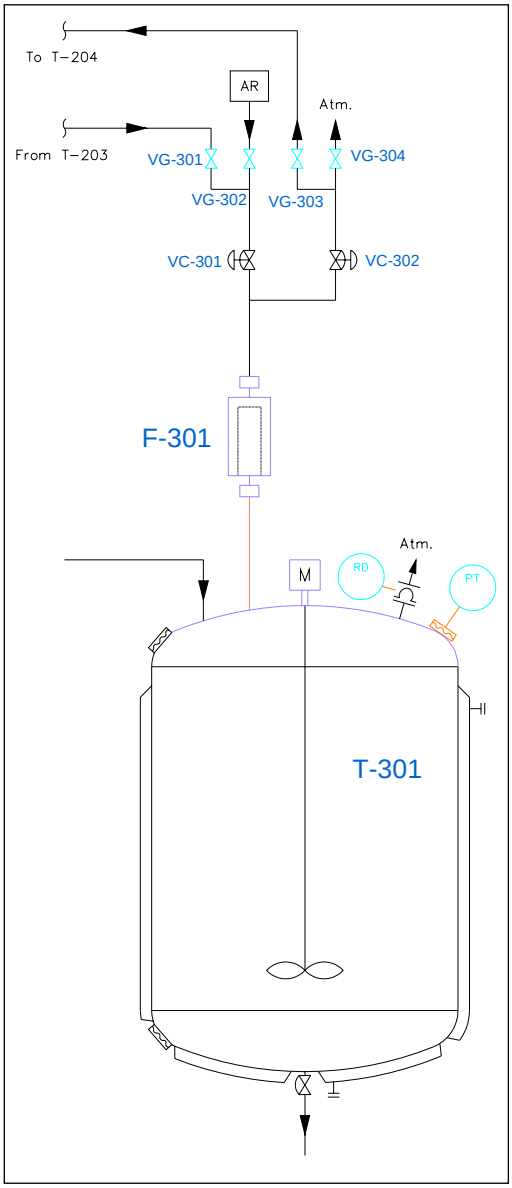


Figure 16: Scheme of the T-301 vessel in the PID.

3.6 Instrumentation and control loops

In order to guarantee a satisfactory performance of compartment II, the main physical and chemical parameters such as temperature, pH, pressure, liquid and gas flow rates, etc will be controlled.

In the following paragraphs, the instrumentation and control loops of the PBR, T-101, and T-301 vessels are detailed.

3.6.1 FEEDING VESSEL

The parameters controlled in the inlet fresh medium vessel, T-101 are the following:

- Pressure
- Temperature
- Liquid level

In figure 17 the instrumentation and the control loops needed for the satisfactory performance of the feeding vessel are.

3.6.1.1 Pressure control loop in T-101

As it has been described previously, the pressure in this vessel is the driving force to propel the liquid from this tank to the PBR. In this way, no pumps are required, sterilization is easier and the performance of the system is more robust. Since the liquid outlet flow requires to maintain a certain tank overpressure, the pressure in T-101 is a key parameter for the global performance of C-II. It is controlled adapting the inlet and outlet gas flow to liquid level variations.

The liquid outlet of T-101, that is the liquid fed to the PBR, is regulated by means of the control valve VC-103, its action depends on the measure of a liquid mass flowmeter. This control loop is the responsible for the regulation and adjustment on the dilution rate of the PBR. When the pressure decreases in T-101, the pressure controller acts over the control valve

VC-101, allowing the inlet of gas in the vessel. The inlet gas can be either Ar or gas from T-203 vessel (gas from the gas loop of the PBR).

If the pressure increases in T-101, the controller acts over the control valve VC-101 and gas is discharged out of the tank. With the valves VD-107 and VD-108 it is possible to drive the gas to the vessel T-204 (that means to feed this gas in the gas loop of the PBR) or alternatively to discharge it to the atmosphere, a disposal gas line or a different pilot plant gas storage tank.

In the top of the vessel a safety disc will be installed which, in case of considerable overpressure (>2 atm), it will break relieving the gas to the atmosphere. This situation would occur if for example the filter F-102 were clogged.

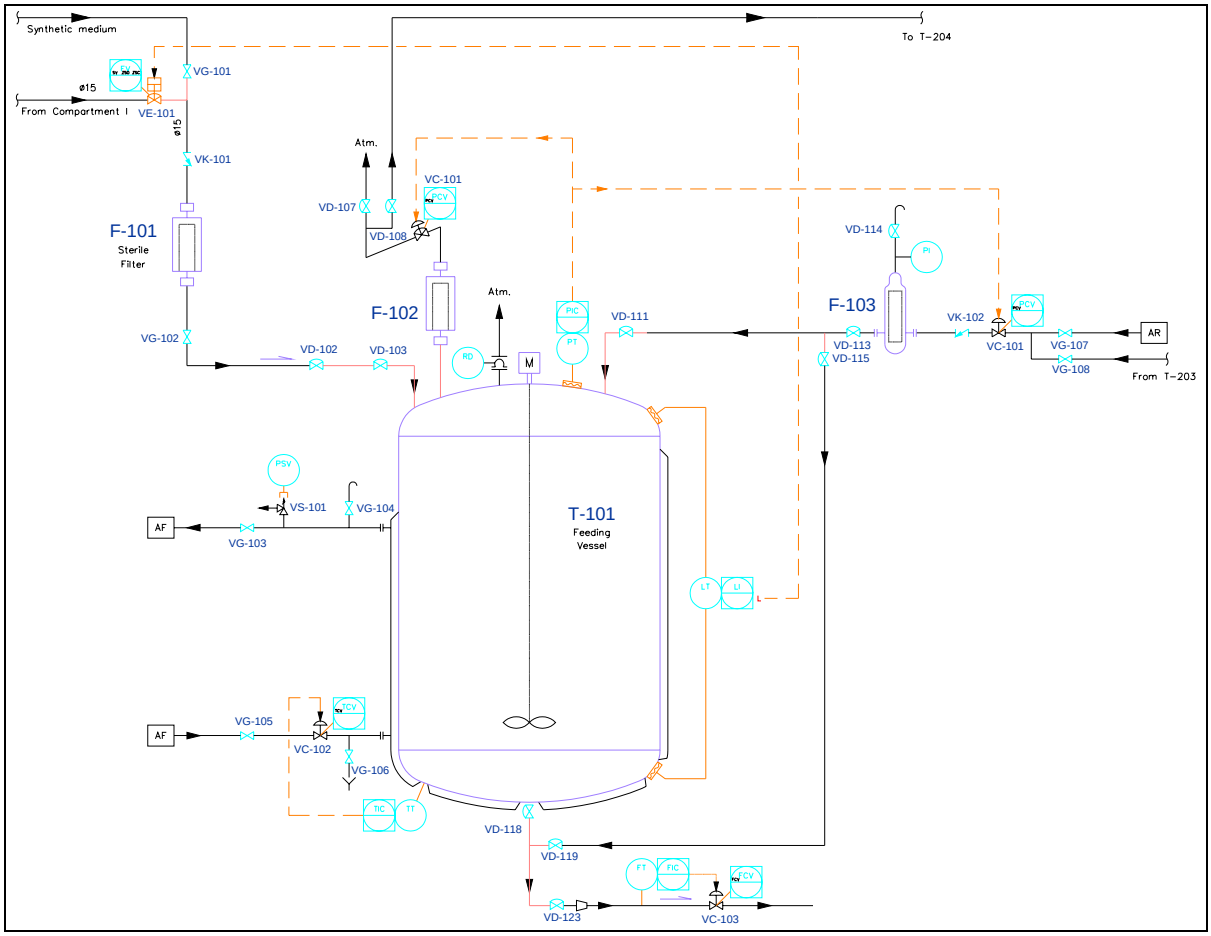


Figure 17: Scheme of the pressure, level, temperature and flow rate control loops in the T-101 vessel.

3.6.1.2 Temperature control loop in T-101

As this is a storage tank culture medium will be maintained at low temperature. The temperature in the T-101 vessel is measured by means of a Pt-100 probe. The controller receives the signal from the probe and acts over the control valve VC-102 that regulates the cold water (4ºC) flow rate that pass through the double jacket of the vessel (figure 17).

3.6.1.3 Liquid level control loop in T-101

The liquid level in the vessel T-101 is measured by means of two pressure sensors located one in the top of the vessel and the other in the base of it. The difference between both measures is proportional to the volume of liquid inside the tank (figure 17).

If compartments I and II are operating interconnected and the T-101 is filled of liquid, the level controller will close the electrovalve VE-101 to prevent the liquid overflow in T-101 and the central control system will have to suspend the liquid outflow from C-I.

3.6.2 PHOTOBIOREACTOR

The parameters controlled in the PBR are the following:

- Pressure
- Temperature
- pH
- Biomass concentration
- Liquid level
- Inlet and outlet liquid flow rate
- Gas flow rate

Moreover, there are parameters that are not controlled but only monitored, such as the redox potential, the dissolved oxygen concentration in the culture (pO_2) or the CO_2 and VFA concentration into the gas loop.

In figure 18 a scheme of the control loops of the PBR can be found.

3.6.2.1 Pressure control loop in the PBR

The pressure both in the PBR and in the gas loop is a parameter of key of importance, since pressures higher or lower than the set point pressure can cause a malfunction of the whole system and the sterility would be compromised.

There is a pressure sensor installed in the head of the PBR with a pressure indicator, which allows to visually check the pressure on the top of the PBR (practical during the sterilization). Humidity and the VFA of the gas phase in the E-201 are retained in the bioreactor by means of a properly sized gas condenser. The gas exiting the PBR is filtered by F-201. After this step, the gas is driven to the vessel T-204, where the gas pressure is stabilized and measured. The filter F-201 is duplicated (A and B) in order to be able to change the working filtering unit if one of them is clogged. When the pressure difference measured in the vessel T-204 (after the

filter F-201) and at the top of the PBR (before the filter F-201) is higher that a certain value, it indicates that the working filter is clogged and it must be replaced by the other filter. Therefore, when the pressure controller detects this situation, it changes the filter in operation (from F-201A to F-201B or in reverse) acting on the electrovalves VE-201, VE-202, VE-203, and VE-204. In this way, the working filter is always in satisfactory conditions while the other unit can be replaced.

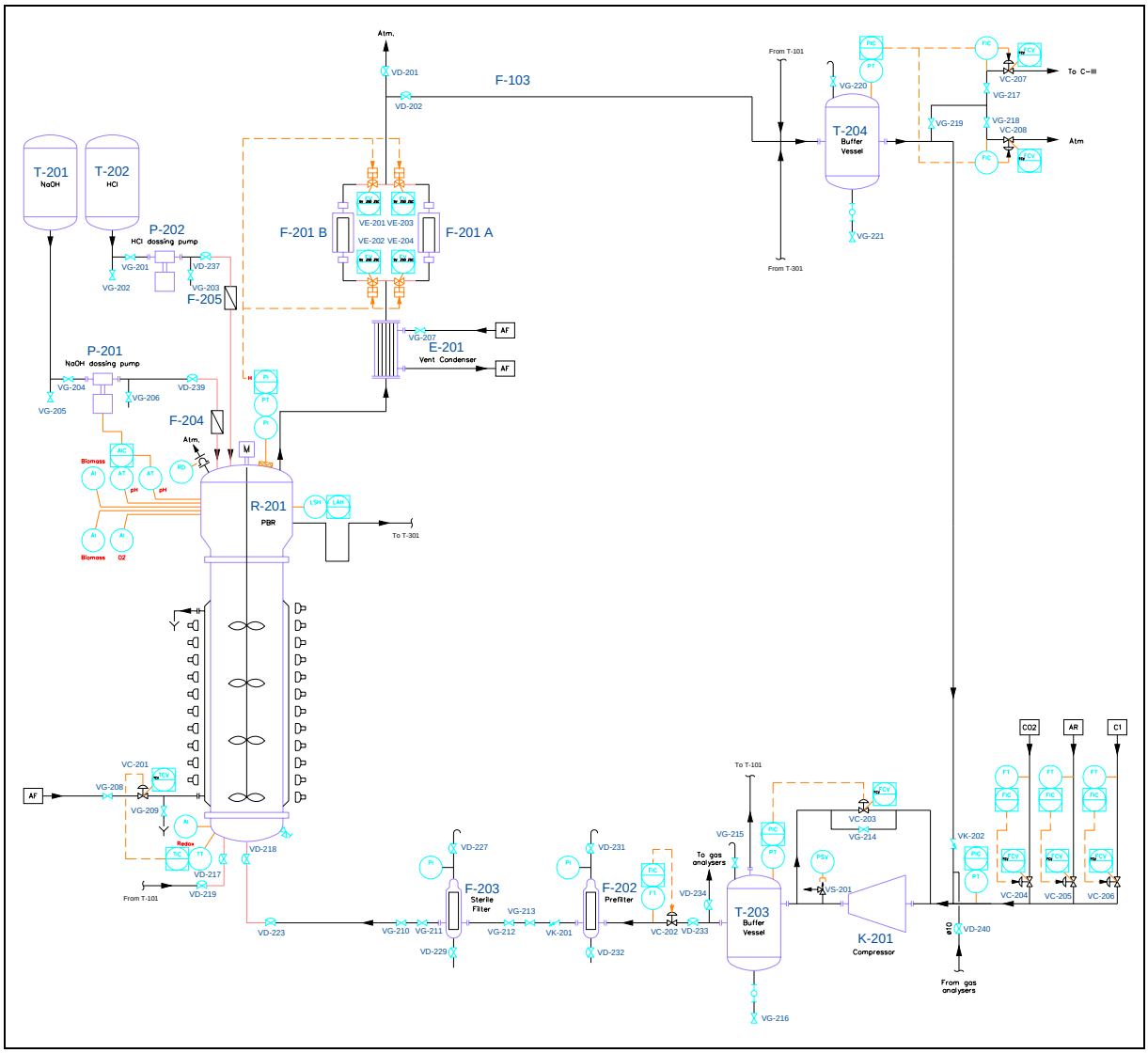


Figure 18: Scheme of the PBR control loops in the PID.

In case of having the two filters F-201 (A and B) clogged, the pressure will increase until the control system reacts (limiting the input flow) or in an emergency case the pressure will overcome the limit pressure of the safety disc that is located at the top of the PBR.

In the vessel T-204, the PBR gas outlet is mixed with the gas proceeding from the vessels T-101 and T-301. In T-204, the pressure is controlled by discharging gas according to the overpressure set point. If the pressure is slightly higher than the pressure set-point, the pressure controller will adjust the pressure by opening the regulating valve VC-207, and driving the excess of gas to C-III. If the overpressure is considerably higher than the pressure set-point and would compromise C-III performance, the pressure controller will act over the control valve VC-208 and the gas will be discharged to the atmosphere, or to a properly installed laboratory gas line, as an emergency procedure.

The gas from T-204 is added to any external gas required, which can be either gas incoming from C-I, or stored gases as Ar or CO_2. Each external gas pipe has a mass flowmeter, to measure and adjust the inlet gas flow.

After this point, the gas pressure is increased by means of the compressor K-201, which is located at the gas recirculation loop. After the compressor, the buffer vessel T-203 stabilizes the pressure, which is measured and controlled. The pressure controller acts over the regulating valve VC-203 (this valve is located in the compressor gas recirculation line) to kept constant the pressure in the vessel. In the gas outlet of T-203 towards the PBR, there is a flowmeter installed, which measure is used by the flow rate controller to act over the regulating valve VC-202. In this way it increases or decreases the gas flow rate to the PBR.

If the vessels T-101 and T-301 require extra gas to compensate a pressure decrease, this gas is taken from the vessel T-203, which is the higher pressure point in the PBR gas loop.

The gas exiting T-203 is filtered (F-202 and F-203) and go into the PBR by means of the sparger, which distribute the gas in small bubbles (proposed size around 0.5 mm of diameter).

3.6.2.2 Temperature control loop in the PBR

As the PBR is illuminated its temperature tends to continually increase and the main action to perform is to cool the bioreactor. Only in special cases, for example for sterilization, heating with a steam line is foreseen. At design time it is assumed that steam will be available in the laboratory only during certain periods of time but not continuously at any time.

The temperature in the PBR is measured by means of a Pt-100 probe that is located in the base section. The controller receives the signal from the temperature probe and modifies the position of a control valve that regulates the cold water (4ºC) flow rate that passes through the glass double jacket of the PBR.

3.6.2.3 pH control loop in the PBR

The pH of the culture is a key factor on the performance of the compartment. Because of that, in the head section of the PBR there are two pH probes located. Those are retractable to allow its substitution during the operation of the compartment. If one of them losses its calibration, it will be observed by comparison with the other probe, the other operation variables and the previous knowledge of the process.

Depending on the pH value of the culture, the pH controller will act over the acid or the alkali pumps (4-20 mA). The *alkali* (NaOH) is in the tank T-201 and the acid (HCl) in T-202.

3.6.2.4 Biomass concentration control loop in the PBR

In the head section of the PBR there are located two retractable biomass probes in order to be able to follow the cell concentration in the culture. Since it is an important parameter for the performance of the compartment and even for the whole Melissa loop, this kind of probe is duplicated. It can be studied to install a biomass probe cleaning system, for example a small gas blowing line on their glass surface if no cleaning system is foreseen by the manufacturer.

The Melissa Control System (MCS) receives the signal and, similarly as it is done for compartment IVa, it will command operational variables such as light intensity or liquid flow of the PBR in order to attain the required biomass productivity.

3.6.2.5. Liquid level control loop in the PBR

In this case no electronic control is used but a physical one, which only requires proper bioreactor/tank location and maintenance of constant gas pressures. The liquid level in the PBR is controlled by means of a combination of a gravity, pressure and an hydraulic seal located in the liquid output. Pressure in the PBR and in the T-301 have to be nearly equal, with a slight increment (~2%, but smaller than the one necessary to overcome the hydraulic seal) in the PBR side. The maximum liquid level in T-301 is located slightly below the minimum bioreactor level. This set up assures that as the liquid enters the bioreactor and reaches the output liquid tube it is immediately displaced towards T-301.

The liquid output line has a 'U' form were the overflow liquid is retained sealing the gas inside the bioreactor and becoming a first barrier between the PBR and T-301. Of course any hydraulic or gas overpressure inside the bioreactor higher that the force due to the hydraulic column in the 'U' loop plus the one due to the T-301 pressure will overcome the hydraulic seal. This is seen as an initial safety measure in front of an overpressure. However this dos not preclude to install a safety overpressure system in the PBR because once the pressures in PBR and T-301 will be equilibrated, the internal pressure could eventually continue to increase. Thus, the liquid level is kept constant while at the same time there is no gas leak from the PBR to the output liquid line. However, there is also a level sensor proposed in order to allow an alarm activation in case of malfunction.

3.6.2.6 Inlet and outlet liquid flow rate control loop in the PBR

The fresh medium flows from the vessel T-101 to the PBR because the pressure is higher in the T-101 vessel. In the liquid line between T-101 and the PBR a control valve and a liquid flowmeter are installed. Depending on the value of the liquid flow rate measured, the controller

acts over the regulating valve VC-103 and modifies the flow rate. As there are no mechanical systems to propel the liquid, such as pumps, the contamination risk is decreased considerably, recovered biomass is not damaged and the risk of system malfunction due pump mechanical failure or due to biomass retained in the pump is eliminated.

As the liquid level is kept constant in the PBR, the same amount of fresh medium that incomes the PBR is the amount of culture medium that overflows from the PBR to the vessel T-301.

3.6.3 DISCHARGE VESSEL

The parameters controlled in the outlet culture vessel (T-301) are the following:

- Pressure
- Temperature
- Liquid level

In the figure 19, a scheme of the control loops involved in the performance of the vessel T-301 can be observed.

3.6.3.1 Pressure control loop in T-301

A pressure sensor measures the pressure inside the vessel and sends the electrical signal to the pressure controller, which can act over two regulating valves, VC-301 and VC-302.

The valve VC-302 is opened when overpressure in the vessel T-301 is detected. The outlet gas can be either driven to T-204 vessel (located in the PBR gas loop) or relieved to the atmosphere or a proper laboratory disposal gas line.

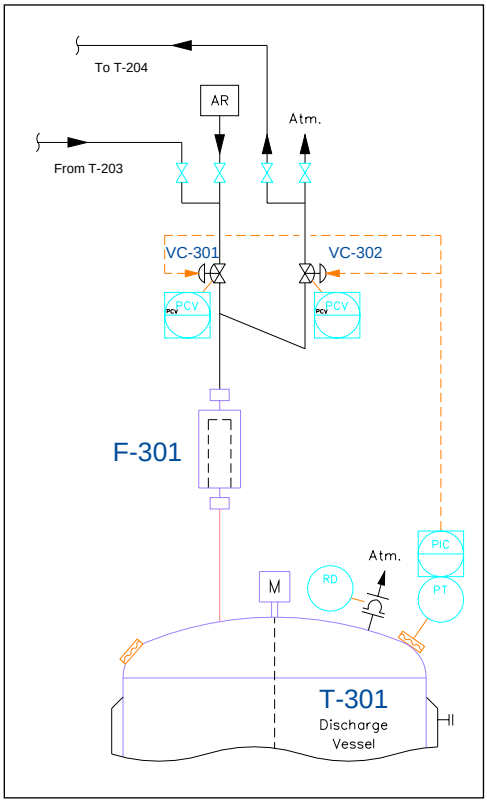


Figure 19: Scheme of the pressure control loop in the vessel B-2

The control valve VC-301 regulates the T-301 gas inlet, which is required when the gas pressure is lower than the pressure set point. The inlet gas can income from the vessel T-203 (gas incoming from the PBR gas loop) or alternatively it can be argon from an external gas line.

Both the inlet gas and the outlet gas pass through the filter F-301, so the sterility of the vessel is maintained.

As a safety measurement, in the top of the T-301 vessel, there is safety disc, which would relieve to the atmosphere a possible overpressure, if this is higher than the tare pressure of the safety disc (2 atm).

3.6.3.2 Temperature control loop in T-301

The temperature in the vessel T-301 is measured by means of a Pt-100 probe. The controller receives the signal from the probe and acts over a control valve that regulates the cold water (4ºC) flow rate that pass through the double jacket of the vessel.

3.6.3.3 Liquid level control loop in T-301

The liquid level in the vessel T-301 is measured by means of two pressure sensors located one in the top of the vessel and the other in the base of it. The difference between both measures is proportional to the volume of liquid inside the tank.

3.7 Sterilization system

The sterilization of the system is carried out by pressuring the system with steam at 121 ºC and 1.2 atm. Sterilization time depends on the time to reach the sterilization temperature by the different parts involved and also if liquid remains in the equipment or not. In any case 20 minutes is the minimum time to maintain any part under wet, high pressure and temperature conditions to consider it sterilized. In the PID diagram (Appendix 6.1) it can be observed the several steam circuits that allow to sterilize the different parts of the equipment as it has to operate under sterile conditions. Sterilization procedure can be pre-programmed in the controllers or manually performed. In any case the equipment has the necessary steam lines to proceed with the sterilization.

The equipments are divided into independent zones, which will be sterilized independently. In each zone, there will be a filtered steam inlet and a steam trap to collect the condensates.

As a general guideline, to achieve an optimal result it is required to input the steam from the upper point of the equipment and to collect the condensed steam in the lower point. In cases on the spatial configuration is of key importance, a detailed scheme in the PID has been prepared showing the 3D configuration.

4 ABBREVIATIONS

AI: Analytical measure indicator

AIC: Analytical measure indicator and controller

AR: Argon

AT: Analytical measure sensor

B: Vessel

C: Condensates

E: Condenser

F: Filter

FCV: Flow control valve

FIC: Flow indicator and controller

FT: Flow sensor

FV: Flow valve

HPC: Higher plant chambers

IVF: Illuminated volume fraction

K: Gas compressor

LAH: High level alarm

LI: Level indicator

LSH: High level sign

LT: Level sensor

M: Propeller engine

MCS: Melissa control system

P: Dosing pump

PBR: Photobioreactor

PCV: Pressure control valve

PI: Pressure indicator

PIC: Pressure indicator and controller

PID: Process and instrumentation diagram

PSV: Pressure safety valve

PT: Pressure sensor

R: Photobioreactor

RD: Safety disc

S: Steam trap

T: Vessel

TCV: Temperature control valve

TIC: Temperature indicator and controller

TT: Temperature sensor

UAB: Universitat Autònoma de Barcelona

VA: Angle valve

VB: Ball valve

VC: Control valve

VD: Diaphragm valve

VE: Electrovalve

VF: Filtered steam

VFA: Volatile fatty acids

VG: Gate valve

VK: Check valve

VS Pressure safety valve

5 REFERENCES

Masot, A.; Albiol, J.; Gòdia, F.; Waters, G.; Dixon, M. A. (2004). Static Mass Balance Studies of the MELiSSA Pilot Plant: Integration of a Higher Plant Chamber. 2004-01-2579. ICES 2004. SAE International.

Creus N. ; Perez J.; Mengual X.; Albiol J. ; Godia F. (2001) Connection of compartments III and IV at pilot scale. MELISSA Technical Note TN 47.6. ESTEC/Contract13292/98/NL/MV.

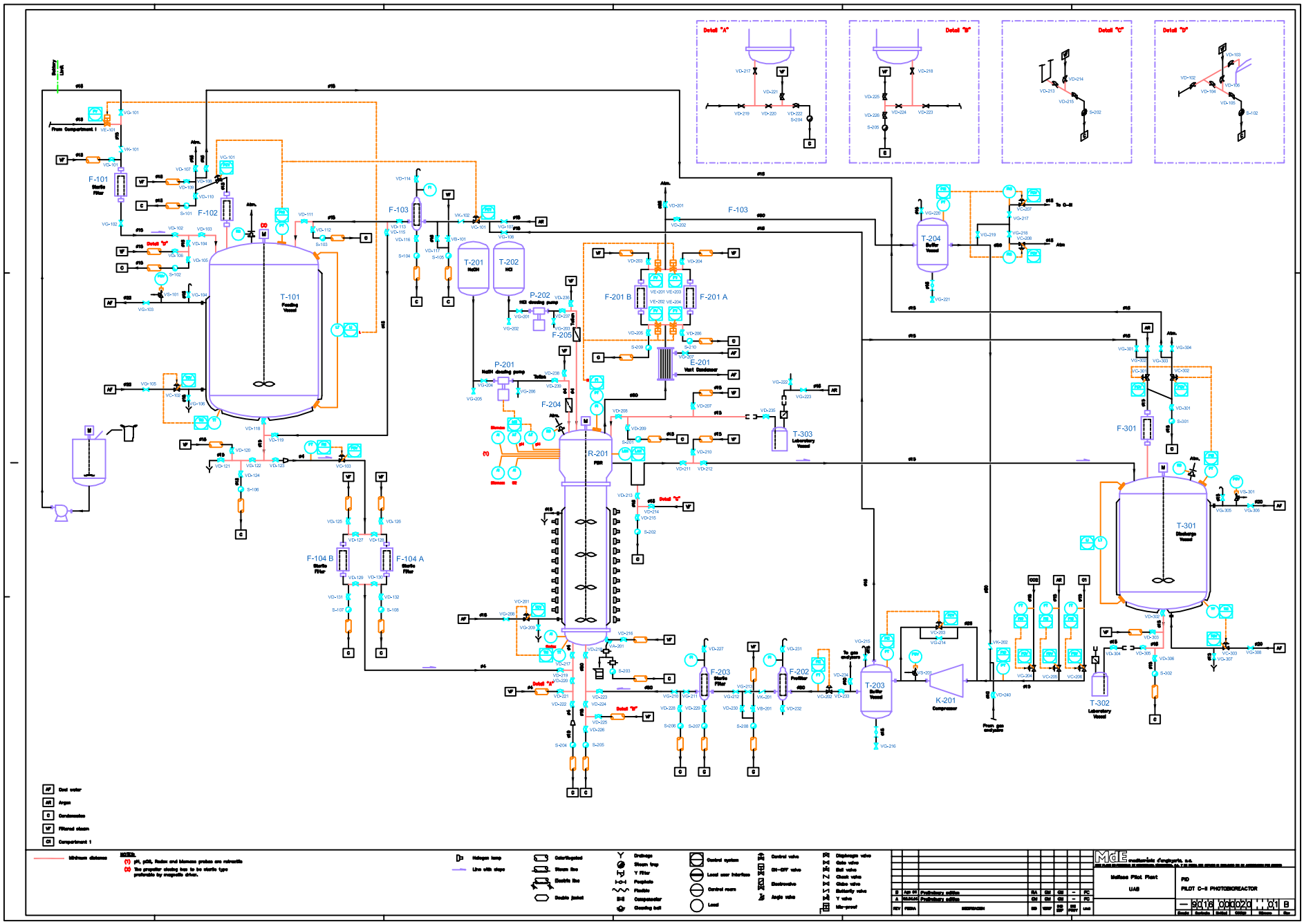
Creus N.; Albiol J. ; Godia F. Preliminary Connection Between 3 Compartments (2000). MELISSA Technical Note TN 43.8. ESTEC/CONTRACT11549/95/NL/FG.

Masot A. Albiol J. Godia F. (2004) Higher plant chamber preliminary requirements document. MELISSA Technical Note TN 65.5. ESTEC/CONTRACT11549/95/NL/FG.

Cabello F. ; Albiol J. ; Godia F. (2001) Photoheterotrophic Compartment. Light limitation tests.. MELISSA Technical Note TN 37.7. 2001 European Space Agency. ESTEC/CONTRACT11549/95/NL/FG .

6 APPENDIXES

6.1: PID of Compartment II


Detail "A"
Detail "B"
Detail "C"
Detail "D"
F-101
F-102
F-103
T-101
T-201
T-202
P-202
F-205
P-201
F-204
F-201 B
F-201 A
E-201
R-201
T-204
T-303
F-301
T-301
F-104 B
F-104 A
F-203
F-202
T-203
K-201
T-302
Air
Argon
Condensate
Filtered steam
Compartment 1
Minimum distance
Halogen lamp
Line with steps
Cylindrical
Steam line
Electric line
Double jacket
Drainage
Steam trap
Y Filter
Peephole
Flexible
Compensator
Cleaning ball
Control system
Local user interface
Control room
Local
Control valve
On-Off valve
Electrovalve
Angle valve
Diaphragm valve
Gate valve
Ball valve
Check valve
Globe valve
Butterfly valve
Y valve
Mix-proof
Mallorca Pilot Plant
UAB
PID
PILOT C-II PHOTOBIOREACTOR
90118 000020 01 B

6.2: Equipment and instrumental lists

AREA	EQ	ID	No.	Element	Service	Pos	Width (mm)	Height (mm)	Material	Flowrate (L/min)	Pressure (kg/cm2)	Volume (L)	Power (kW)	Remarks
100	F	1		F-101	Liquid filter: T-101 inlet				Polyester	8.3				Sterile with prefilter
100	F	2		F-102	Gas filter: PBR inlet				Teflon	2				Sterile
100	F	3		F-103	Gas filter: T-101 inlet				Teflon	0.1				Sterile
100	F	4	A	F-104A	Liquid filter: T-101 outlet				Polyester	0.08				Sterile with prefilter
100	F	4	B	F-104B	Liquid filter: T-101 outlet					0.08				
100	S	1		S-101	Ster. gas outlet T-101									
100	S	2		S-102	Ster. liquid inlet T-101									
100	S	3		S-103	Ster. gas inlet T-101									
100	S	4		S-104	Ster. F-103									
100	S	5		S-105	Ster. gas inlet T-101									
100	S	6		S-106	Ster. liquid outlet T-101									
100	S	7		S-107	Ster. F-104B									
100	S	8		S-108	Ster. F-104A									
100	T	1		T-101	Feeding vessel	V	700	700	AISI 316		-1/+3	250		With sterile agitator
100	VB	1		VB-101	Ster. inlet Ar line T-101									
100	VC	1		VC-101	Gas inlet in T-101									
100	VC	2		VC-102	Cool water input T-101									
100	VC	3		VC-103	Flow rate CL inlet R-201									
100	VD	1		VD-101	Stream inlet in F-101									
100	VD	2		VD-102	Ster. inlet liquid line T-101									
100	VD	3		VD-103	Ster. inlet liquid line T-101									
100	VD	4		VD-104	Ster. inlet liquid line T-101									
100	VD	5		VD-105	Ster. inlet liquid line T-101									
100	VD	6		VD-106	Ster. inlet liquid line T-101									
100	VD	7		VD-107	Ster. outlet gas line T-101									
100	VD	8		VD-108	Ster. outlet gas line T-101									
100	VD	9		VD-109	Ster. outlet gas line T-101									
100	VD	10		VD-110	Ster. outlet gas line T-101									
100	VD	11		VD-111	Ster. inlet Ar line T-101									
100	VD	12		VD-112	Ster. inlet Ar line T-101									
100	VD	13		VD-113	Ster. inlet Ar line T-101									
100	VD	14		VD-114	Pressure relief F-103									
100	VD	15		VD-115	Ar bypass of T-101									
100	VD	16		VD-116	Ster. F-103									
100	VD	17		VD-117	Ster. inlet Ar line T-101									
100	VD	18		VD-118	Liquid oulet T-101									
100	VD	19		VD-119	Ar bypass of T-101									
100	VD	20		VD-120	Ster. liq. line T-101&R-201									
100	VD	21		VD-121	Ster. liq. line T-101&R-201									
100	VD	22		VD-122	Ster. liq. line T-101&R-201									
100	VD	23		VD-123	Ster. liq. line T-101&R-201									
100	VD	24		VD-124	Ster. liq. line T-101&R-201									
100	VD	25		VD-125	Ster. liq. line F-104									
100	VD	26		VD-126	Ster. liq. line F-104									
100	VD	27		VD-127	Switch between F-104 A/B									
100	VD	28		VD-128	Switch between F-104 A/B									
100	VD	29		VD-129	Switch between F-104 A/B									
100	VD	30		VD-130	Switch between F-104 A/B									
100	VD	31		VD-131	Ster. liq. line F-104									
100	VD	32		VD-132	Ster. liq. line F-104									
100	VE	1		VE-101	CI liquid inlet in T-101									
100	VG	1		VG-101	Liquid inlet T-101									
100	VG	2		VG-102	Liquid inlet T-101									
100	VG	3		VG-103	Cool water output T-101									
100	VG	4		VG-104	Cool water output T-101									
100	VG	5		VG-105	Cool water input T-101									
100	VG	6		VG-106	Cool water input T-101									
100	VG	7		VG-107	Ar inlet in T-101									
100	VG	8		VG-108	Output K-201 gas inlet T-101									
100	VK	1		VK-101	Liq. inlet T-101									
100	VK	2		VK-102	Gas inlet T-101									
100	VS	1		VS-101	Cool water output T-101									
200	E	1		E-201	Condenser: outlet PBR				AISI 316		-1/+2			Tubes and shell
200	F	1	A	F-201A	Gas filter: PBR outlet				Teflon	2				Sterile with prefilter
200	F	1	B	F-201B	Gas filter: PBR outlet					2				
200	F	2		F-202	Gas prefilter: PBR inlet				Teflon	2				Sterile
200	F	3		F-203	Gas filter: PBR inlet				Teflon	2				Sterile
200	F	4		F-204	NaOH filter				Teflon	0.001				Sterile
200	F	5		F-205	HCl filter				Teflon	0.001				Sterile

AREA	EQ	ID	No.	Element	Service	Pos	Width (mm)	Height (mm)	Material	Flowrate (L/min)	Pressure (kg/cm2)	Volume (L)	Power (kW)	Remarks
200	K	1		K-201	Gas compressor				Cast iron	2				
200	P	1		P-201	NaOH Dosing pump				Teflon					With 4-20 mA signal for regulation
200	P	2		P-202	HCl Dosing pump				Teflon					With 4-20 mA signal for regulation
200	R	1		R-201	Photobioreactor (PBR)	V	400	2000	Glass/AISI 316					With sterile agitator, SS heads
200	S	1		S-201	Cond. outlet R-201 inoculum line									
200	S	2		S-202	Cond. outlet R-201 liq. outlet line									
200	S	3		S-203	Cond. outlet R-201 liq. sample line									
200	S	4		S-204	Cond. outlet R-201 liq. outlet line									
200	S	5		S-205	Cond. outlet R-201 gas inlet line									
200	S	6		S-206	Cond. outlet R-201 gas inlet line									
200	S	7		S-207	Cond. outlet R-201 gas inlet line									
200	S	8		S-208	Cond. outlet R-201 gas inlet line									
200	S	9		S-209	Cond. outlet F-201B									
200	S	10		S-210	Cond. outlet F-201A									
200	T	1		T-201	Vessel of NaOH	V			Glass		Atm	2		
200	T	2		T-202	Vessel ofHCl	V			Glass		Atm	2		
200	T	3		T-203	Buffer vessel: K-201 disc.	V	200	350	AISI 316		-1/+3	10		
200	T	4		T-204	Buffer vessel: PBR disc.	V	200	350	AISI 316		-1/+3	10		
200	VA	1		VA-201	Liquid sample outlet R-201									
200	VB	1		VB-201	Ster. gas loop									
200	VC	1		VC-201	Temp. CL in R-201									
200	VC	2		VC-202	Pressure CL in K-201 disc.									
200	VC	3		VC-203	Gas flow rate CL in gas loop									
200	VC	4		VC-204	Gas flow rate CL CO2 inlet									
200	VC	5		VC-205	Gas flow rate CL Ar inlet									
200	VC	6		VC-206	Gas flow rate CL C-I inlet									
200	VC	7		VC-207	Gas outlet from C-II to C-III									
200	VC	8		VC-208	Gas outlet from C-II to atm									
200	VD	1		VD-201	Gas F-201 outlet to atm									
200	VD	2		VD-202	Gas F-201 outlet to T-204									
200	VD	3		VD-203	Ster. stream inlet in F-201B									
200	VD	4		VD-204	Ster. stream inlet in F-201A									
200	VD	5		VD-205	Ster. stream outlet in F-201B									
200	VD	6		VD-206	Ster. stream outlet in F-201A									
200	VD	7		VD-207	Ster. stream inlet in R-201									
200	VD	8		VD-208	Ster. stream inlet in R-201									
200	VD	9		VD-209	Ster. stream inlet in R-201									
200	VD	10		VD-210	Ster. liq. line R-201&T-301									
200	VD	11		VD-211	Ster. liq. line R-201&T-301									
200	VD	12		VD-212	Ster. liq. line R-201&T-301									
200	VD	13		VD-213	Ster. liq. line R-201&T-301									
200	VD	14		VD-214	Ster. liq. line R-201&T-301									
200	VD	15		VD-215	Ster. liq. line R-201&T-301									
200	VD	16		VD-216	Ster. liq. sample line									
200	VD	17		VD-217	Liquid inlet in R-201									
200	VD	18		VD-218	Gas inlet in R-201									
200	VD	19		VD-219	Liquid inlet in R-201									
200	VD	20		VD-220	Ster. liq. inlet in R-201 line									
200	VD	21		VD-221	Ster. liq. inlet in R-201 line									
200	VD	22		VD-222	Ster. liq. inlet in R-201 line									
200	VD	23		VD-223	Gas inlet in R-201									
200	VD	24		VD-224	Ster. gas inlet in R-201 line									
200	VD	25		VD-225	Ster. gas inlet in R-201 line									
200	VD	26		VD-226	Ster. gas inlet in R-201 line									
200	VD	27		VD-227	Pressure relief F-203									
200	VD	28		VD-228	Ster. gas loop									
200	VD	29		VD-229	Ster. gas loop									
200	VD	30		VD-230	Ster. gas loop									
200	VD	31		VD-231	Pressure relief F-202									
200	VD	32		VD-232	F-202 liquid purgue									
200	VD	33		VD-233	Gas analysers line on/off									
200	VD	34		VD-234	Gas analysers line on/off									
200	VD	35		VD-235	Lab vessel valve									
200	VD	36		VD-236	Ster. HCl line									
200	VD	37		VD-237	Ster. HCl line									
200	VD	38		VD-238	Ster. NaOH line									
200	VD	39		VD-239	Ster. NaOH line									
200	VD	40		VD-240	Gas analysers inlet									
200	VE	1		VE-201	F-104B on/off									

AREA	EQ	ID	No.	Element	Service	Pos	Width (mm)	Height (mm)	Material	Flowrate (L/min)	Pressure (kg/cm2)	Volume (L)	Power (kW)	Remarks
200	VE	2		VE-202	F-104B on/off									
200	VE	3		VE-203	F-104A on/off									
200	VE	4		VE-204	F-104A on/off									
200	VG	1		VG-201	Ster. HCl line									
200	VG	2		VG-202	Ster. HCl line									
200	VG	3		VG-203	Ster. HCl line									
200	VG	4		VG-204	Ster. NaOH line									
200	VG	5		VG-205	Ster. NaOH line									
200	VG	6		VG-206	Ster. NaOH line									
200	VG	7		VG-207	Cool water inlet in E-201									
200	VG	8		VG-208	Cool water inlet in R-201									
200	VG	9		VG-209	Drainage cool water line R-201									
200	VG	10		VG-210	R-201 Gas inlet line									
200	VG	11		VG-211	R-201 Gas inlet line									
200	VG	12		VG-212	R-201 Gas inlet line									
200	VG	13		VG-213	R-201 Gas inlet line									
200	VG	14		VG-214	Bypass VC-203									
200	VG	15		VG-215	Venting valve in T-203									
200	VG	16		VG-216	Drainage line in T-203									
200	VG	17		VG-217	T-204 gas oulet to C-III									
200	VG	18		VG-218	T-204 gas oulet to atm									
200	VG	19		VG-219	T-204 gas oulet to C-III/atm									
200	VG	20		VG-220	Venting valve in T-204									
200	VG	21		VG-221	Venting valve in T-204									
200	VG	22		VG-222	Venting valve in lab vessel									
200	VG	23		VG-223	Ar inlet in lab vessel									
200	VK	1		VK-201	R-201 Gas inlet line									
200	VK	2		VK-202	Check valve before gas inlet									
200	VS	1		VS-201	Safety valve in K-201 discharge									
300	F	1		F-301	Gas filter: T-301 inlet/outlet				Teflon	2				Sterile
300	S	1		S-301	Cond. outlet T-301 gas inlet/outlet									
300	S	2		S-302	Cond. outlet T-301 liquid discharge									
300	T	1		T-301	Discharge vessel	V	700	700	AISI 316		-1/+3	250		With sterile agitator
300	T	2		T-302	Lab vessel	V			AISI 316		-1/+3	10		
300	T	3		T-303	Lab vessel	V			AISI 316		-1/+3	10		
300	VC	1		VC-301	Gas inlet in T-301									
300	VC	2		VC-302	Gas outlet in T-301									
300	VC	3		VC-303	Cool water input T-301									
300	VD	1		VD-301	Ster. outlet gas line in T-301									
300	VD	2		VD-302	Liquid discharge in T-301									
300	VD	3		VD-303	Ster. stream inlet liq. disc. line T-301									
300	VD	4		VD-304	Lab vessel liq inlet									
300	VD	5		VD-305	T-301 liq. outlet to lab vessel									
300	VD	6		VD-306	Cond. outlet liq. disc. line T-301									
300	VG	1		VG-301	Gas inlet in T-301									
300	VG	2		VG-302	Gas inlet in T-301									
300	VG	3		VG-303	Gas outlet in T-301									
300	VG	4		VG-304	Gas outlet in T-301									
300	VG	5		VG-305	Venting cool water T-301 line									
300	VG	6		VG-306	T-301 cool water outlet line									
300	VG	7		VG-307	T-301 cool water drainage									
300	VG	8		VG-308	T-301 cool water inlet line									
300	VS	1		VS-301	Safety valve in T-301 cool water									

AREA	LC	EQUIP	F	INS	ID	NAME	LOOP	TAG	INSTRUMENT	SERVICE	E/S	RANGE	UNITS	ALL	ALH
100	P	T-101	1	PT			PLC T-1011	PT T-1011	Pressure sensor	Pressure in T-101	AI	-1/+3	kg/cm²	0.45	0.55
100	P	T-101	1	PCV	A	VC-101	PLC T-1011	PCV T-1011A	Regulating valve	Gas outlet flow rate from T-101	AO	0-100	%	10	90
100	P	T-101	1	PCV	B	VC-102	PLC T-1011	PCV T-1011B	Regulating valve	Gas inlet flow rate from T-101	AO	0-100	%	10	90
100	L	T-101	2	LT			LLC T-1012	LT T-1012	Level sensor	Level in T-101	AI	0-100	%	15	85
100	L	T-101	2	FV		VE-101	LLC T-1012	FV T-1012	Electrovalve	Inlet liquid coming from C-I	AO	0-100	%	10	90
100	T	T-101	3	TT			TLC T-1013	TT T-1013	Temp. sensor	Temperature in T-101	AI	0-150	°C	4	10
100	T	T-101	3	TCV		VC-102	TLC T-1013	TCV T-1013	Regulating valve	Cool water flow rate through T-101 jacket	AO	0-100	%	10	90
100	F	T-101	4	FT			FLC T-1014	FT T-1014	Mass flow sensor	Mass flow rate from T-101 to R-201	AI	0-5	L/h	--	--
100	F	T-101	4	FCV		VC-103	FLC T-1014	FCV T-1014	Regulating valve	Mass flow rate from T-101 to R-201	AO	0-100	%	10	90
200	pH	R-201	1	pHT	A		pHLC R-2011	pHT R-2011A	pH sensor	pH in R-201	AI	0-14	upH	6.5	7.5
200	pH	R-201	1	pHT	B		pHLC R-2011	pHT R-2011B	pH sensor	pH in R-201	AI	0-14	upH	6.5	7.5
200	pH	R-201	1	P	A	P-201	pHLC R-2011	P R-2011A	Peristaltic pump	NaOH pump	AO	0-1	L/h	--	--
200	pH	R-201	1	P	B	P-202	pHLC R-2011	P R-2011B	Peristaltic pump	HCl pump	AO	0-1	L/h	--	--
200	O2	R-201	2	O2T			O2LC R-2012	O2T R-2012	O_2 sensor	O_2 in R-201	AI	0-100	%	0	5
200	X	R-201	3	XT	A		XLC R-2013	XT R-2013A	Biomass sensor	Biomass concentration in R-201	AI	0-3	g/L	--	--
200	X	R-201	3	XT	B		XLC R-2013	XT R-2013B	Biomass sensor	Biomass concentration in R-201	AI	0-3	g/L	--	--
200	RD	R-201	4	RDT			RDLC R-2014	RDT R-2014	Redox pot. sensor	Redox potential	AI	-1000/+1000	%	--	--
200	T	R-201	5	TT			TLC R-2015	TT R-2015	Temp. sensor	Temperature in R-201	AI	0-150	°C	28	32
200	T	R-201	5	TCV		VC-201	TLC R-2015	TCV R-2015	Regulating valve	Cool water flow rate through R-201 jacket	AO	0-100	%	10	90
200	L	R-201	6	LT			LLC R-2016	LT R-2016	Level sensor	Level in R-201	AI	0-100	%	--	--
200	FI	R-201	7	PT			FILC R-2017	PT R-2017	Pressure sensor	Pressure in R-201 head	AI	-1/+3	kg/cm²	--	--
200	FI	F-201	7	VE	A	VE-201	FILC F-2017	VE F-2017A	Electrovalve	Open/close F-201B line	AO	0-100	%	--	--
200	FI	F-201	7	VE	B	VE-202	FILC F-2017	VE F-2017B	Electrovalve	Open/close F-201B line	AO	0-100	%	--	--
200	FI	F-201	7	VE	C	VE-203	FILC F-2017	VE F-2017C	Electrovalve	Open/close F-201A line	AO	0-100	%	--	--
200	FI	F-201	7	VE	D	VE-204	FILC F-2017	VE F-2017D	Electrovalve	Open/close F-201A line	AO	0-100	%	--	--
200	P	T-204	8	PT			PLC T-2048	PT T-2048	Pressure sensor	Pressure in T-204	AI	-1/+3	kg/cm²	--	--
200	P	T-204	8	PCV		VC-207	PLC T-2048	PCV T-2048	Regulating valve	Gas outlet from T-204 to C-III	AO	0-100	%	10	90
200	P	T-204	8	PCV		VC-208	PLC T-2048	PCV T-2048	Regulating valve	Gas outlet from R-201 to atm	AO	0-100	%	10	90
200	P	T-204	8	FIC	A		PLC T-2048	FIC T-2048A	Mass flow sensor	Gas outlet from T-204 to C-III	AO	0-0.5	L/min		
200	P	T-204	8	FIC	B		PLC T-2048	FIC T-2048B	Mass flow sensor	Gas outlet from R-201 to atm	AO	0-0.5	L/min		
200	F	R-201	9	FT			FLC R-2019	FT R-2019	Mass flow sensor	Mass flow rate in the R-201 gas inlet	AI	0-5	L/h	--	--
200	F	R-201	9	FCV		VC-203	FLC R-2019	FCV R-2019	Regulating valve	Flow rate in the R-201 gas inlet	AO	0-100	%	10	90
200	P	R-201	10	PT			PLC R-20110	PT R-20110	Pressure sensor	Pressure in R-201 gas inlet	AI	-1/+3	kg/cm²	--	--
200	P	R-201	10	PCV		VC-202	PLC R-20110	PCV R-20110	Regulating valve	Pressure in R-201 gas inlet	AO	0-100	%	10	90
300	P	T-301	1	PT			PLC T-3011	PT T-3011	Pressure sensor	Pressure in T-301	AI	-1/+3	kg/cm²	0.45	0.55
300	P	T-301	1	PCV	A	VC-301	PLC T-3011	PCV T-3011A	Regulating valve	Air/gas inlet flow rate in T-301	AO	0-100	%	10	90
300	P	T-301	1	PCV	B	VC-302	PLC T-3011	PCV T-3011B	Regulating valve	Gas outlet flow rate from T-301	AO	0-100	%	10	90
300	L	T-301	2	LT			LLC T-3012	LT T-3012	Level sensor	Level in T-301	AI	0-100	%	--	--
300	T	T-103	3	TT			TLC T-1033	TT T-1033	Temp. sensor	Temperature in T-101	AI	0-150	°C	4	10
300	T	T-103	3	TCV		VC-303	TLC T-1033	TCV T-1033	Regulating valve	Cool water flow rate through T-301 jacket	AO	0-100	%	10	90

EQUIPMENT		
EQ	AREA	Total
Condensers	200	1
Total Condensers		**1**
Filters	100	5
	200	6
	300	1
Total Filters		**12**
Compressors	200	1
Total Compressors		**1**
Pumps	200	2
Total Pumps		**2**
PBRs	200	1
Total PBRs		**1**
Steam traps	100	8
	200	10
	300	2
Total Steam traps		**20**
Vessels	100	1
	200	4
	300	3
Total Vessels		**8**
Angle valves	200	1
Total Angle valves		**1**
Ball valves	100	1
	200	1
Total Ball valves		**2**
Control valves	100	3
	200	8
	300	3
Total Control valves		**14**
Diaphragm valves	100	32
	200	40
	300	6
Total Diaphragm valves		**78**
Electrovalves	100	1
	200	4
Total Electrovalves		**5**
Gate valves	100	8
	200	23
	300	8
Total Gate valves		**39**
Check valves	100	2
	200	2
Total Check valves		**4**
Safety pressure valves	100	1
	200	1
	300	1
Total Safety pressure valves		**3**
Total equipment		**191**

INSTRUMENTATION		
INSTRUMENT	AREA	Total
Biomass sensors	200	2
Total Biomass sensors		**2**
Electrovalves	100	1
	200	4
Total Electrovalves		**5**
Level sensors	100	1
	200	1
	300	1
Total Level sensors		**3**
Mass flow sensors	100	1
	200	3
Total Mass flow sensors		**4**
O2 sensors	200	1
Total O2 sensors		**1**
Peristaltic pumps	200	2
Total Peristaltic pumps		**2**
pH sensors	200	2
Total pH sensors		**2**
Pressure sensors	100	1
	200	3
	300	1
Total Pressure sensors		**5**
Redox pot. sensors	200	1
Total Redox pot. sensors		**1**
Regulating valves	100	4
	200	5
	300	3
Total Regulating valves		**12**
Temp. sensors	100	1
	200	1
	300	1
Total Temp. sensors		**3**
Total instrumentation		**40**

Signals		Input/Output	
INSTRUMENT	AREA	AI	AO
Biomass sensors	200	2	
Total Biomass sensors		**2**	
Electrovalves	100		1
	200		4
Total Electrovalves			**5**
Level sensors	100	1	
	200	1	
	300	1	
Total Level sensors		**3**	
Mass flow sensors	100	1	
	200	1	2
Total Mass flow sensors		**2**	**2**
O2 sensors	200	1	
Total O2 sensors		**1**	
Peristaltic pumps	200		2
Total Peristaltic pumps			**2**
pH sensors	200	2	
Total pH sensors		**2**	
Pressure sensors	100	1	
	200	3	
	300	1	
Total Pressure sensors		**5**	
Redox pot. sensors	200	1	
Total Redox pot. sensors		**1**	
Regulating valves	100		4
	200		5
	300		3
Total Regulating valves			**12**
Temp. sensors	100	1	
	200	1	
	300	1	
Total Temp. sensors		**3**	
Total general		**19**	**21**