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MELISSA

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General Laboratory Layout

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Draft	0	23/03/03	Preliminary Version
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Table of Contents

1 INTRODUCTION

The MELISSA project is a tool for the study and development of Advanced Biological Life Support Systems. Since the beginning it was realized the necessity of a physical loop implementation to verify the successful research results, obtained by the different MELISSA partners, in a common location. This implementation will generate inestimable results and provide information for further improvements. The final goal is the experimental demonstration of the MELISSA concept by means of long-term tests on a physical implementation of the loop design. The location for those tests is known as the MELISSA Pilot Plant.

Since the beginning, a laboratory for this purpose was foreseen and was initially located in ESTEC. In this initial location it was mainly devoted to compartment IVa and its control system and later to compartment III. Also, small scale bioreactor bench test were usually performed. In 1995 an agreement was reached with Universitat Autònoma de Barcelona (UAB) to take over the managing of the Pilot Plant and with the Spanish and Catalan authorities to co-finance the lab. After the agreement, the ESTEC laboratory was moved to the UAB premises. Since its first removal, the Pilot Plant laboratory has continued its task of developing, testing and interconnecting the MELISSA compartments. Among the main tasks performed several can be highlighted such as the scale up of compartment IVa and validation of its control software, the upgrade and characterization of compartment III, the basic tests for compartment II or the interconnected operation of several compartments, either at bench scale (compartments II, III and IVa) as well as III and IVa at pilot scale.

In the Initial agreement it was already foreseen that the laboratory would be expanded as more room would be needed for incorporation of the rest of the compartments. At present time it is foreseen the incorporation of compartments I, V and IVb along the year 2005. Taking advantage of the removal of different Engineering studies to a new building, a new expanded laboratory has been foreseen for relocation of the present units in operation and installation of the new ones.

This report describes the preliminary characteristics of the new location and foreseen compartments with more emphasis in the characteristics of the actual laboratory building under construction at UAB. A more detailed engineering design will be described in TN-75.6.

2 GENERAL LAYOUT OF THE LABORATORY

The new MELISSA Pilot Plant laboratory will be located in a new wing of the recently constructed Escola Tècnica Superior d'Enginyeries (ETSE) building, nearby the actual location of the MELISSA Pilot Plant. A general overview of the building and location of the new wing and Pilot Plants is shown in figure 1. Inside the building it will share the ground floor and services with two other plants dedicated to biotechnology and waste water treatment as described in the following pages. A closer view of its location inside the expanded wing of the new building is shown in figure 2.

After consultation with the future users of the building the architect's office has issued a first design of the distribution and services that will be built by the company finally awarding the contract. In this section this present architects proposal for the plant will be revised. After revision of the present project, some modifications appear to be necessary to better fulfil the MELISSA Pilot Plant needs. Those modifications will be commented were necessary.

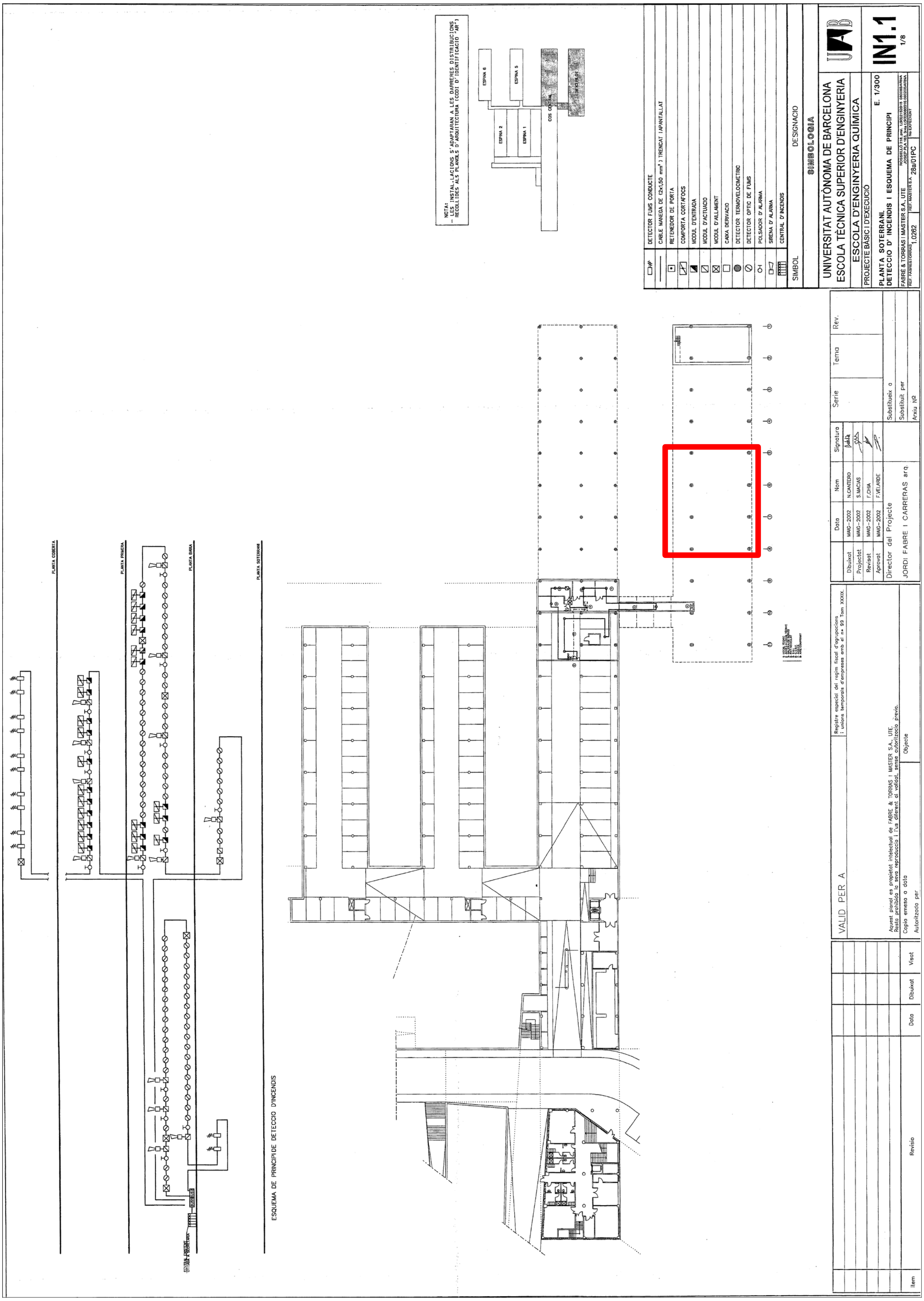

Figure 1 General view of the ETSE building The red line identifies the location of the MELISSA Pilot Plant. Also shown part of the basic scheme of the fire detection system.

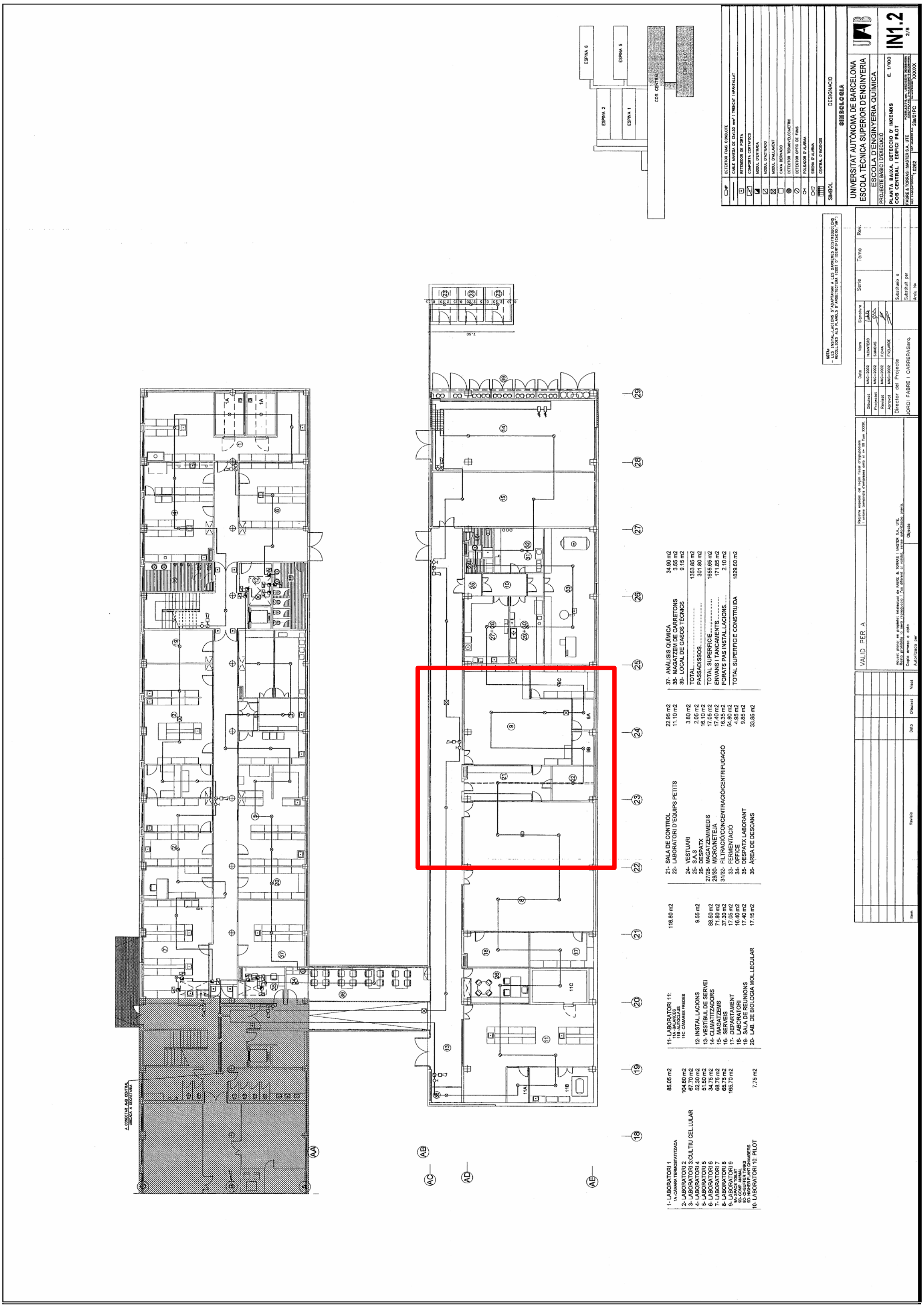

Figure 2: Location of the MELISSA Pilot Plant (red line) in the new wing of the ETSE building. Also shown part of the fire detection system.

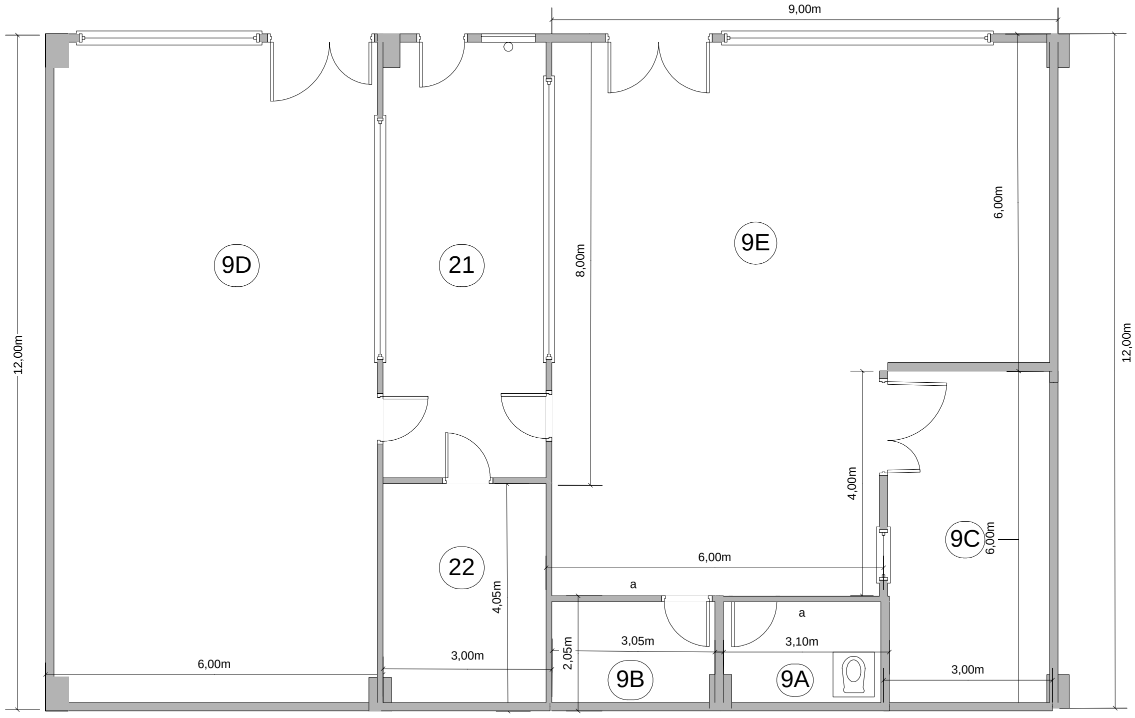

Figure 3. General layout of the new MELISSA Pilot Plant laboratory.

The general layout of the laboratory is represented in figure 3. It has a total surface of 216 m^2 (including walls) distributed in 7 dedicated areas:

- Higher plant area for installation of the higher plant chambers and its accessory equipment (72 m^2). (**9D**)
- Area for bioreactors of compartments II, III and IVa and its accessory equipment (78 m^2). (**9E**)
- Compartment I area that will contain the bioreactor as well as the equipment for preparation of the feeding (18 m^2). (**9C**)
- Animal compartment area for containing the rats cage as well as any other equipment required for breeding (6 m^2). (**9B**)
- Computer room area with visualization to the plant for the location of the computer services to control the plant and associated services which can include some instrumentation (24 m^2). (**21**)
- Toilet Area for location of an automatic system for collection of the human waste donations from voluntary people (6 m^2). (**9A**)

- Library/meeting room area for storage of all the Pilot Plant documentation and meetings related to the MELISSA project (12 m^2). (**22**).

Height of the lab will be of 4.5 metres in the laboratory areas except in the computer (area 21) and library (area 22) rooms were the ceiling will be of 2,5 metres. In this case the extra room above the ceiling of the areas 21 and 22 will be prepared to be used as a storage space.

In the following a more detailed description of the services available can be found.

3 MAIN SERVICES OF THE LABORATORY

The laboratory will be equipped with a number of general services in a similar way as the ones already available in the present location. However, cooling systems and air recirculation have been improved and some of the services depend on centralised systems.

3.1 Power supply

The new wing were the MELISSA laboratory will be located has its power supply grid lines distributed in tree main areas as can be seen in figure 4. One area corresponds to a normal supply without any backup system (figure 4A). A second area is connected to a diesel power generator which switches on after one minute of a power failure (figure 4B). A third area includes an unbreakable power supply system to maintain in operation the more sensible systems to a power failure, such as computers or PLC's, during the time it takes the diesel power generator to take over (figure 4C). The cost of each line implementation is different and therefore they do not have the same power assigned.

To calculate the power supply necessary for the laboratory an initial estimation based on the foreseen equipment was done resulting in a requirement of about 47kW of 220-350 V. After evaluation of all the power requirements of the other labs the final power assigned to the MELISSA was distributed as follows.

The common power supply line (line code LN/LAB9) without backup systems for the MELISSA labs are calculated for a total of 43.6 A and a surplus of 10A adding to a 53.6 A (30 KW) see figure 5. This line will be distributed to sub lines FL9.1, Fl9.2, FL 9.3, FL9.4, FL9.5. For a distribution of those lines in the labs see figure 8. Lines FL9.1 and FL 9.2 correspond to the lines located at the ceiling of laboratories 9D and 9E (higher plant and C-II, C-II and C-Va labs).

The power line connected to the diesel generator system (line code LP/LAB9) has the same main characteristics as the previous one. That is a calculated total consumption of 43.6 A and a surplus of 10A adding to a 53.6 A (30 KW) see figure 6. This line will be distributed in sub lines GL9.1 (ceiling line for the lab 9E) and line GL9.2 (wall sockets in labs 9E, and 9C). It will be proposed to extend that line to labs

9a, 9B and 9D. Nevertheless due to the high power consumption foreseen for the lamps in compartment IVb (lab 9D) those lamps will not be connected to this line.

The power line connected to the diesel generator and the UPS, foreseen for the most sensible equipments (line code LS/LAB9) is foreseen for total consumption of 14 A and a surplus of 3A adding to a 18 A (3.3 KW) see figure 7. This line will be distributed in sub lines SL9.1 (computer room, lab 21) and line SL9.2 (wall sockets of labs 9E and 9C).

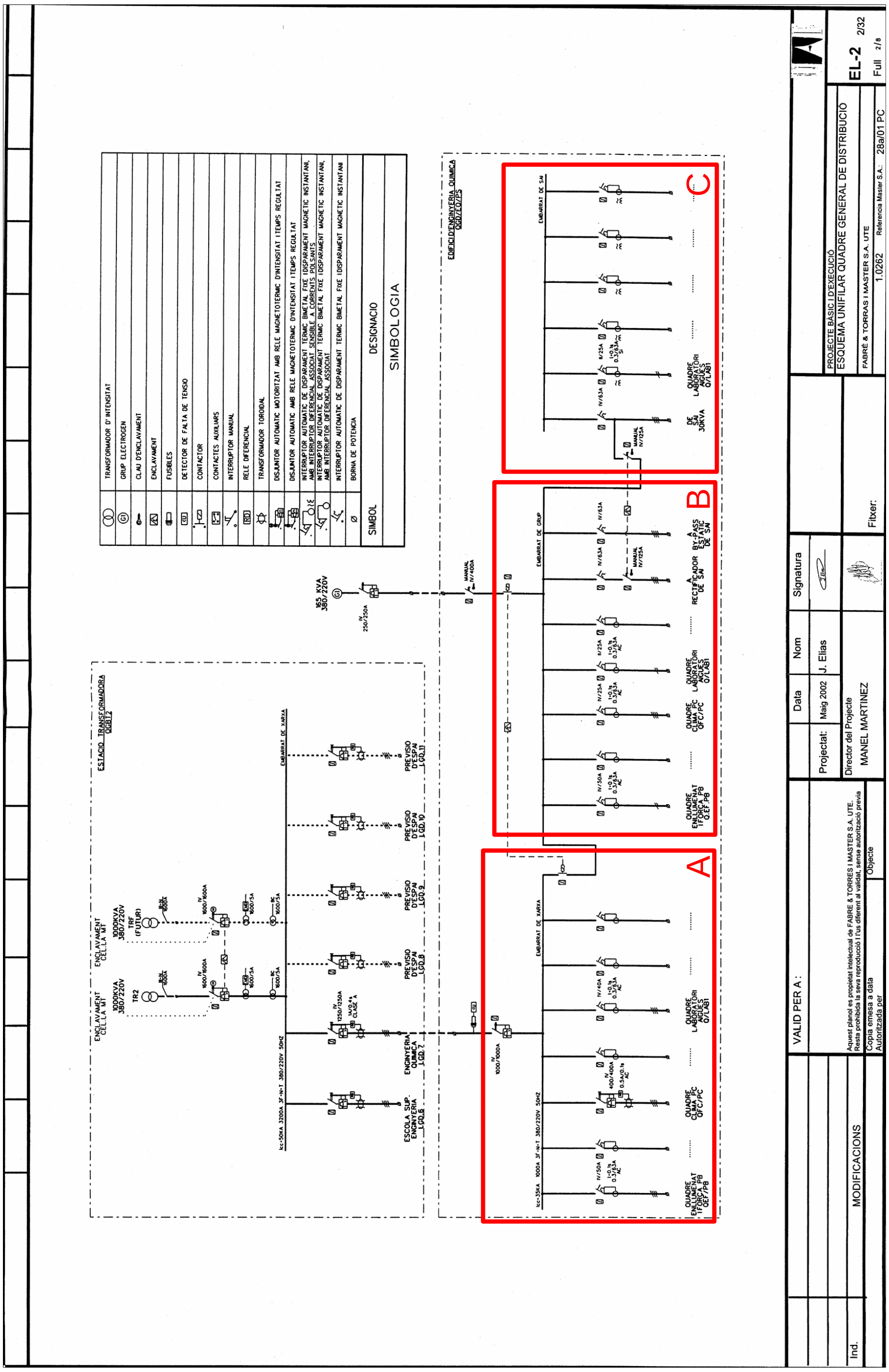

Figure 4: General power distribution. A: power lines without backup system. B: Power lines with diesel power generation. C: Power lines with diesel power generation and UPS sytem.

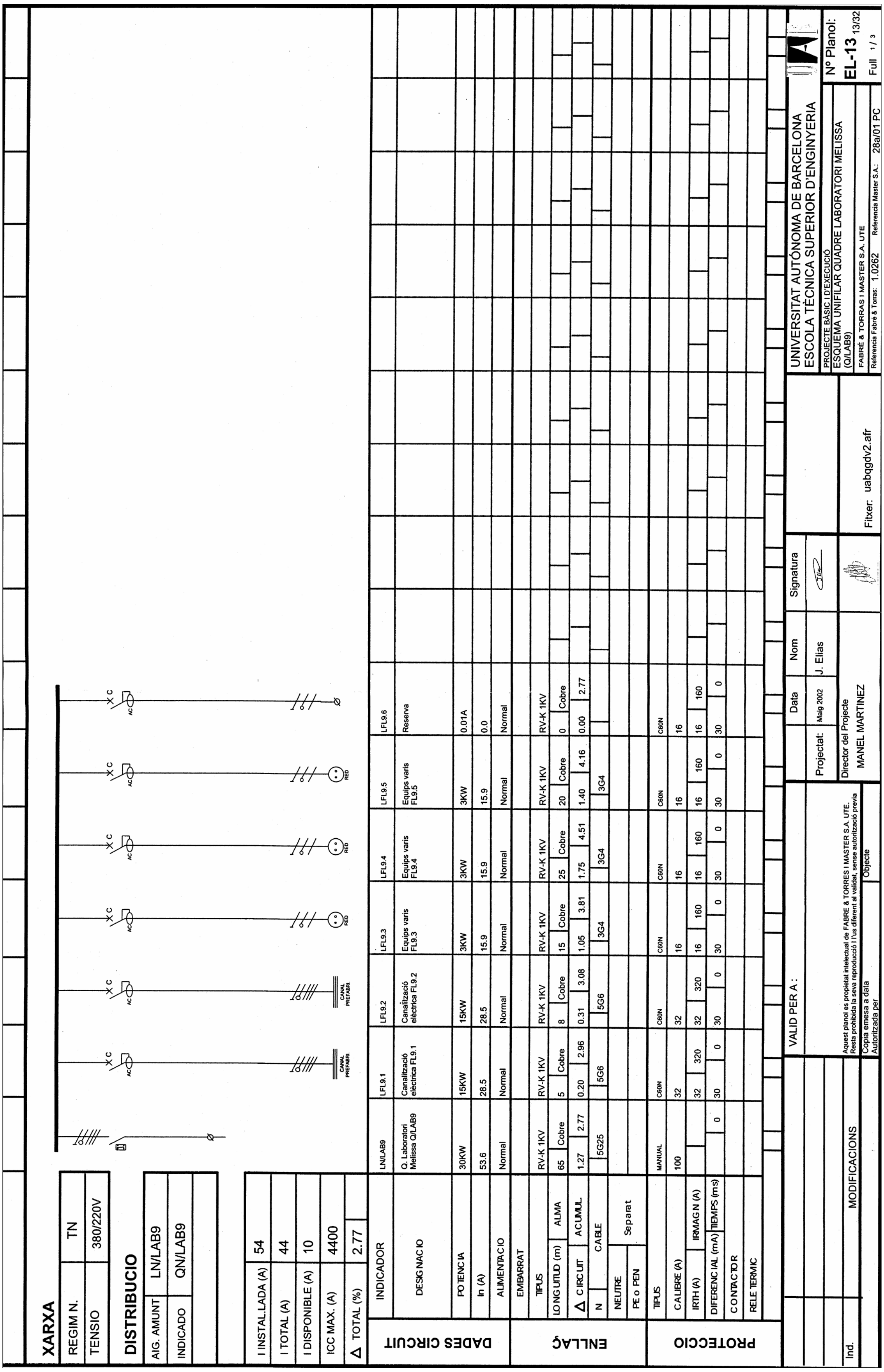

Figure 5: Description of power line LN/LAB9

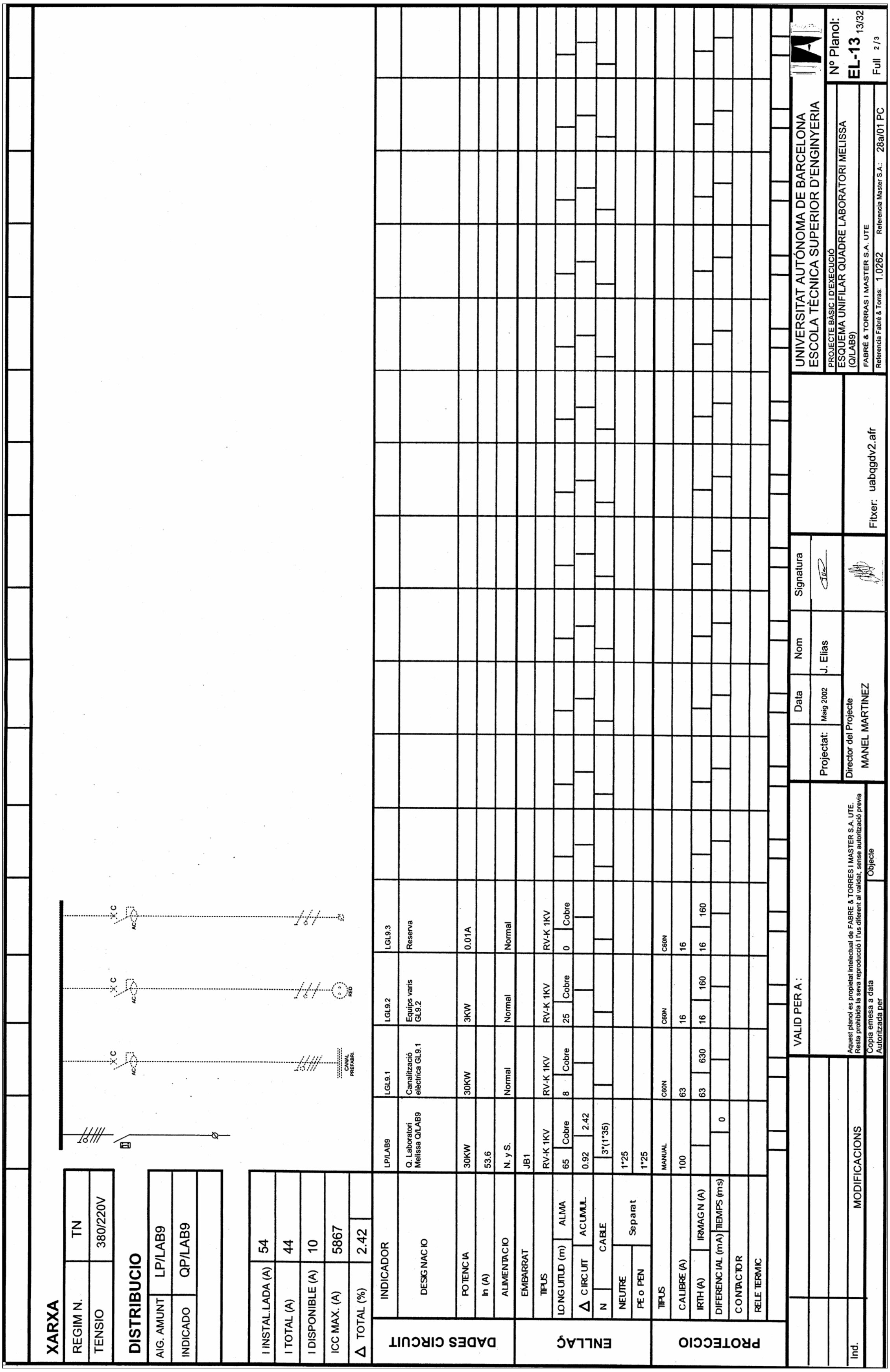

Figure 6: Description of power line LP/LAB9

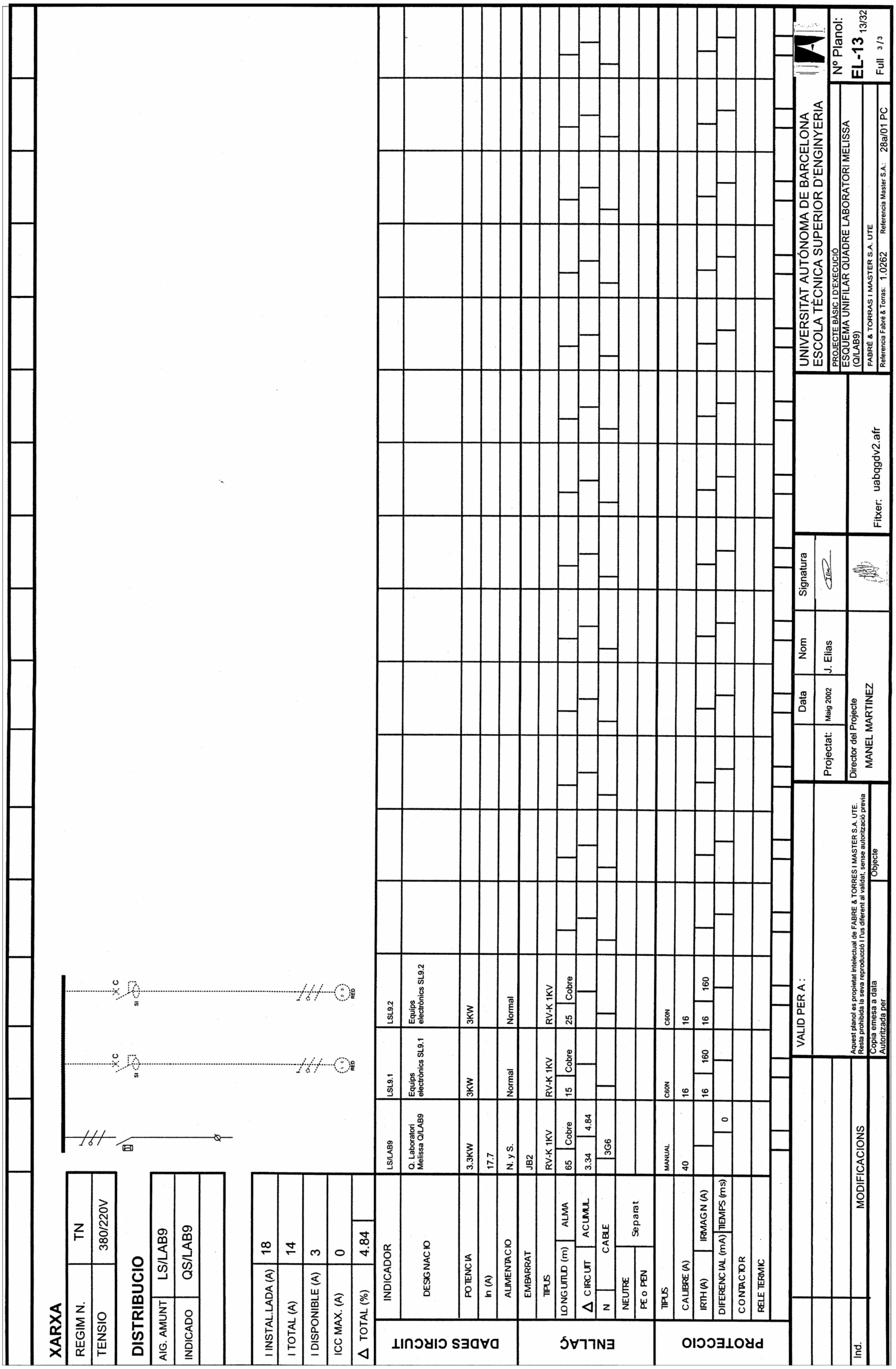

Figure 7: Description of power line LS/LAB9

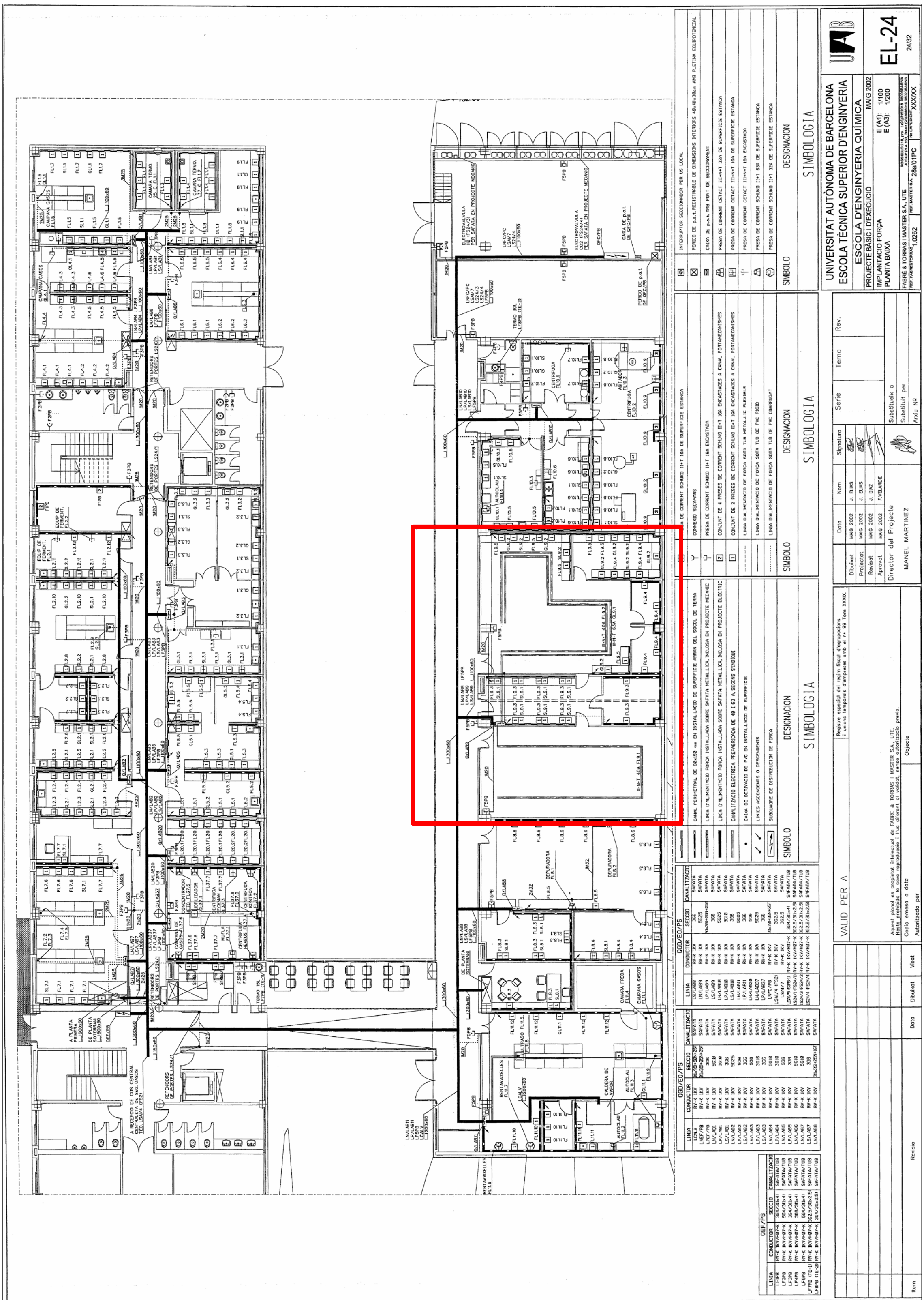

Figure 8: Physical distribution of the power lines on each lab.

3.2 Water supply and drains

Due to the possibility of leaks in the bioreactors, the floor of rooms 9E, 9C and 9D will require drains in the floor. The laboratory for the bioreactors of compartments II, III and IVa (9E) has slow slopes, corresponding to the floor drains, to allow for water elimination in case of leaking or to easy cleaning of the bioreactors.

The laboratory will be connected to the general tap water lines. In figure 8 it can be seen the tap water connections for rooms 9E and 9D. It will be proposed to extend those lines to labs 9a, 9B and 9C.

Demineralised water for its use in bench equipment for temperature control and for some maintenance tasks will also be available. In figure 8 it can be seen the connection to the general demineralised water lines (provided by a demineraliser Vivendi model Selectron 30 380 l/h). It is foreseen 4 connection points in lab 9E and one in 9D. It will be proposed to extend the line to lab 9C.

Distilled water type I will also be provided at the sink level in lab 9E (provided by Millipore Milli-RX-75 Plus 75L/h better than 5 MOhms) to allow for a easy culture medium preparation.

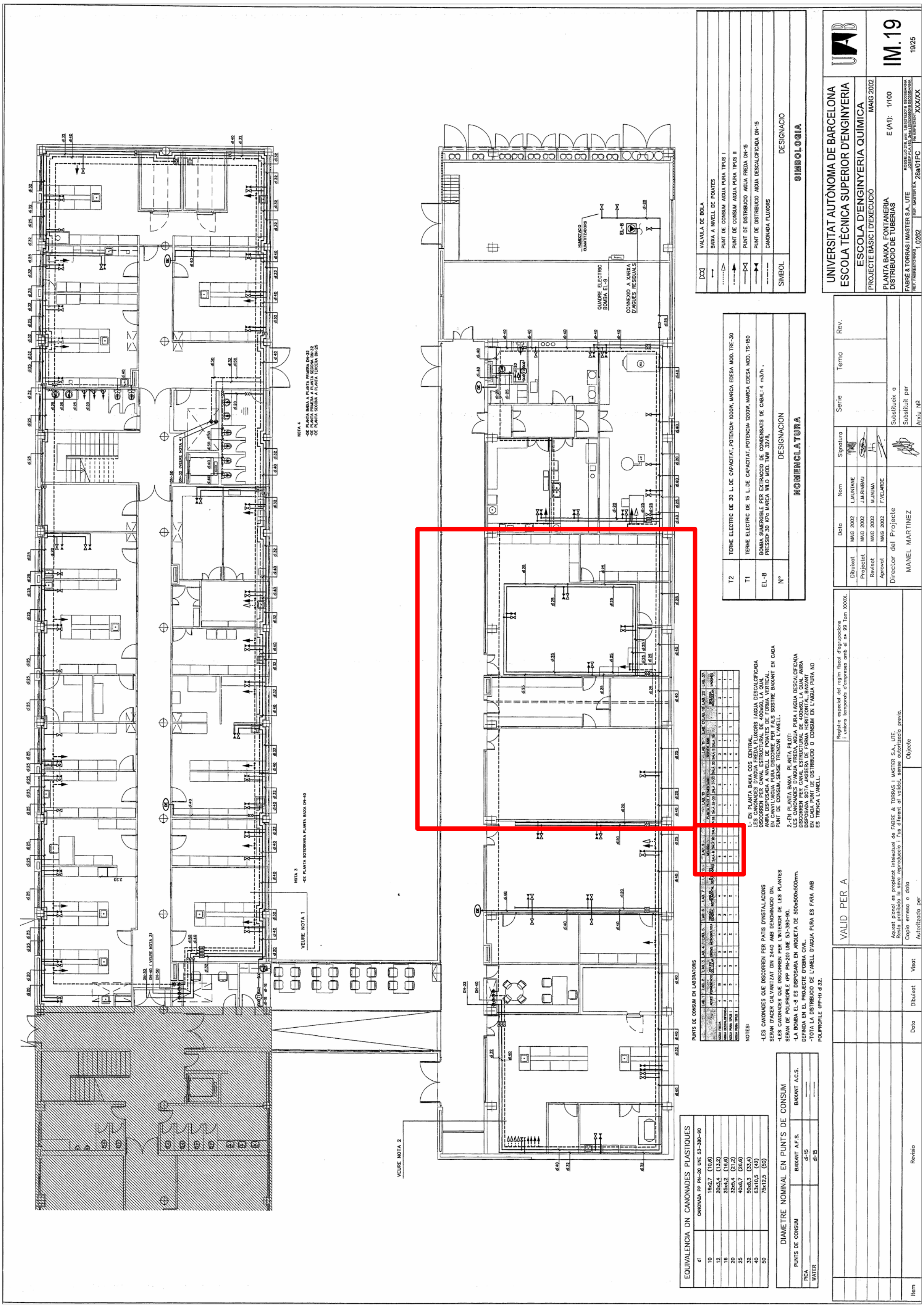

Figure 9 : Distribution scheme of tap water and demineralised water.

3.3 Gas services

The laboratory will be provided with a connection to the centralised supply of the following gases:

N_2,: Usually used for calibration of gas equipment, degasification or removing of CO_2 for pH control, mixing for artificial air generation or for nitrogen supply to certain fixing organisms.

O_2,: Usually used to supply oxygen to certain cultures.

CO_2, Usually used as carbon source or for pH control.

He_2,: Usually used as carrier gas for gas chromatographs and also as inert gas for mixing or de-aeration. For de-aeration Ar is less expensive and its addition will be studied.

H_2,: combustion gas for FID detectors in gas chromatography. It is also consumed by certain bacteria.

Gas containers are located outside the building in location 39 (fig 2).

Compressed air: Used either as cheap oxygen supply in certain applications, and as a pressure source for pneumatic equipment. The line will be provided with oil filtering and moisture condensing devices to avoid effects from the central compressor system on the equipment. Supplied by Worthington Mod. RLR 550M/300/T 36m^3·/h at 8bar and a 300L buffer tank with a cold drier and oil purge. Gas supply line will be pressurized at 4 bar and filtered with activated carbon, followed by 1 micron and 0.01 micron filters.

For in situ sterilization purposes steam will be provided by a shared line by an electrical heater (ATTSU Mod. GE-140/8/6) providing up to 142 kg/h at 6 bar.

As shown in figure 10 in the present configuration there are 4 connection points in lab 9E for He, N_2, O_2, CO_2, compressed air and steam. There are also 3 connection points in lab 9D for N_2, O_2, CO_2. In view of the possible installation of gas chromatographs it will be proposed to install a H_2 line. Also in lab 9C connections for N_2, O_2, CO_2, He and compressed air should be desirable. Steam has not bee foreseen in 9C because it is not foreseen to sterilize C-I but its installation is possible.

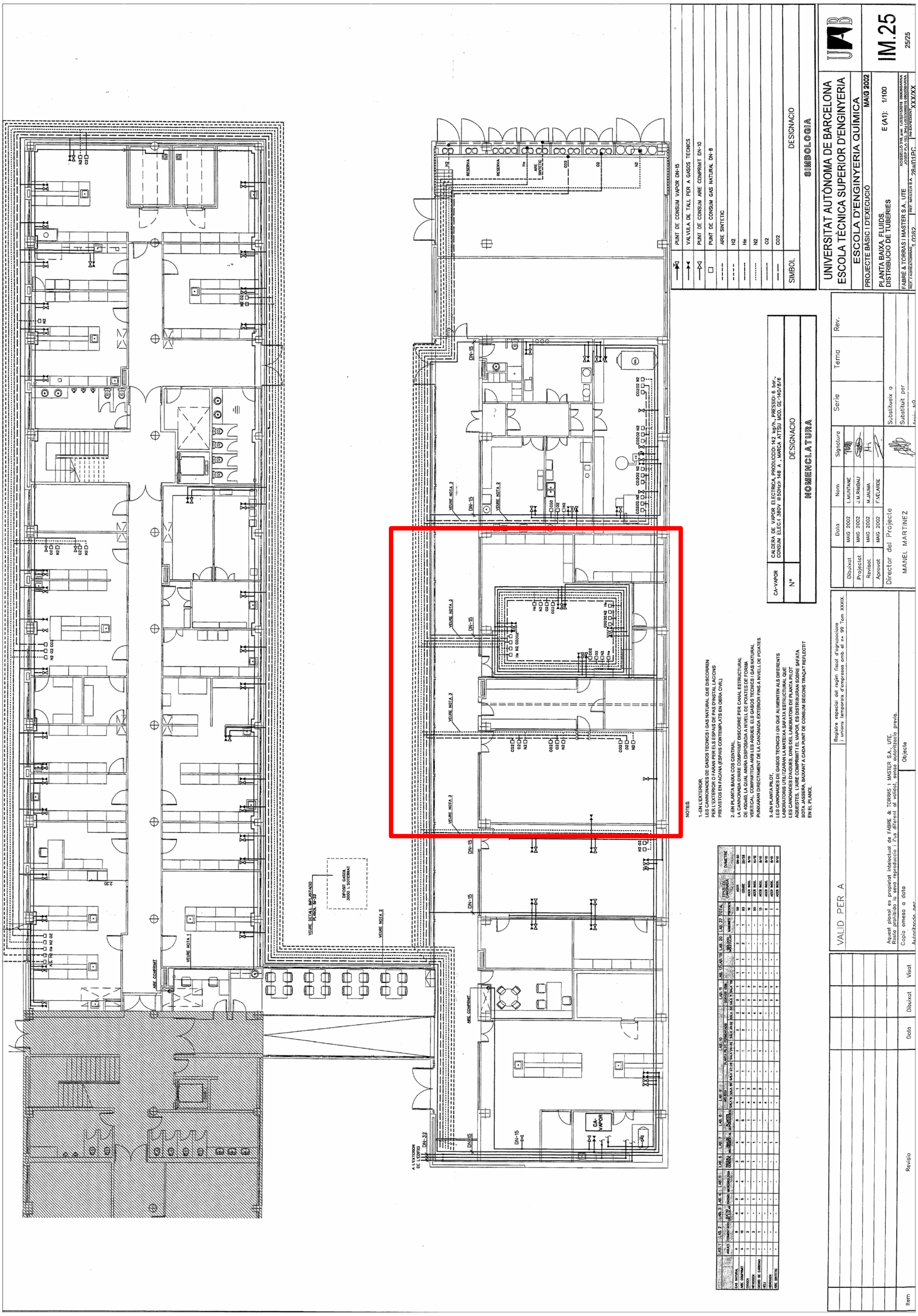

Figure 10: Gas lines distribution scheme.

3.4 Liquid and Air Cooling services

Cooling services in the laboratory will be distributed into a cooling liquid line and a cool air recirculation system.

Cool liquid supply will consist in an isolated piping system conveying 4ºC chilled water. Each bioreactor in room 9E can be interconnected to this network. It should also be possible to use the cooling fluid in lab 9D. The cooler will be centralized in an external services area. The cooling line will also be used to refrigerate the incoming air in the labs using a heat exchanger.

Cool air recirculation is foreseen for all laboratories. It will have independent circuits for each laboratory in view of the foreseen heat generation due to the use of the illumination systems, and also to avoid the spread of smells from compartment I cabinet. The total cooling power for this lab, used in the calculations together with all the labs, was calculated from the expected power consumption and taken to be of 37 KWatts.

Figure 11 shows the distribution of the air supply and removal system. The air removed from the labs will be used to equilibrate the temperature of the incoming air by means of a heat exchanger system outside the lab and rejected. The newly incoming air will be conveyed to each lab and heated/cooled as necessary at the entrance of each lab.

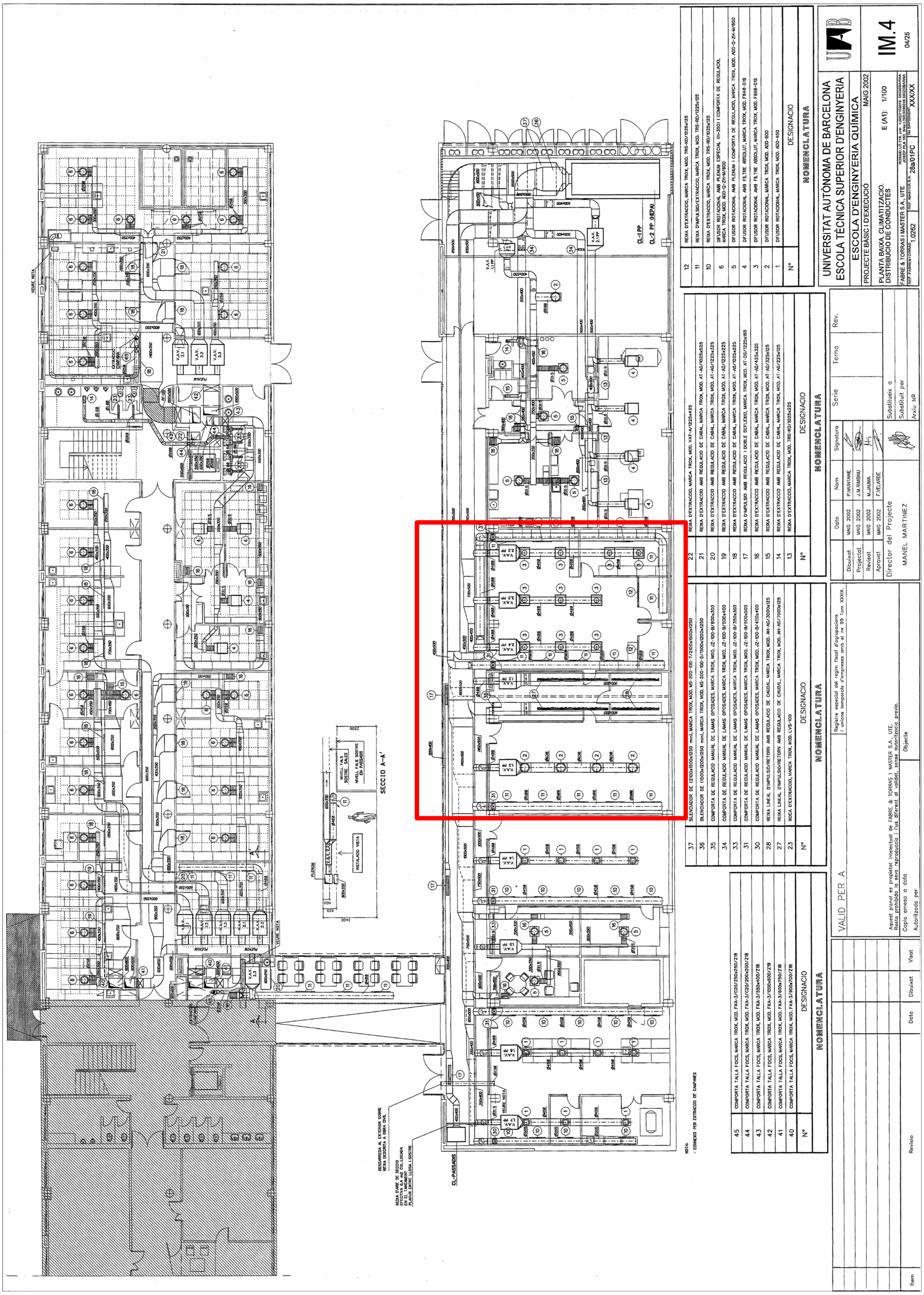

Figure 11: Location of the air conditioning input/output system.

3.5 Computer Services

The laboratory will have an internal ethernet network were the main control computers and related equipments will be connected. Especifications for this network and the complete control system have already been reviewed by NTE (TN 72.2). The laboratory will also have connection to the local UAB LAN, and therefore to internet, for easy communication with the rest of the partners.

All the computer services will be located in a dedicated area (lab 21) with a view to the lab trough glass windows and independent temperature control. Computer power supply lines will be independent from the laboratory ones and with UPS as explained above.

3.6 Safety

All the new areas are provided with security surveillance systems allowing to detect dangerous gas accumulation and fire generation.

Gas detection system is prepared to detect accumulation of natural gas, used in lab burners, and CO_2. If an H_2 line is provided the system will also incorporate its corresponding detection system. Also taking into account the possible generation of H_2S in compartment 1 it will be proposed to install a detector of this gas in room 9C.

All the laboratories are interconnected to a network of fire detectors and centralised alarm system as shown in figure 2.

3.7 Ancillary services

For its operation the laboratory will require other services such as microbial culture nursery area, chemical product storage, glassware cleaning area, chemical analysis service, etc… all those services will be located outside the main laboratory depicted in figure 3 and will be shared with the rest of the laboratories of the Chemical Engineering department. This way the cost in materials, room and maintenance personnel is minimized. Common ancillary services will be located in lab 11 (fig 2). Analytical services will be located in lab 37.

Air conditioning equipment and central cooler will be located in lab 14 (fig 2). Auxiliary power supply systems, water purification systems, and compressed air equipment will be located in the underground under location 35. Steam generator will be located in Lab 11B.

4 GENERAL COMPARTMENT REQUIREMENTS DESCRIPTION

As mentioned before a more detailed engineering description of each compartment in the new Pilot Plant will be done in TN 75.6. Nevertheless the foreseen status and general description of points to take into account for installation of each compartment in the Pilot Plant will be described below.

The general compartment interconnection has been already the subject of previous initial descriptions and summarized in technical notes 62.3, 62.4 and 62.5. Those interconnections are the basis of the present foreseen interconnections in the Pilot Plant. A general graphical overview of the previously proposed liquid and gas interconnection among compartments can be seen in figures 12 and 13. Although future MELISSA decisions might result in changes onto the foreseen interconnections.

4.1 General considerations for the interconnection hardware.

For the implementation of the Pilot Plant a number of interconnections is already foreseen and it has been decided to set up a permanent network of pipes for the interconnection of compartments. To physically set up those interconnections it is also necessary to decide beforehand the location of the equipment in the Pilot Plant. To this purpose an initial proposal for the location of the equipment has been done. With this basis, figure 14 shows a first proposal for equipment location and the interconnections among compartments. The Piping will be installed in the ceiling. It is not the intention of figure 14 to indicate the exact path of the piping but only to indicate the interconnections among compartments. It has been decided to contact a specialized company for the installation of the piping. The proposed interconnections here and their characteristics will be used as the starting point with the selected company for the decision of the best implementation and the selection of the necessary equipment.

The piping system has to allow steam sterilization and therefore provide for steam input and output connections, and condensed water drains. The pipes will have to be isolated from the ambient temperature in order to minimize the waste of energy and to limit the risk of injury on the operators. It will also incorporate safety valves to avoid overpressures and the installed pressure sensors will allow to monitor the sterilization procedure.

The piping system will also incorporate the stainless steel cases for the exchangeable sterility filters (for example stainless steal 316 cartridge housings such as for example Millipore Acerliner or Millipore Optiseal). It will have to incorporate appropriate systems for liquid or gas flow impulsion, flow measurement and regulation, gas or liquid mixing and sampling ports for analysis.

The piping system will consider the proper procedure for substituting the filtering and pumping devices in case of malfunction, without compromising the sterility and operational conditions of the rest of the equipment. If required twin units, that can substitute the failing one, can be foreseen. In this case, automatic detection of the failing device and switch to use an alternative one, can be taken over by the control system. Similar consideration will have to be done for the substitution of key probes and sensors.

More detailed specification of this equipment will be done in TN 75.6.

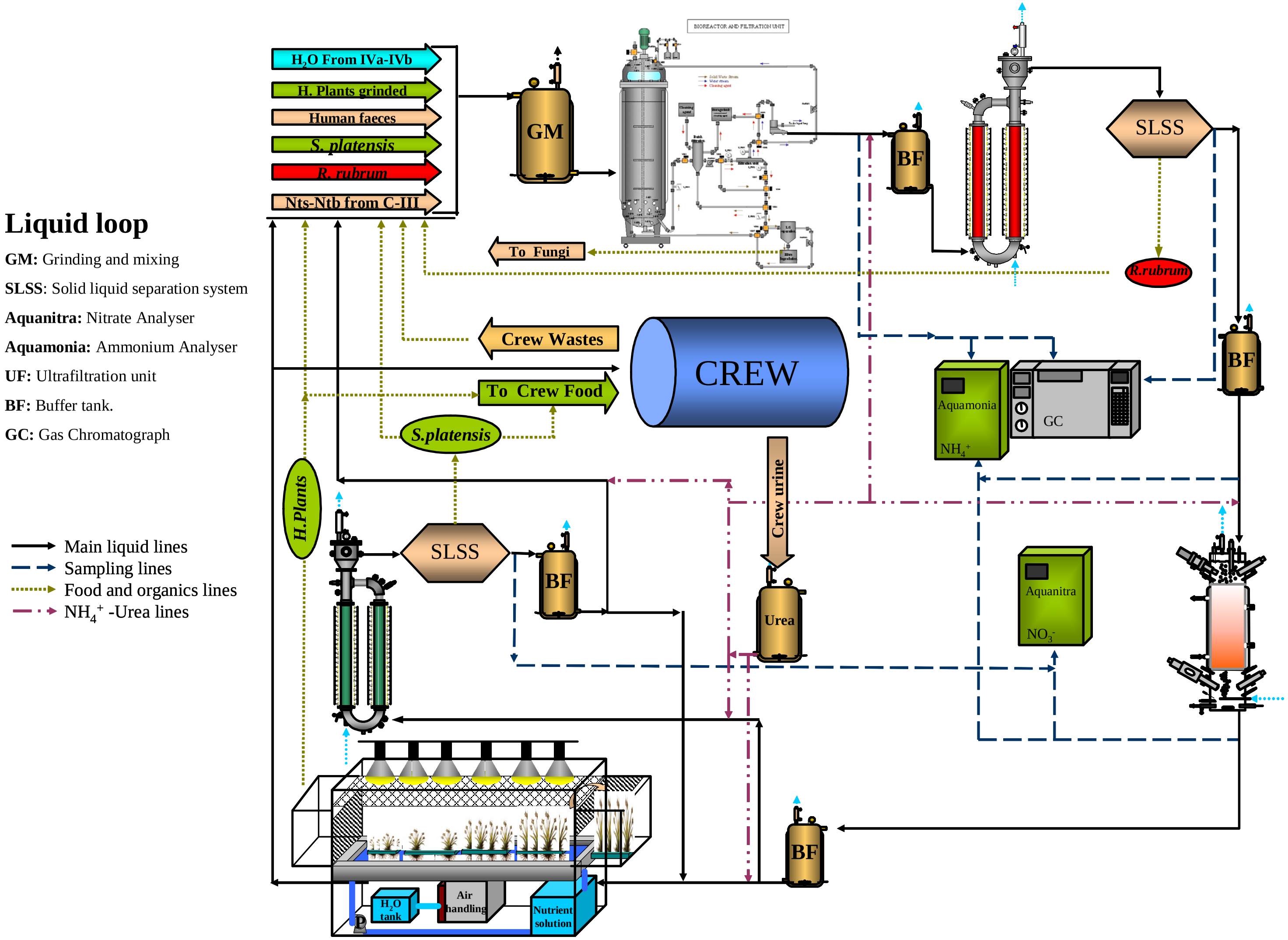

Figure 12: Schematic overview of the MELISSA liquid loop.

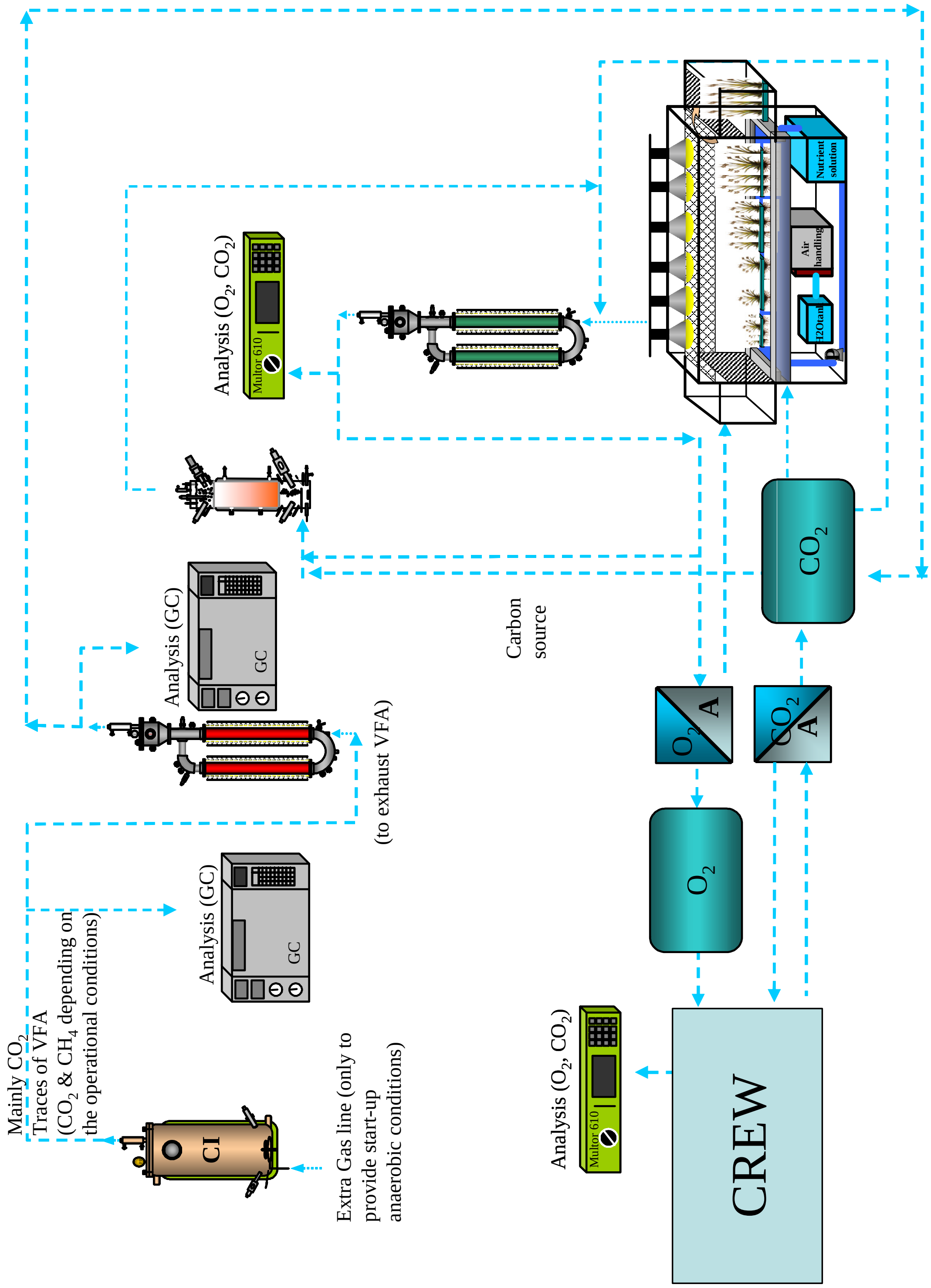

Figure 13: Schematic overview of the MELISSA gas loop.

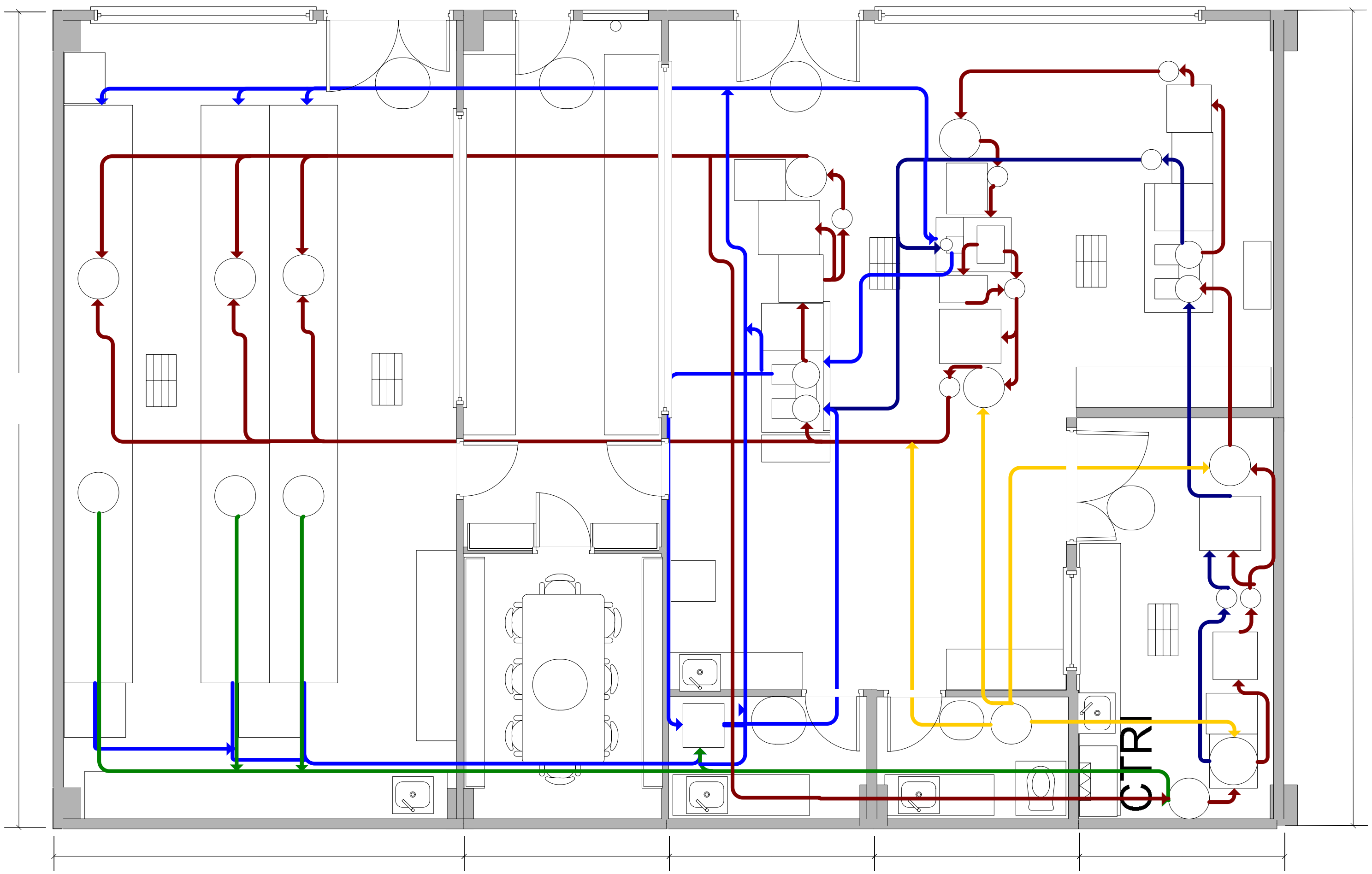

Figure 14: Schematic view of the proposed location of the equipment and the pipes that will have to interconnect the compartments. C-I: compartment I reactor. Ctrl: control rack. SLSS: Solid liquid separation system. AN: Analyzers. BF: Buffer tank. C-II reactor compartment II, F: Stainless steel filter case. RT: recirculation tank. Ev.: Evapotranspiraiton tank. HPC: Higher plant chamber. Frdg: Refrigerator. Brown lines: liquid loop. Blue lines: gas loop. Yellow lines: Urine pipes. Green lines: purified water. Cab: Cabinet with air extraction system.

4.2 Compartment I

The bioreactor for compartment I is at present time under scale up and test at EPAS. It is foreseen to install the EPAS developed and tested bioreactor in the Pilot Plant as soon as the construction is finished. Nevertheless it has been preliminary described in TN 71.1 and the Pilot Plant layout concerning traits will be briefly summarized.

The foreseen bioreactor, depicted in figure 15, will be of an approximate volume of 100 litres and will have attached a tangential filtration unit. The filtration unit allows for periodic removal of liquefied material while the particulate matter remains in the reactor.

For the preparation of the reactor input substrate it will be necessary to install a grinder equipment and a tank were to mix the components and add water if necessary. After the mixture is done anaerobiosis can be established (e.g. flushing with a gas such as argon) in the mixing tank and connected to the main C-I tank for transferring the substrate. If this is done periodically, for example every 2 days, all the tank content will be transferred to the bioreactor. Other wise, if continuously done, equipment to measure and maintain a constant flow will have to be installed.

Either if the bioreactor is fed periodically or continuously, filtration will surely be discontinuous and therefore it will be necessary to install a buffer tank to collect the liquid output of the bioreactor after it is separated by means of the filtration unit. As this will be the input for compartment II it has to be possible to autoclave the buffer tank by steam sterilization and therefore a stainless steel tank is proposed, unless corrosion forces to use an alternative. If sterility is to be maintained, the liquid input and output lines have to be sterilizable stainless steel tubes with sterility filtration devices and steam sterilization of the lines installed. Filtration devices should be stainless steal 316 cartridge housings such as for example Millipore Acerliner or Millipore Optiseal, depending on volume, with exchangeable and sterilizable in place cartridges.

Gas lines for recovery of the gas produced by the first compartment will have similar characteristics as the liquid lines. Therefore they will have to be stainless steal sterilizable in place and with sterility filtration devices to prevent cross contamination through gas phase using also stainless steal sterilizable in place filter housings. Also gas lines for the maintenance of anaerobiosys in C-I bioreactor and filtration unit will have to be provided.

Liquid lines will have to incorporate pumps for liquid transfer and liquid flow measuring devices. Gas lines will have to incorporate gas flow meters, pressure meters and compressors. Gas and liquid lines will have to foresee appropriate sampling points for liquid and gas composition and valves. At present time it is foreseen the measurement of ammonium, and volatile fatty acid composition either in liquid and probably as well in the gas lines. Detailed specification of each item will be done in TN-75.6.

Installation of this reactor and its filtration unit, together with grinders, mixing tank and buffer tank is foreseen in lab 9C. Although in some of the presented UAB original drawings, lab 9C is depicted with a single door and all the walls are with a surrounding bench, a wider double door will be installed and all the benches will be removed except one to allow cleaning and manipulation of the bioreactor equipment. Figure 14 shows the foreseen distribution in room 9C. Gas and liquid gas outputs will have to be connected to the bioreactors in lab 9E and therefore connections through the wall will have to be mounted.

Safety considerations

Taking into account the nature of the fermentations taking place in compartment I it is necessary to take into account the safety and welfare of the operators of the laboratory. Although the risks for the operators health are considered low they nevertheless have to be taken into account. The risks can come from the interaction of the operator with the content of the bioreactor, which can be either the gas phase of the solid/liquid phase.

Interaction with the gas phase can take place either by an accidental leak or when opening the bioreactor or associated equipment for cleaning, sampling or maintenance reasons. The gasses produced in compartment I are derived from the biological fermentation activities of the bacteria and in general result in bad smells even at low concentrations and so are easily detected by a human. For this reason the laboratory for compartment I is enclosed in an independent room with and autonomous air recirculation and air conditionining system. This will assure the elimination of the volatile compounds generated.

Nevertheless, the generation of H_2S is of importance because its accumulation can result in the death of the operators. For this reason, the installation of a H_2S detection system interconnected to the general detection system for burning gas or H_2 will be included. This way any inadvertent accumulation of this gas, for example due to a gas leak of an improper operation of the gas recirculation procedure or of inadequate operator procedure, can be detected. H_2S levels will be maintained below 40 µg/m^3 (24 hours average) or 100 µg/m^3 (30 min. peaks).

From the point of view of the liquid and solid material in the bioreactor and due to the consortium characteristics of the strains used it can not be ruled out the possibility that pathogen anaerobic or falcultative bacteria survive in the tank (Clostridium, Salmonella,…). To completely discard this possibility the best option would be to follow a heat sterilization protocol before opening or dismounting the equipment. For this operation it is necessary to include as a requirement for the first compartment bioreactor, the compatibility with in situ steam sterilization. The same consideration can be done for the filtration system. This is already a requirement for the rest of the interconnection equipment of the Pilot Plant. Once autoclaved the content of the bioreactor can be discharged to the sink using a foreseen direct connection.

Before opening or dismounting the equipment for the cleaning operations or maintenance operations, venting of the interior of the equipment with compressed air will remove the accumulated gasses to the outside of the laboratory, using the pipes installed in the laboratory for this purpose. The introduction of oxygen with the air also has the effect of decreasing the viability of the strictly anaerobic bacteria but not of the facultative ones and for this a previous steam sterilization procedure is preferred.

Sampling operations of the bioreactor should be performed using small containers that can be connected directly to the bioreactor and filled using a valve system. Upon removing the sampling container from the bioreactor it should remain closed and isolated from the laboratory. This will allow to avoid the introduction of air in the samples, which is convenient for some analysis taking into account the anaerobic nature of the process. This will also protect the operator from contamination and bad smells.

To perform analysis on the content of the sampling containers it will nevertheless be necessary to open the container. To this purpose it is proposed to install a bench cabinet equipped with an air extraction system as the ones used to manipulate in the laboratory the toxic chemical compounds. This one will also be useful for preparing culture mediums containing volatile fatty acids.

Besides the C-I equipment, laboratory 9C should be equipped with:

- Inert gas line to establish anaerobiosis (He, Ar, …).
- In case of installation of gas chromatograph He and H_2 lines will be required. This can probably be shared with C-II.
- Air cooling/venting system.
- Steam line (optional).
- Cool liquid line for temperature control and gas condensation system.
- Demineralized water.
- Tap water
- Compressed air in case of using pneumatic devices.
- Data connection to computer room 22.

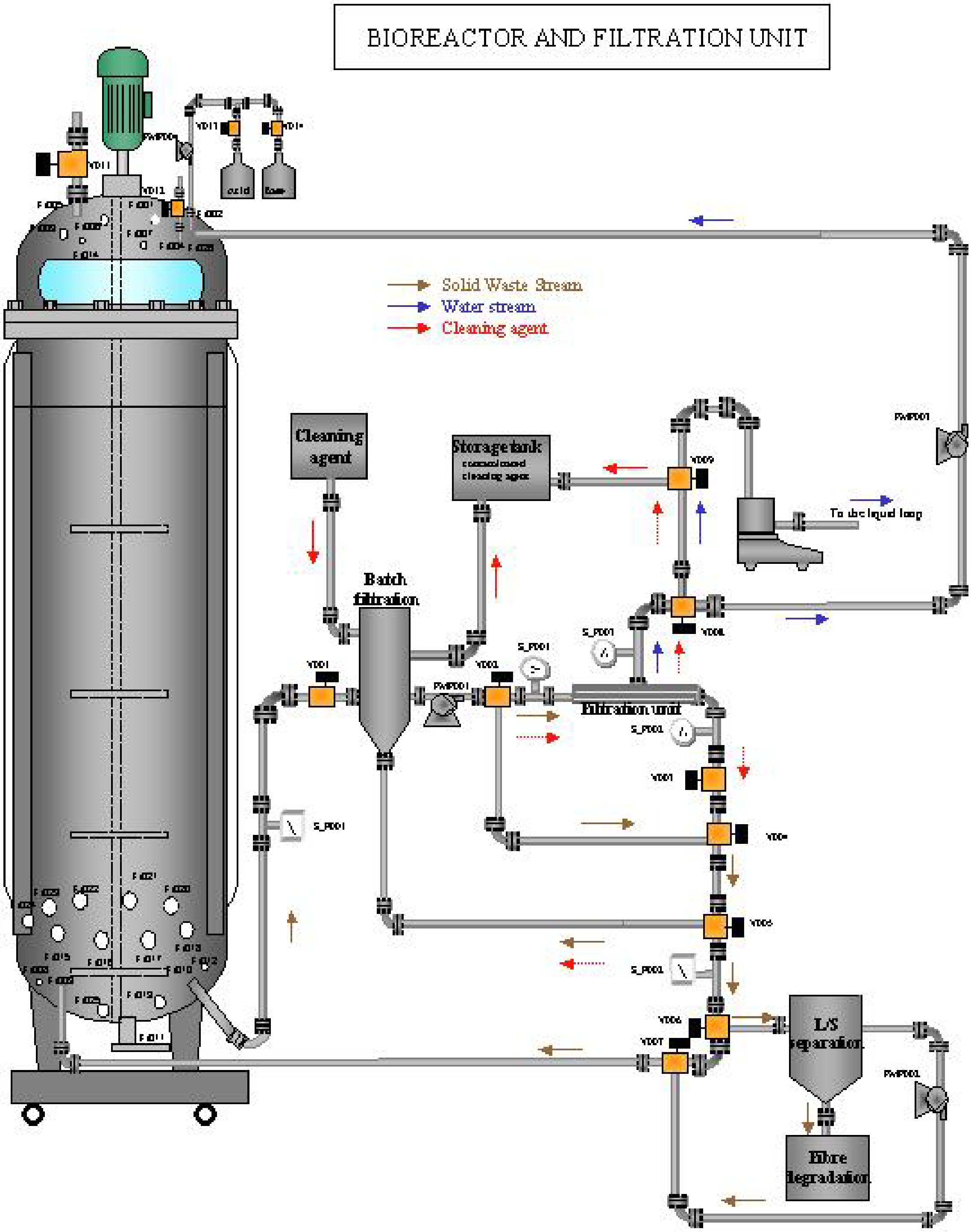

Figure 15: Schematic design of compartment I and its filtration unit.

4.3 Compartment II

Compartment II bioreactor will be located in lab 9E and interconnected to the gas and liquid lines from compartment C-I in lab 9C. Therefore a close location to this lab will be preferred. Interconnnection will be done directly to the stainless steel lines from lab 9C although appropriate valves and steam sterilizable connections will be provided to allow independent start up.

The bioreactor for this compartment is still under design at present time. However it will incorporate basically the same items of equipment as previously used in the MELISSA Pilot Plant but with new equipment. It will also incorporate new analyzers. A visual description of the equipment composing this compartment can be seen in figure 14. In the new version the main bioreactor will be different from the one seen in figure 14 but the gas and liquid interconnections and composing elements will be identical.

As in the case of compartment C-I, the bioreactor input gas and liquid pipes will be stainless steel, *in-situ* sterilizable lines with *in situ* sterilizable filtration units similar to the ones proposed for compartment C-I. Gas and liquid lines will have flow measuring devices incorporated, as well as the pumps and compressors and sampling points as shown in figure 16.

The piping system will adhere to the general considerations of sterility, measurements, or redundancy already described in for the general piping system.

The output of C-II bioreactor contains biomass and therefore the biomass separation device will have to be installed besides the bioreactor. This solid liquid separation system is at this moment under study. Whatever the type of system is finally selected it will have to be connected to the sterile lines of the output of C-II without compromising its operation. Therefore an stainless steel interconnection, in situ sterilizable, liquid line can be foreseen. The line will incorporate a pumping device that has to be sterilizable and its operation not amenable to be affected by the presence of the biomass in the pumped medium.

As already mentioned if the solid-liquid separation system (SLSS) finally selected is to be operated in a semicontinuous way it will be necessary to store the output of compartment C_II in a storage tank waiting for the separation system to be operated. This will also allow to operate it only during working hours when the plant operators are present and to avoid its operation during weekends or in the event of malfunction of the separation system until it is repaired. The SLSS system selected should be compatible with the sterile operation of compartment C-II and subsequent steps. Whatever the SLSS is selected for the output of C-II it is convenient that the liquid output is stored in a buffer tank. This will allow to account for temporal differences in flow as well as allow the discontinuous operation of the SLSS wile C-IVa is continuously operated.

The output gas line will be interconnected to compartments IVa and/or IVb as described in TN-62.4. This line will also have to assure sterility of compartment C-II bioreactor and therefore will have to be *in situ* sterilizable stainless steel line with filtration device as described before for the gas input to the compartment. The line will also have flow measurement devices, sampling points and remotely controlled valves to direct the gas flow to the required compartment.

Compartment C-II in room 9E will require the following services:

- Demineralized water.
- Tap water
- Inert gas line to establish anaerobiosis (He, Ar, …).
- In case of installation of gas chromatograph He and H_2 lines will be required. This can probably be shared with C-I.
- Air cooling/venting system
- Liquid cooling supply system.
- Steam line
- Data connection to computer room 22.
- Compressed air in case of using pneumatic devices.

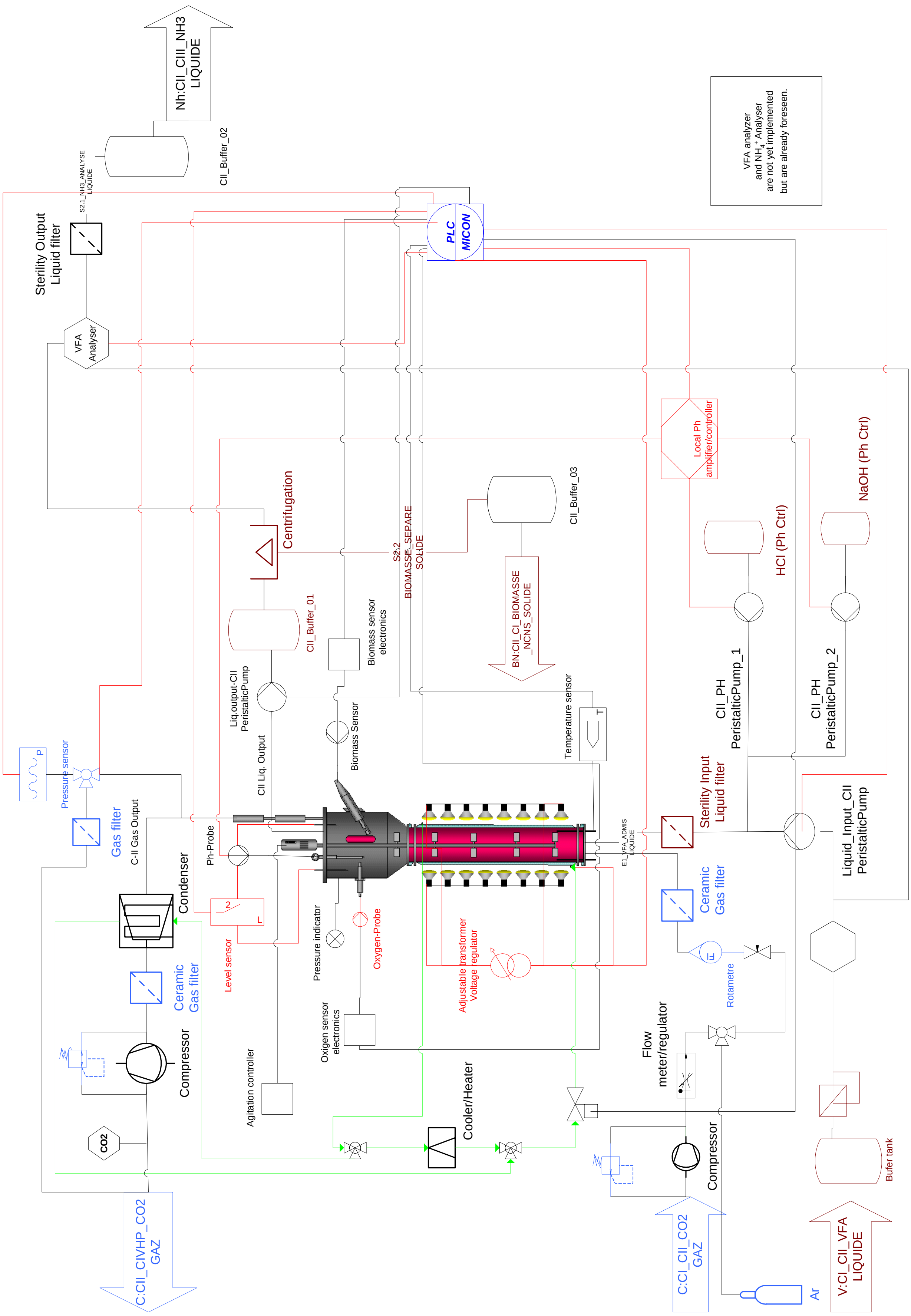

Figure 16: Configuration scheme of compartment C-II.

4.4 Compartment III

Compartment III bioreactor will be located in lab 9E and interconnected to the gas and liquid lines from compartment C-II in the same lab. A close location to this bioreactor will be preferred. All lines should be prepared for steam sterilization.

Input liquid phase interconnection will be done directly to the stainless steel lines from C-II buffer tank or SLSS, although appropriate valves and steam sterilizable connections will be provided to allow independent start up.

The output liquid line of this compartment contains very low levels of biomass. Nevertheless some biomass can leak the bioreactor. Also the bioreactor will be periodically submitted to a liquid backflow to improve hydrodynamic characteristics and release excess of biomass in the fixed bed. Therefore the liquid lines will have to foresee the periodic backflow and provide a container to collect the excess of biomass released from the fixed bed. It will have to be studied if a SLSS can be shared with other compartments such as compartments II or IVa in the same room but initially they will be considered different.

The biomass free liquid output of C-III has to be used in compartments IVa and IVb. Therefore it is proposed to incorporate an intermediate buffer tank to allow better flow control to the compartments. Alternatively each subsequent compartment can have its own buffer tank if it is required for example to mix and homogenise each input with liquid from other sources, such for example ammonium from urea or diluted with water recovered in the HPC C-IVb.

Besides being steam sterilizable gas phase interconnections will incorporate air filtering devices to assure gas sterilization. Also, connections have to allow either the use of a mixture of pure gasses, for operation in isolation, or the incorporation of gas from compartments VIa and /or IVb for oxygen enriched air supply. Minor amounts of carbon dioxide are also needed in C-III that, in case it is observed that the content of this gas in the output from compartments IVa and IVb is insufficient, addition of gas from compartments C-I/C-II line should be possible.

Liquid and gas lines will have to foresee flow measurement and sampling lines for composition analysis. Compartment III is foreseen to require Ammonia, Nitrite and

Nitrate liquid content analysis and at least oxygen and carbon dioxide gas content for process control and monitoring.

The present bioreactor in the Pilot Plant will probably be upgraded. Nevertheless the present configuration of the available equipment, (see figure 17 for a graphical overview), can be used as a reference to evaluate the equipment that will be part of this compartment in the Pilot Plant. A more detailed engineering description of this compartment will be provided in TN-75.6.

Compartment C-III in laboratory 9E will require the following services:

- Demineralized water.
- Tap water
- Gas lines for independent operation O_2, CO_2, N_2.
- Compressed air as base for mixing with other gasses for independent operation and also in case of using pneumatic devices
- Liquid cooling line for output gas lines condensation.
- Steam line
- Data connection to computer room 22.

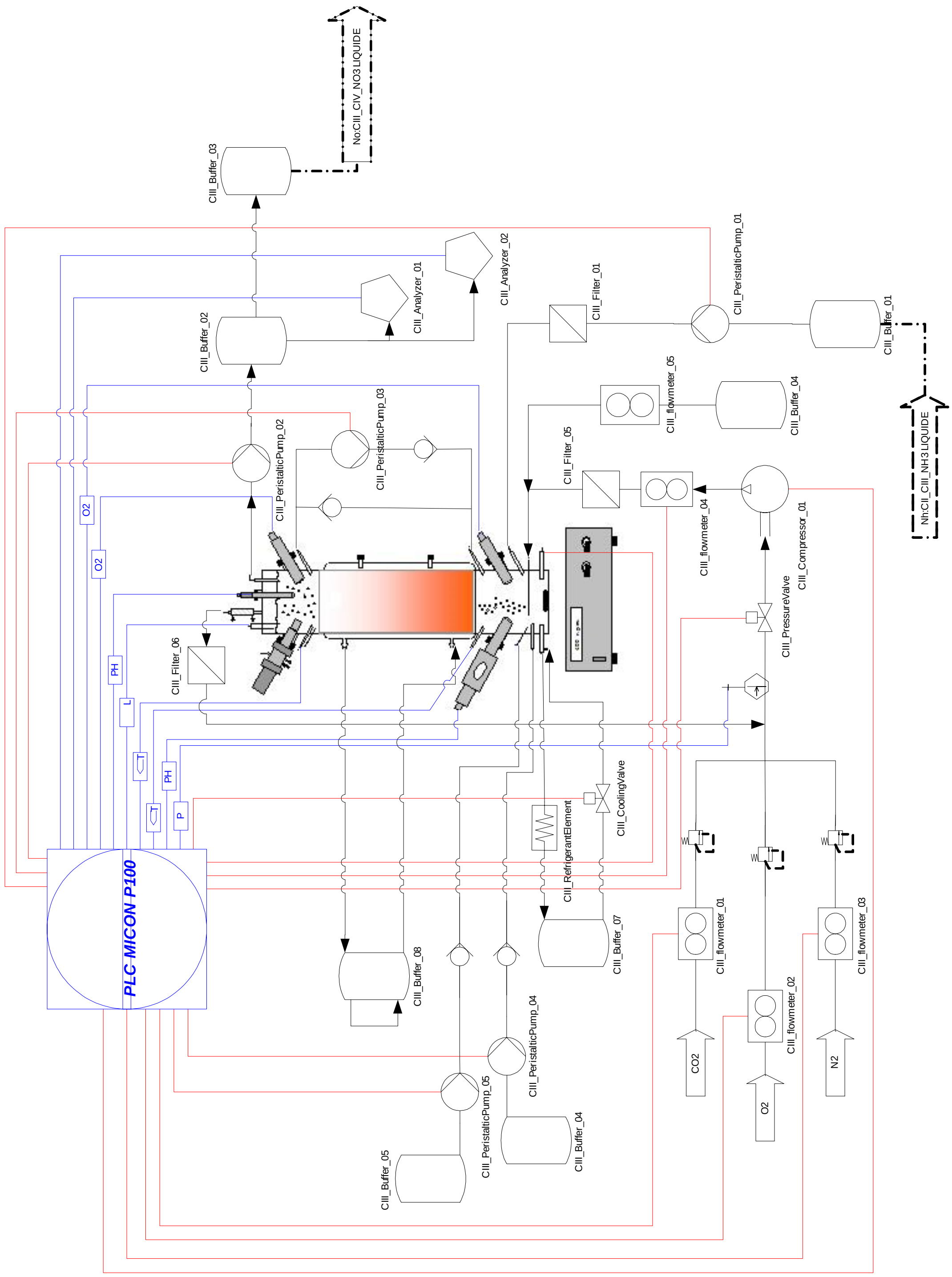

Figure 17: Schematic overview of compartment III

4.5 Compartment IVa

Compartment IVa bioreactor will be located in lab 9E and interconnected to the gas and liquid lines from compartment C-III in the same lab. A close location to this bioreactor will be preferred. All lines should be prepared for steam sterilization.

The bioreactor foreseen to be used is the one at present time in the pilot plant. A graphical overview of this compartment and the equipment involved can be seen in figure 18.

The input liquid lines of this compartment will be prepared for steam sterilization. An input buffer tank would be convenient allowing for mixing and homogeneization with liquids from various sources and allow for a more stable flow control. The liquid from the buffer tank will be filter sterilized and introduced in the bioreactor. Pumps, sampling points and flow measurement devices have to be foreseen in this line.

The output of the bioreactor will contain biomass and therefore a SLSS system will have to be foreseen as well as a pumping system not affected by the biomass presence. The extracted biomass will also have to be stored appropriately. The liquid output of C-IVa can be sent to compartment IVb in case it contains nitrite. Otherwise it can be stored as a source of clean water for other compartments. Nevertheless it may contain more salts than the water recovered from the higher plants evapotranspiration and therefore have other uses. Sampling points for the composition analysis will have to be foreseen.

Input and output gas lines will be steam sterilizable and allow for filtering of gas to assure sterility. Input gas can be either a gas mixture from artificial source for start-up or the gas coming from compartments C-I/C-II, C.III and C-V mixed according to requirements. Initially the interconnection is foreseen mainly with the animal compartment but mixing it with the gas from other MELISSA sources will probably have to be studied and gas lines foreseen. Output gas lines can be interconnected with compartments C-V and C-III for an air supply enriched in oxygen.

Compartment C-IVa in laboratory 9C will require the following services:

- Demineralized water.
- Tap water

- Gas lines for independent operation O_2, CO_2, N_2.
- Compressed air as base for mixing with other gasses for independent operation and also in case of using pneumatic devices
- Liquid cooling line for temperature control and output gas lines condensation.
- Air cooling for lamp heat elimination.
- Steam line
- Data connection to computer room 22.

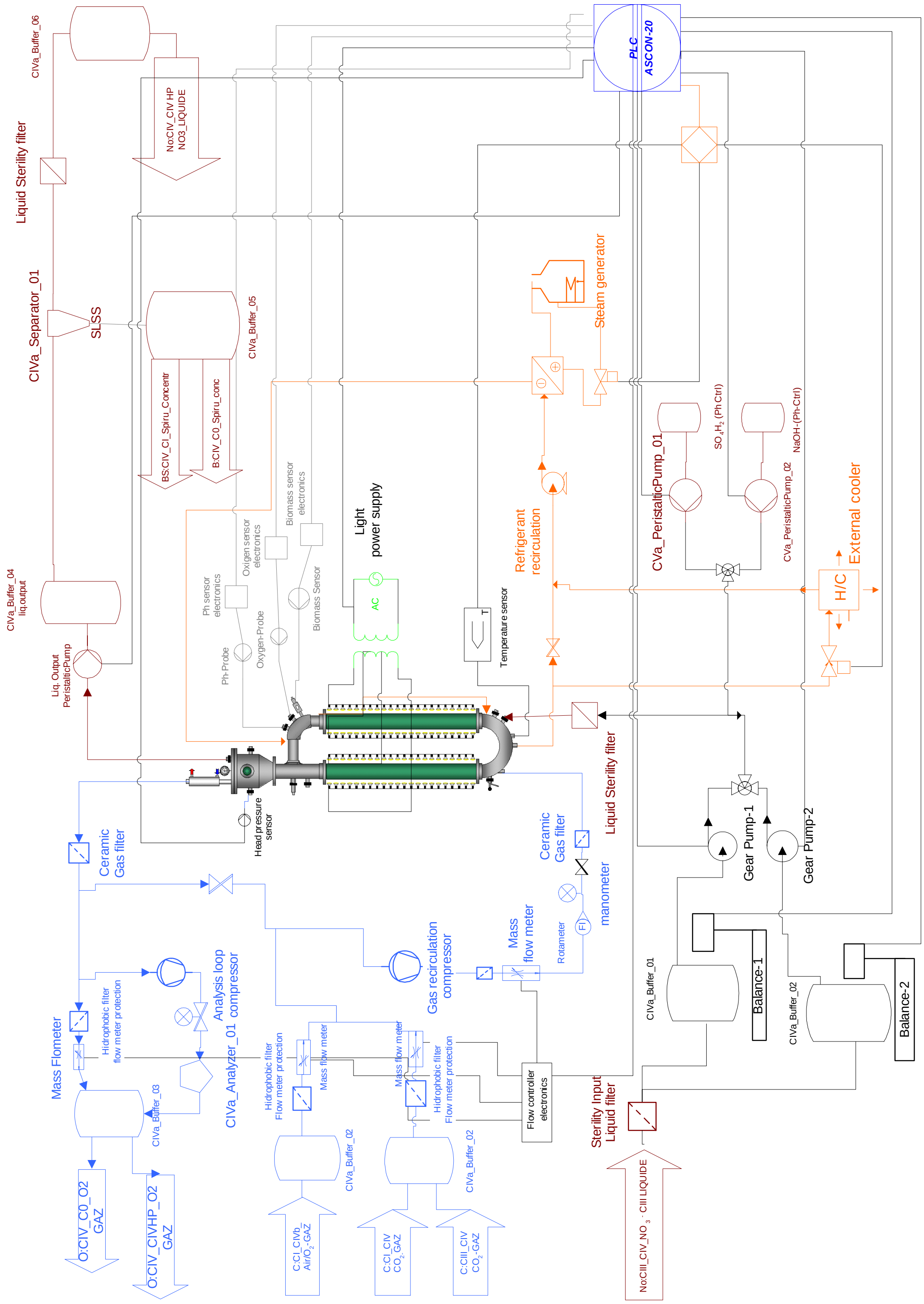

Figure 18: Schematic view of compartment IVa.

4.6 Compartment IVb

The higher plant compartment C-IVb will be installed in room 9D. Design of the higher plant chambers has not finished at this time. Nevertheless as a starting point the design discussed in a recent stage of one member of our group in Guelph can be considered (see figure 19 as one possible design), until Univ. of Guelph provides a final design. This together with the higher plant chamber preliminary requirements proposed in TN 65.5 should allow an initial evaluation of requirements of the compartment. Their dimensions are still uncertain but we will consider them of being about 0.8 m with and between 5/6 metres long. In principle 3 chambers of this type will be considered. If 4 chambers of the preliminary design are used it could be necessary to move the door interconnecting rooms 9D and 21.

Sterile operation is in principle not a requirement. However a minimum of safety, for example air filtration, might be desirable in order to avoid contamination by fungi spores or air travelling diseases or to avoid cross contamination among chambers in case one is affected.

Higher plant chambers will have to be operated initially in isolation prior to its interconnected operation. Therefore they will require appropriated gas supply and artificial liquid medium input and output lines. Input gasses can be a mixture of CO_2 and common air with provision of nitrogen if it is desired to dilute the air or chamber gas concentrations. An oxygen supply will allow to perform tests with increased oxygen concentrations o prepare artificial air together with the other gas lines.

Once the chambers are in operation interconnected with the rest of the compartments, mixing with the liquid supply from other sources might be necessary. For example with the nitrogen source from the urea tank or with the liquid output of compartment IVa. If the chambers have an external recirculation tank, were for example pH is adjusted, this tank might be used as a buffer tank and to mix the liquid sources from other compartments or from urea. Therefore a separated independent buffer tank for the chambers might not be necessary.

The higher plant chambers illumination will generate an increased heat generation in the room that has been taken into account in the design of the air recirculation system.

Maintenance of the internal chamber temperature and condensation of evapotranspiration water will require an internal refrigeration system. The operation of this is still not decided. If necessary the lab will be provided with a 4 ºC chilled water supply that might be used by the refrigeration system,.

Inoculation of the chambers will require also to foresee a nursery area for plant breeding which if small will be included besides the chambers. Otherwise it will be installed in another laboratory.

According to this preliminary overview compartment C-IVb in laboratory 9D will require the following services:

- Demineralized water.
- Tap water
- Gas lines for independent operation O_2, CO_2, N_2.
- Compressed air as base for mixing with other gasses for independent operation and also in case of using pneumatic devices.
- Air cooling for lamps heat elimination and temperature control.
- Liquid cooling line for temperature control and maybe for evapotraspiration condensation depending on chamber design.
- Data connection to computer room 22.

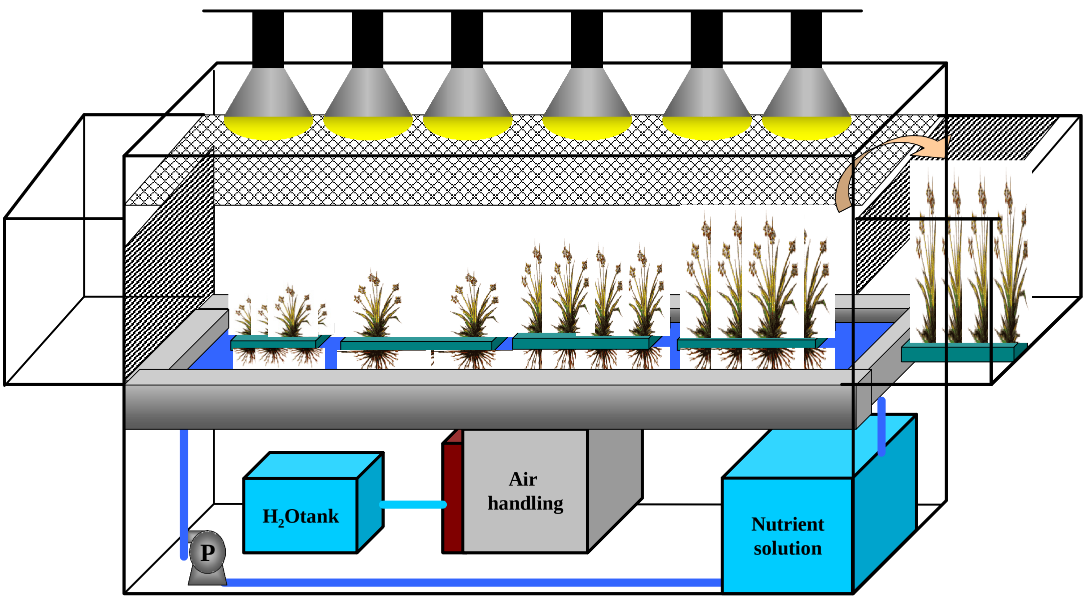

Figure 19: schematic view of one possible design for the HP chamber.

4.7 Animal compartment

The animal compartment will be installed in room 9B. Design of this compartment has not yet been done but will be analyzed in TN 75.5. At present time it can be foreseen that it will consist in a sealed container allowing to feed the animals and recover their wastes. To allow to perform the interconnection gas tests with compartments IVa and IVb it will have to be gas tight. When operated independently, for example for animal breathing tests, gas will have to be supplied from external sources and properly mixed. Therefore gas connections including compressed air, O_2, CO_2 and N_2 might be in principle proposed for installation.

No liquid connection is foreseen at present time with other compartments although water consumption from the MELISSA sources might be possible without major problems.

Cleaning, mounting and dismounting of the cages can be foreseen as an activity to be done in this room and therefore a bench and a sink will be needed.

According to this short preliminary overview of the animal compartment (C-V), the following services will be required in laboratory 9B:

- Demineralized water.
- Tap water
- Gas lines for independent operation O_2, CO_2, N_2.
- Compressed air as base for mixing with other gasses for independent operation and also in case of using pneumatic devices.
- Liquid cooling for humidity of breath air condensation.
- Data connection to computer room 22.

4.8 Space Toilet

The operation of compartment C-I will require to collect samples of wastes from human voluntaries. In principle faeces and urine will be collected separately.

Due to the special characteristics of the sampling recovery, an automatic system to collect the samples is preferred. Automatic systems such as the one used in the Japanese Institute of Environmental Sciences can be used. Those systems collect the faeces and either dry or freeze the samples depending on the design. In any case human intervention is minimized. This automatic faeces collection system will be installed in room 9A. As the model has not been selected it is difficult to foresee its requirements. The surface to occupy might be of 1 or 2 m^2 and it will probably require a tap water connection. If it dries the samples a heavy power consumption can be expected. If it freeze dries the samples, as it has been proposed in other MELISSA meetings, it will be provided with a vacuum pump and a means of freezing the sample. In this case it will require a liquid nitrogen tank that at this moment it is foreseen to be periodically filled from another tank. UAB has a central liquid nitrogen supply and its periodic filling is not expected to be a problem.

Urine samples will also have to be collected and treated separately. Its collection appears much more simple than the faeces collection and does not necessarily have to be done in the lab but can also be supplied in bottles. In any case the collected urine will have to be stored appropriately and probably treated to decompose its main components, such as urine and uric acid, into simpler compounds such as ammonium or CO_2. This step has not been studied yet but it is foreseen to be simple and performed in a common bioreactor. Alternatively some MELISSA compartments might accept directly the supply of urine and only the storage will be necessary. This equipment can also be located in this room. Its requirements in terms of power and data connection will probably be identical to the one small bench bioreactor.

For cleaning and service of the equipment in this room a bench and a sink will also be necessary.

According to this short preliminary overview of the space toilet, the following services will be required in room 9A:

- Demineralized water.

- Tap water
- Compressed air in case of using pneumatic devices.
- Liquid cooling for humidity condensation of output air in urea tank.
- Data connection to computer room 22.

5 CONCLUSION

In the previous chapters the general configuration and layout of the upgraded MELISSA Pilot Plant has been presented. I shows the equipment and services included in the UAB architecture office design and proposes modifications to that design to better fulfil the expected tasks of the pilot plant. It also proposes an initial configuration for the equipment location and the interconnection lines to install.

The document does not intend to be the definitive Pilot Plant layout design but to show the actual status and serve as the discussion reference document until the new lab is finally installed.

5.1 REFERENCES

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