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Pilot Plant Integration Strategy

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1 INTRODUCTION

The MELISSA project was conceived as a tool for the study and development of Advanced Biological Life Support Systems. Since the beginning it was realized the necessity of the implementation of the successful research results, obtained by the different MELISSA partners, in a common location for evaluation. This implementation will generate inestimable results and also 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.

One preliminary key milestone towards the final demonstration, will be the set up of an integration loop. This implementation will allow to close the gas loop using experimental animals as a crew. It also has to generate 20% of the dry weight of a human diet and recycle all the biomass generated in the loop and the faeces generated by one man per day. Urine will be recycled as necessary. The fact that human faeces are used instead of the faeces of the experimental animals impedes complete closure. This restriction is imposed by the previously demonstrated fact, that the bacteria strains necessary for the proper liquefaction of human faeces, are different from the ones able to properly liquefy the animal's faeces. As the final goal is human sustainability, the use of human faeces is preferred from the beginning.

To reach such integration milestone a step by step approach will be followed. It will include the incorporation of new equipment as well as the removal of the equipment today present in the Pilot Plant in Barcelona to a nearby expanded laboratory with capacity to incorporate all the equipments.

This report describes the integration strategy that will be followed to reach the above mentioned goal and will take into account the sequence of steps to follow as well as the requirements for each step and any possible foreseen difficulty.

For the description of the integration strategy, major and minor steps have been identified. In the following each of those steps will be described. A complete Gantt diagram is provided at the end and partial diagrams are provided between the text for an easier overview of the time frame.

2 CONNECTION OF COMPARTMENTS III AND IVA AT PILOT SCALE.

2.1 Liquid interconnection of compartments III and IVa

The present status of the MELISSA Pilot Plant operation is geared towards the accomplishment of this milestone. Liquid interconnection of compartments III and IVa at pilot scale was already performed and the results were summarized at TN 47.6. It is considered the starting point of the integration.

After the successful interconnection of those compartments the next step is to advance in the set up of an integrated control system for those compartments. Indeed, compartment IVa control system operation has already been tested in a number of occasions. Next step is the successful incorporation of the control system for compartment III.

2.2 Installation of ammonia and nitrite on-line analysers

Successful operation of the control system of compartment III will depend on instant availability of reliable data about its operation. To this purpose it is necessary to incorporate proper on-line analysers for ammonia and nitrate, as the nitrogen source is in this compartment the energy source. Therefore it is a key variable for controlling the compartment. This step has already been performed and results are summarized in TN 52.2.

2.3 Data collection for nitrite estimator development.

Nitrite is an intermediate product between *Nitrosomonas* and *Nitrobacter* species in this compartment. It is not desired to be obtained in the water for human consumption. Its appearance reflects a non proper operation of compartment III, and it is not readily consumed in the following compartments if another nitrogen source is available. Therefore determination and control of its generation is necessary.

Due to the difficulty to found a proper on-line analyser for nitrite it was decided to start by performing a software estimation of its generation. To this purpose ADERSA has been developing a subroutine that allows to estimate its production. It will also allow the control system to take appropriate actions even before its content increases significantly. In order to fine tune this software to the Pilot Plant process it is necessary

to collect experimental data under defined test conditions. It has been agreed that this procedure will take place until June 2003 (TN 52.4). This will allow ADERSA to supply the control software at the end of July 2003 with the predictor included.

Nevertheless the possibility to incorporate a nitrite measurement system is still being considered a desirable option and at present time the search for an appropriate method of measuring it continues. The results of this activity will be provided in TN 66.1.

2.4 Integration of nitrite predictor and control software for compartment III.

In parallel with the development of the nitrite predictor subroutine, ADERSA is also developing the first version of the corresponding control software that will allow to properly control compartment III. Once this software and the nitrite predictor subroutine will be finished, they will be supplied to the Pilot Plant in a C source code in order to be incorporated in the Melissa Control System MCS. The control software is expected to be received at July 2003.

Once the software is received it will be incorporated in the MCS software framework that allows to receive the values of the variables and send the corresponding commands. The resulting integrated software will be tested following a previously accorded protocol with ADERSA. The results obtained will allow to evaluate the performance of the nitrite predictor and the control algorithms. The description of the software integration in MCS, evaluation of the nitrite predictor and of the control of compartment III will be described in technical notes 52.4, 65.3 and 65.4.

After upgrade of the MCS by NTE the last tests will be repeated as a verification of its correct operation (see below).

2.5 Gas and liquid phase interconnection of compartments III and IVa.

After the successful implementation and test of the liquid interconnections of compartments III and IV it will be possible to attempt the liquid and gas interconnection of these compartments. Indeed as compartment III requires oxygen for its operation it would be interesting to evaluate the supply of oxygen from compartment IVa. It must be mentioned that the loop will not be completely closed because of the CO_2 requirement of both compartments. Nevertheless, it will allow to initiate the interconnection of gas

phases among different compartments which presents different difficulties than those found in liquid loops because of the particular nature of gases, wich require special attention for pressures, flows leaks, etc... This will be a precursor step in gas interconnection until the other compartments are incorporated.

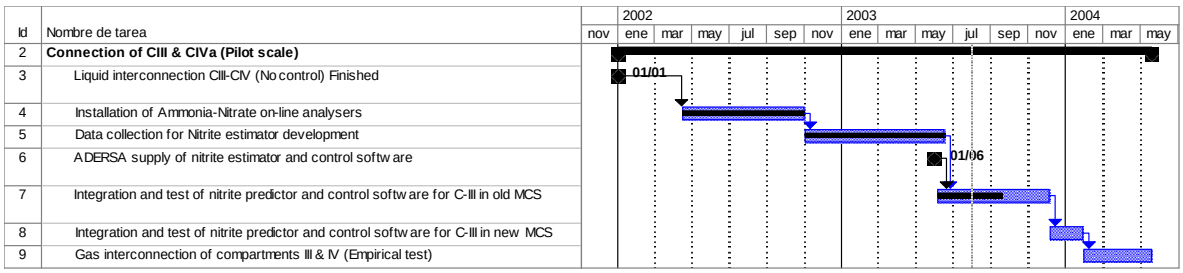


Figure 1: Planned schedule for connection of compartments III and IVa at pilot scale

3 UPGRADE OF PILOT PLANT CONTROL SYSTEM

The control system presently used in the Pilot Plant was installed in 1990. After the first installation it has received only minor upgrades. As a result it is a completely outdated control system that requires an urgent upgrade to XXI century standards.

Due to this fact NTE (Nuevas Tecnologias Espaciales), with the advice of ADERSA and UAB, was committed to the study and installation of an upgraded version of the control software. It is foreseen that after a period of analysis and after agreement with ESA and the advisors, the new control system will be purchased and installed. The hardware and software to control compartments III and IVa is foreseen to be installed during the period between February-march 2003.

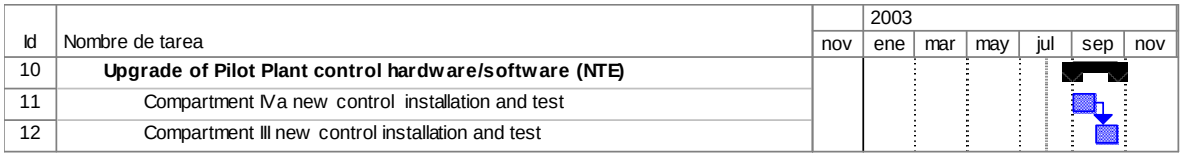


Figure 2: Planned schedule for upgrade of Pilot Plant control hardware.

The upgrade will be divided in two steps. In the first one the hardware and software for compartment IVa will be upgraded. In the second one the same procedure will be followed for compartment III.

Once the upgraded system will be installed, it is foreseen to repeat the control tests of compartments III and IVa interconnected as a verification of proper operation. Upgrade will be summarized in TN 47.4.

4 CONNECTION OF COMPARTMENTS II, III AND IVA

After successful implementation of the interconnection of compartments III and IVa at pilot scale, the next logical step will be to incorporate compartment II to the connection as was previously done in the bench loop tests. To this purpose the following steps are necessary:

4.1 Upgrade of compartment II bioreactor

4.1.1 Design of upgraded compartment II bioreactor

At present time the bioreactor for compartment II is a 7 litres illuminated CSTR. In order to advance towards the integration loop, it will be necessary to upgrade this bioreactor. The new calculated volume has been of the order of 50 litres illuminated volume. Nevertheless the volume necessary is heavily dependent on the performance of the bioreactor which in turn depends on the efficacy of the light energy supply and therefore on final design. Therefore the given volume is only an approximation and will depend on the final design agreed with the manufacturer. The specifications for the new reactor will be described in TN 62.2.

4.1.2 Construction, set-up and characterization of compartment II bioreactor

Construction time for the bioreactor will depend on the manufacturer, but it is considered that at least will take 6 months.

Once built, the bioreactor will be installed, tested and characterized. Installation will include the set up of compartment II control system for the upgraded MCS. The installation details and the results of its test and characterization will be described in TN 65.1 and 65.2.

4.1.3 Design and installation of VFA measurement system.

Proper operation of compartment II requires to continuously assure its efficiency by determination of the consumption of the volatile fatty acids. To this purpose it will be necessary to know the VFA's entering the compartment. This will allow to adjust the optimum operational conditions. It will also be necessary to determine the consumption of those VFA's by analysis of the output flows composition. It was therefore decided to install an automatic VFA measurement system for this purpose. Among the desired

capacities it is preferred to be able to multiplex different sample sources in order to measure different lines and if possible different bioreactors (e.g. C-I and C-II). The results of the study for the requirement of those analysers will be performed in TN 62.1.

Once the technology will be decided the analysers will be purchased and installed in the Pilot Plant. Purchase and installation is foreseen to take place during the second half of 2003. Besides the initial operational tests they will be further tested during the set up and characterization of the new bioreactor of compartment II.

4.2 Interconnection of compartment II with compartments III and IVa.

Once the upgraded bioreactor for compartment II will be operational, the next step will be its interconnection with compartments III and IVa. To this purpose the following is required:

- It is desirable that the control system for compartment II has been previously installed and tested in the isolated bioreactor (about 1 month).
- It will be required to be able to automatically separate the biomass from the liquid effluent. At this moment the methodology is under study but it is not decided if UAB will have to built one system based on the results of the study or will be supplied with one system for testing.
- The liquid influent to compartment II will have to be a synthetic medium able to supply all the compounds needed by the 3 compartments. Elaboration of this medium will be based on the results of the previous tests performed on the bench loop and all available results of the ICP analysis performed on the liquid output medium of the first compartment.

With all the previous results available the following milestones will be performed:

4.2.1 Liquid phase interconnection of compartments II, III and IVa

Once the bioreactor, the medium composition and the biomass separation system are ready compartments II, III and IV will be connected in their liquid phase. This interconnection will be maintained as long as possible but the target will be of around 3 months of uninterrupted interconnection and a possible total duration of 5 months.

4.2.2 Liquid and gas phase interconnection of compartments II, III and IVa

According to present calculations, compartment II will require a carbon dioxide supply. As it is interesting to polish the carbon dioxide generated in compartment I from minor contaminants before introducing it into compartment IV it was proposed to pass the gas generated in compartment I through compartment II. The carbon dioxide not completely used in compartment II can be used in compartment IVa. Therefore as a new step towards the integration of the loop, the interconnection of liquid and gas phases at the same time can be assayed.

For those tests, carbon dioxide will be introduced into compartment II and the output gas will be transferred to compartments III and IVa. As CO_2 has an important paper in pH maintenance, the tests will allow to verify its impact. An appropriate length for this test would be around one month of uninterrupted connection which will probably require a total time of about 4 months for proper installation of interconnecting tubing together with pressure and flow regulation devices and their coordination.

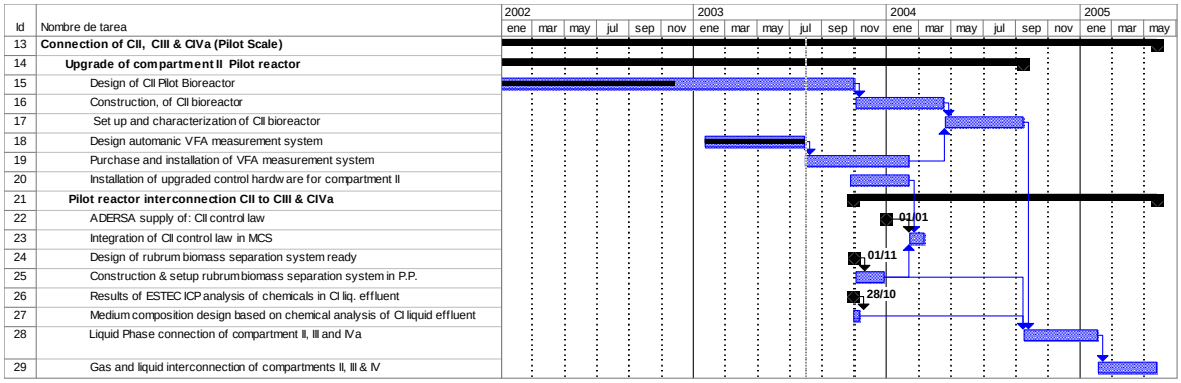


Figure 3: Planned schedule for interconnection of compartments II, III and IVa at pilot scale.

5 INCORPORATION OF HIGHER PLANT COMPARTMENT (IVB).

The MELISSA integration test will allow to produce the oxygen necessary to maintain alive a certain amount of laboratory animals and consume the produced CO_2.

However the final objective of the project is to provide food for human sustainability. Therefore to obtain a balanced human diet, higher plants are necessary. In preparation of this event a higher plant compartment will be installed, already in this integration test. Therefore new higher plant chambers have to be designed, built and installed according to the Pilot Plant objectives.

5.1 Design requirements specification for the higher plant chambers

The first step towards the integration of new higher plant chambers in the MELISSA Pilot Plant will be the development of new units adapted for its dedicated use in the pilot plant. To this purpose an agreement of collaboration was reached with university of Guelph. According to it UAB and UoG will provide a preliminary requirements document with specification of the desired performance of the Pilot Plant chambers (TN 65.5). Based on it UoG and UAB will provide a more detailed design document based on which the higher plant chambers can be built (TN 75.3). In order to improve the communication between the two involved groups, one UAB master student did spend a training period of 2 months at UoG. At the end of 2003 it is expected to receive one member of UoG at UAB to continue with the specifications document of the HPC. This will allow a better harmonization of the requirements. At the end of the process, around April 2004, the requirements for a higher plant chamber for the Pilot Plant will be ready.

5.2 Design and construction of higher plant chambers.

Once the requirements documents will be issued, UoG will provide a final design document. The future plant chambers will be built based on this document. During 2004-2005 UoG will attempt to obtain the required additional funding for construction of the higher plant chambers and build them. At present time the delivery is not expected until removal of the Pilot Plant to the new location is completed which would not take place before end 2005.

5.3 Set up of higher plant chambers in Pilot Plant

Following the construction and delivery of the new higher plant chambers they will be installed at UAB Pilot Plant. The installation time will be dependent on the

delivery status of the hardware, but the installation would take place in about 2 months. After installation the first test will take place without biomass, for about 1 month.

5.4 Higher plant chambers control system installation and test

Once the higher plant chambers will be installed the next step, will be to install an appropriate control subroutine, for its operation in coordination with the other MELISSA compartments. It is therefore expected to receive the routines, ready to be inserted into MCS, before the tests.

For this tests the chambers will be started, growing real plants and using the agreed staggered plantation method. At the same time, the proper operation of the local control system can be verified. The procedure for the control tests will be agreed with ADERSA as usual and therefore the length of the tests is not yet determined. However at least a 2 months test can be expected.

Once the tests will be finished the chambers will be ready for interconnection with the other compartments.

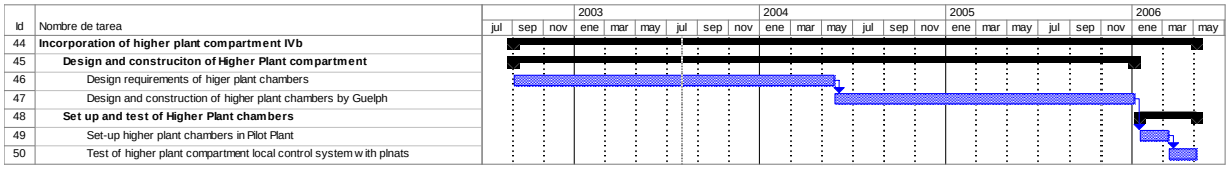


Figure 4: Planned schedule for development and installation of higher plant chambers for the Pilot Plant.

6 RELOCATION OF PILOT PLANT LABORATORY TO A NEW LABORATORY.

Since its previous re-installation from ESTEC, the MELISSA Pilot Plant has been operating compartments II, III and IVa at bench and pilot scale. In 1998 the Pilot reactor for compartment IVa was upgraded to a 77 litres unit. In the near future incorporation of compartments I, IVb and V (crew) is necessary. To this purpose an expansion of the laboratory is required. Taking advantage of the relocation of the complete Chemical Engineering Department, a new expanded location has been assigned for the MELISSA Pilot Plant. The new laboratory is expected to be available on January 2006. Therefore relocation of the hardware present today in the Pilot Plant has to be foreseen around that period.

6.1 Relocation of Pilot reactors

As a first step for the relocation of the hardware it is proposed to move individual units in the reverse order in which they were installed (C-IV, C-II, C-III). The bioreactors will be dismounted, moved and mounted again in the new location. Taking advantage of this opportunity, wiring and piping will be revised and upgraded in order to improve safety and reliability.

Perhaps a special case would be the *Nitrosomonas-Nitrobacter* fixed bed. Due to the difficulty in its start up it is proposed not to move it until other bioreactors have been previously moved and tested in the new location and any possible inconvenience can be foreseen and its fast relocation guaranteed. The bioreactor can be partially dismounted and the fixed bed part maintained for a few hours with oxygenation by an external pump meanwhile the rest of the compartment III equipment is reinstalled.

Id	Nombre de tarea
38	**UAB Pilot Plant relocation to new lab**
39	New Pilot Plant laboratory ready
40	Relocation of CIV
41	Relocation of CII
42	Relocation of CIII
43	Relocation of bench reactors and accessory equipment

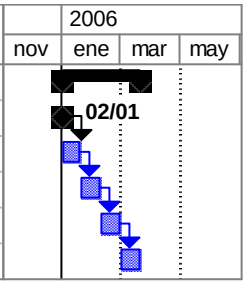


Figure 5: Planned schedule for development the reallocation of the Pilot Plant. To the new lab

At the moment there are 3 pilot bioreactors to move. It is calculated that at least two weeks will be necessary for relocation of each bioreactor. Therefore a total time of about two months can be calculated for this part of the removal. The removal sequence proposed is:

- Relocation of compartment IVa. (3 weeks)
- Relocation of compartment II. (3 weeks)
- Relocation of compartment III. (3 weeks)

6.2 Relocation of bench reactors and accessory equipment

Besides the Pilot reactors the Pilot Plant has also other equipment to relocate such as the top bench bioreactors and accessory equipment, small equipment, analyzers, centrifuges, etc… , nowadays most of them are not located in the same laboratory but are

distributed in different dependencies of the Department. It is considered that at least 3 more weeks will be necessary for relocation and installation of all this equipment.

7 INCORPORATION OF ANIMAL COMPARTMENT.

One of the objectives of the integration loop will be to maintain alive a group of rats using the oxygen generated in compartments IVa and IVb. At the same time those compartments will also consume the CO_2 generated in the loop for example by the animal compartment and compartment I. To this purpose a dedicated containment facility for the rats will have to be built.

7.1 Design of experimental rats containment facility.

In a first approach a special rats cage able to comfortably contain the experimental animals and sealed to allow to close the gas loop with the other compartments, will have to be designed. The design will have to take into account any applicable regulations for animal experimentation. A preliminary design is foreseen to be delivered in TN 75.5 (June-2004).

7.2 Construction and basic test of the animal cage prototype.

Once designed, the animal cage will have to be built and installed in the Pilot Plant. A proper manufacturer will be identified and the design discussed with it with the objective of obtaining a prototype. Once the prototype is built its preliminary installation and test will be done without using animals until its proper operation is assured. Once the proper operation will be verified it can be tested with one animal. All those preliminary tests can possibly be done in the actual facilities, previously to the removal.

7.3 Set up of animal cage in new lab

After the preliminary test of the prototype, any final modifications required will be implemented and after its test the cage will be ready for its interconnection to compartment IV. This will have to be done in the new facility. Therefore its final installation will not be done until the new lab is in operation. Final interconnection will require a proper software routine to be included in the MCS, as commented below.

7.4 Tests of animal atmosphere management system.

Proper integration of the animal compartment in the MELISSA loop will require a dedicated software able to manage the gas atmosphere and coordinate its use with the MELISSA generated O_2 and CO_2 consumed. The software will be incorporated into the MCS and will assure animal survivability. In this case it would be interesting that the software also allows the operation of this compartment disconnected from the others and using external resources. Following the approach previously taken with the other compartments, it is expected that ADERSA will deliver such a software in C code ready to be incorporated into the global control system. Once received the software will be included in MCS and tested. Once basic operation is verified the coordination with the control of other compartments will also be verified.

7.5 Interconnection of animal atmosphere (C-V) with compartment IVa

Once the previous step is finished, the animal compartment will be ready to be interconnected with the other compartments. Its gas phase can be interconnected either to compartment IVa or to compartment IVb, if both are in operation. The obvious difference on oxygen generation capacities can be taken into account by adjusting the number of rats used.

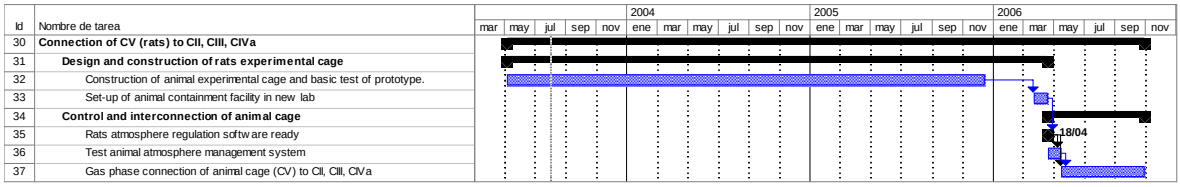


Figure 6: Planned schedule for development, set up and tests of the animal compartment

As a first attempt it is proposed to interconnect compartment V with C-IVa. The system will be slightly simpler and the number of rats that can suffer from an accident are kept to a minimum. On the other hand the difference on the assimilatory and respiratory quotients of compartment IVa and V may result in the gas loop not being completely closed for long periods of time. However oxygen consumption by compartment III has also to be taken into account. Probably an strategy can be found by which, for example, the oxygen is adjusted to what is consumed in compartments III and V meanwhile the CO_2 is adjusted as necessary. That is CO_2 is added from external sources if necessary or air removed if in excess with nitrogen addition. The study of the

necessity and implementation of one strategy like this will be in fact one of the purposes of the study.

This approach will allow to tune and test the system for gas flows and leaks as well as for the ability of the control system to maintain rats alive. A small complement of other external gases, such as N_2, O_2 or CO_2, can be studied to be incorporated by the control system if necessary or for emergency uses. So an emergency system for animals can be developed.

It is therefore proposed that on a first attempt C-V with one rat is interconnected to compartment IVa and the system studied. Once de reliability of the system is demonstrated, interconnection including compartment IVb and using a higher number of rats can be attempted (see below).

8 INCORPORATION OF COMPARTMENT I

This is one of the of the key compartments in the MELISSA loop as it is responsible for the liquefaction of the wastes. It has been developed in EPAS during the past years and an agreement was reached by which they will provide to the Pilot Plant a unit ready to operate. At present time a pre-pilot reactor is being developed and with the results obtained a Pilot reactor of about 100 litres will be built and tested. Once finished it will be delivered to the Pilot Plant (ETA, second half of 2004). In principle it is assumed that it will be installed in the new laboratory. However if at its arrival at UAB the new lab were not ready, it will be preliminary installed in the actual laboratory.

Besides the main tasks for this compartment already considered, other related topics are worth of attention (see 'other considerations and final comments'), such as gas contaminants, CH_4 and H_2S production, or biomass monitoring.

8.1 Installation of EPAS supplied CI hardware.

Once the EPAS developed hardware is delivered to the Pilot Plant it will be installed at the proper location. Gas, liquid and control interfaces will be connected to the corresponding Pilot Plant equipment. Installation time is expected to be around 4 weeks.

8.2 Start-up and preliminary operational tests of installed CI bioreactor.

Once installed the bioreactor will be tested for proper operation of its sensors and local control equipment. To verify its proper operation an anaerobic culture can be started. However for the initial tests it is not considered strictly necessary to perform initial tests using MELISSA strains and nutrients, due to the initial risk of loosing the culture. Nevertheless preparation of the complete MELISSA input waste will depend on the readiness of the human donation collection program (see below) and in the in situ produced higher plants and microbial biomass. In case that any of those is not yet ready an artificial medium or a mixture of the biomass at the time available can be used for the initial tests. Two months are assigned to this task. Once the proper operation is verified the culture following EPAS recipe should be started.

8.3 Tests of CI control software

Once the operation of the local control will be verified the next step, will be to test the ADERSA supplied control software. The received software will be incorporated into MCS and several tests, following ADERSA proposed protocol will be performed. As the protocol is not yet agreed is difficult to foresee its duration. However taking into account previous tests and the high residence time of the bioreactor a 4 months test is believed to be necessary if it is done using the complete MELISSA wastes. Besides, those tests could not start until higher plants and human wastes are continuously available. Therefore it is proposed to start the tests as soon as possible with artificial medium, or with a mixture of artificial and locally produced biomass. In this case a 2 months test is proposed.

9 INTERCONNECTION OF COMPARMENTS I, II, III, IVA AND V

Once compartment one will be ready for operation the next logical step will be to interconnect it to compartments II, III and IVa and V that will have been previously operated in interconnection.

9.1 Set-up a human donation collection program.

The main purpose of compartment I operation is to process the MELISSA generated wastes as well as the corresponding human faeces generated by one man in

one day. Therefore it will be necessary to establish a program to collect the human faeces.

To facilitate the procedure of human faeces collection it is foreseen to habilitate dedicated toilets to collect the faeces from voluntary people. It is desired that the process is as automatic as possible. Therefore it is foreseen to buy automated toilets for human faeces collection similar to the ones used at IES in Japan, unless a special design is done for ESA and the Pilot Plant.

Besides the hardware, human voluntaries will be necessary. Therefore it is expected to set up a program of voluntary people for the donations. It can be established among some students that agree to follow a specific diet. For its contribution, the voluntaries could be awarded a small amount of money. Establishment of the program should start one month before the donations are required, and after the automatic toilets are installed.

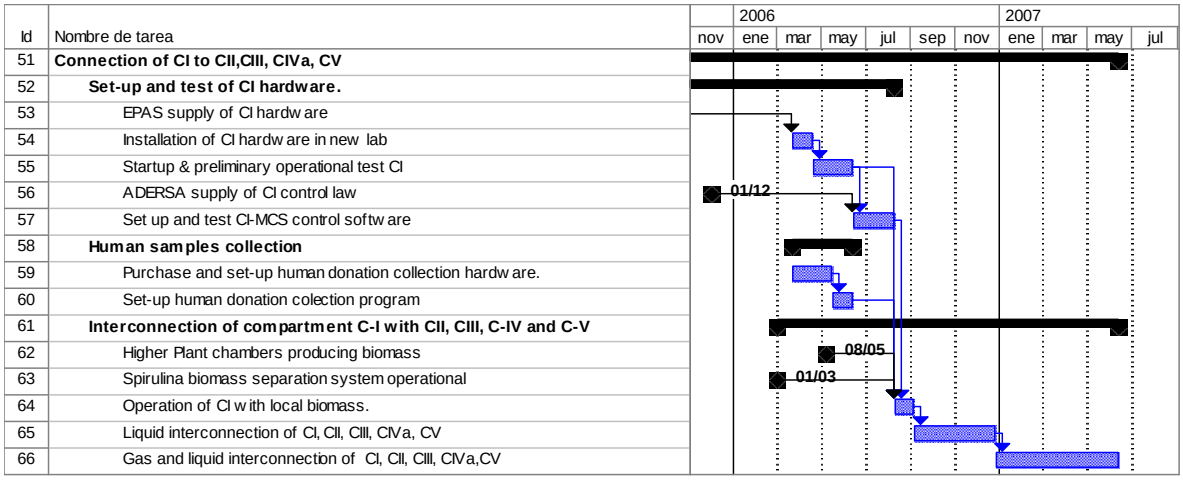


Figure 7: Planned schedule for development, set up and interconnection of compartment I.

9.2 Operation of compartment I using locally produced biomass (plants, microbial, human)

Provided that the higher plant chambers are in operation, compartment I should be fed with the agreed input mixture composition of higher plants, human faeces, *Spirulina* and *R.rubrum* biomass. With this mixture and following the culture conditions optimized by EPAS, this compartment should be started with a view of more long term operation. After start up some time will be required to stabilize the production and to verify the operation of the separation system. For this tests one month should be

assigned. Once its operation and the operation of the biomass separation system is verified its interconnection with the other compartments can be attempted.

9.3 Liquid interconnection of compartment I with compartments II, III and IVa.

Interconnection of compartments I, II, III and IVa will allow to verify in situ the compatibility and performance of the compartments at pilot scale, as it was previously tested at bench scale. It will also be possible to show the coordinated control of all these compartments using the software supplied by ADERSA and integrated in the MCS. If compartments I, II, III and IVa have previously been tested for operation under MCS control but not interconnected, the tests in interconnection should not present major problems. The most important point to verify will be the correct coordination among compartments and the proper operation of the interfaces (SLSS, …).

The time required for the tests is difficult to evaluate, but it should allow at least for one residence time for each compartment, but around five residence times is the optimum. Taking into account that the first compartment is the slowest one, the tests could be done in about 4 months.

9.4 Gas and liquid interconnection of compartments I, II, III, IVa and V.

Once the liquid interconnection has proved to be successful, gas interconnection among compartments should also be assayed. Therefore a gas and liquid interconnection test is proposed.

For this tests, compartments III and V can use the oxygen generated in compartment IVa, by that time already tested. Also compartment IVa can consume the carbon dioxide generated in compartment I, III and V. At present time, the optimization of the degradation capacity of compartment I is not finished. Therefore it is difficult to evaluate and assure that the CO_2 produced by compartments I and V will be completely consumed in compartments III and IVa. However the tests will allow to tune the system and a first had evaluation of its real capacities. The results will allow to better plan the incorporation of the higher plant compartment.

10 INTERCONNECTION OF COMPARMENT IVB WITH I, II, III AND IVA

Up on reaching this point in time, development and test of compartment IVb should have been finished and should be already providing biomass for compartment I. Therefore its interconnection with the rest of the compartments should be performed.

10.1 Liquid interconnection of compartment IVb with compartments II, III and IVa.

In a first step the liquid effluent from the previous compartments should have been analyzed and it should be possible to assure that the majority of the liquid nutrients necessary for the plants are present. It is foreseen the production of dry weight biomass corresponding to the 20% of the calculated consumption of one man in one day, and that only the non edible biomass will be recycled, together with the faeces of one man, and all the biomass produced in the loop. This will also depend on the efficiency of liquefaction of the first compartment. Therefore the availability of nutrients for all the compartments should be assessed.

Special case will be the nitrogen source, as humans reject most of the ingested nitrogen source trough the urine. To this purpose, it was foreseen to recover human urine, to decompose its urea (and uric acid) mainly in ammonium and carbon dioxide and to use this nitrogen source supply at the level of any compartment required. The exact method should be studied but in principle it is an easy step. Therefore a special bioreactor to degrade urine will be set up and its production used at any compartment as needed.

The balance of all nutrients among compartments will be one of the key points to study and optimize.

10.2 Gas and liquid interconnection of compartment IVb with compartments II, III, IVa and V.

The last step in this initial integration tests will be to incorporate together all the compartments, including higher plants, with compartment V only interconnected by its gas phase. After all the previous tests it should be possible a more precise evaluation of the gas flows necessary and an evaluation of the number of rats that the system can

sustain. Therefore the number of rats can be adapted to the O_2/CO_2 produced/consumed. If necessary the number of modules containing rats can be increased.

Interconnection of gas flows will be the initial key point in setting up the hardware (piping, sensors, compressors, etc…) to assure proper pressures and flows in a range of conditions.

At this point the control system will have to coordinate the gas and liquid flows of all the compartments to fulfil the objectives previously defined and therefore its coordination tasks will be the key point even if before it has been proven that it can control the compartments individually.

Once the interconnection and management of compartments will be obtained, a long term test should be targeted and should be no less than 3 months but preferably one year. With this step the integration test can be considered closed and the next goal to be considered should target the maximum percentage of closure possible using man as a crew.

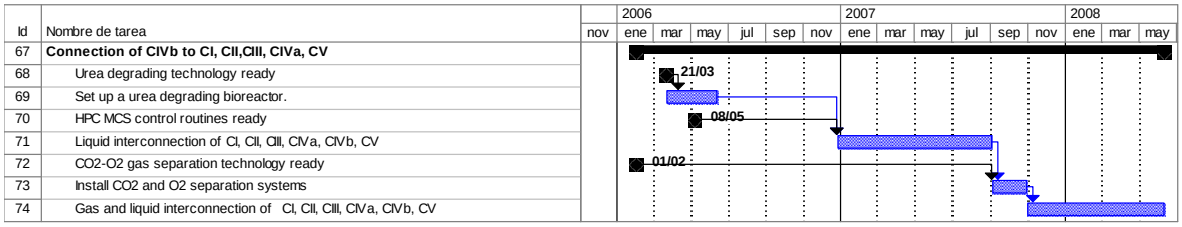


Figure 8: Planned schedule for interconnection of HPC (C-IVb) with compartments I,II, III, IVa, V.

11 OTHER CONSIDERATIONS AND FINAL COMENTS

In this technical note a first schedule of the remaining tasks to reach the integration tests have been done. To reach that goal several Items will have to be delivered to the Pilot Plant or studied in parallel. The following table summarizes the main required items for each Pilot Plant milestone.

Milestone	Required technology for integration	Other technology to consider
Connection III & CIV	• Control software for C_III and C-IV • Upgrade control hardware • On-line Ammonium Nitrate analysers	• Biomass measurement in C-III
Connection C-II, C-III & CIVa	• Upgrade C-II bioreactor • C-II control law • VFA on-line analysers • *R.rubrum* SLSS	•
Connection C-II, C-III, CIVa, C-V	• Dedicated animal cage • Animal atmosphere control software	•
Connection C-I C-II, C-III, CIVa, C-V,	• C-I bioreactor and SLSS • C-I control law • Fungi compartment • Human faeces automatic collector	• Biomass measurement • C-I gas contaminant removal • CH_4 production and alternatives • H_2S recycling • Urine treatment
Connection C-I C-II, C-III, CIVa, C-V, C-IVb	• Higher Plant chambers • Control law for HPC • Urea degradation equipment. • *Spirulina* SLSS • Integrated pH control strategy	• O_2, CO_2 gas separation technology • Ethylene control

Besides the previously described items other topics should be considered, as the ones described below.

11.1 Gas contaminants of CI

In the present MELISSA configuration compartment I is one of the compartments that will possibly generate contaminants in the gas phase. At this point two considerations might be done.

On one side some contaminants might be generated in very small amounts and they will become important as they accumulate in a long term operation of a closed environment. Those are generally responsible of the smells. Elimination of this kind of

contaminants can be done either by using physicochemical technology or a biotechnological approach such as a biological air filter (BAF). Their elimination will become more important as the time of closure of the loop is extended.

On the other side other compounds might be generated in the gas phase of compartment I which may have a more important impact on the mass balance of the major elements. For example if during a transient CH_4 is temporarily generated, it will be required to recover and recycle the carbon into CO_2. Their production depends on the existence and proliferation of methanogenic bacteria in the compartment as well as on the operational conditions. At the moment the compartment will not be operated axenically so the existence of methanogenic strains can not be ruled out. However their proliferation is very limited due to the culture conditions. Anyway their behaviour should be analyzed.

The production of H_2S is another compound which depend on the operational conditions of compartment I. For the MELISSA it would be preferable to generate sulphates. In our present configuration its production can not be discarded and therefore, if it is produced, a recycling method should be further studied. In the original concept, compartment II of the MELISSA loop a subcompartment of compartment II was foreseen to be used for this purpose (*Thiocapsa*). If H_2S is produced in compartment I, Evaluation of the amounts produced and the continuation of research of its recovery for the loop should also be pursued.

11.2 Urine treatment

It is foreseen to collect urine separately from human faeces. Urine can be used directly in several compartments. Anyway it is convenient to evaluate up to which level urine can be directly delivered to the MELISSA compartments or alternatively treated in a bioreactor in order to decompose it in more simples components. For example, into carbon dioxide and ammonium as main compounds. In the process, some salts will also be recovered. Those salts can be used in several compartments directly or separated and used as a source of chemicals useful for example for an integrated pH control scheme.

11.3 Integrated pH control strategy

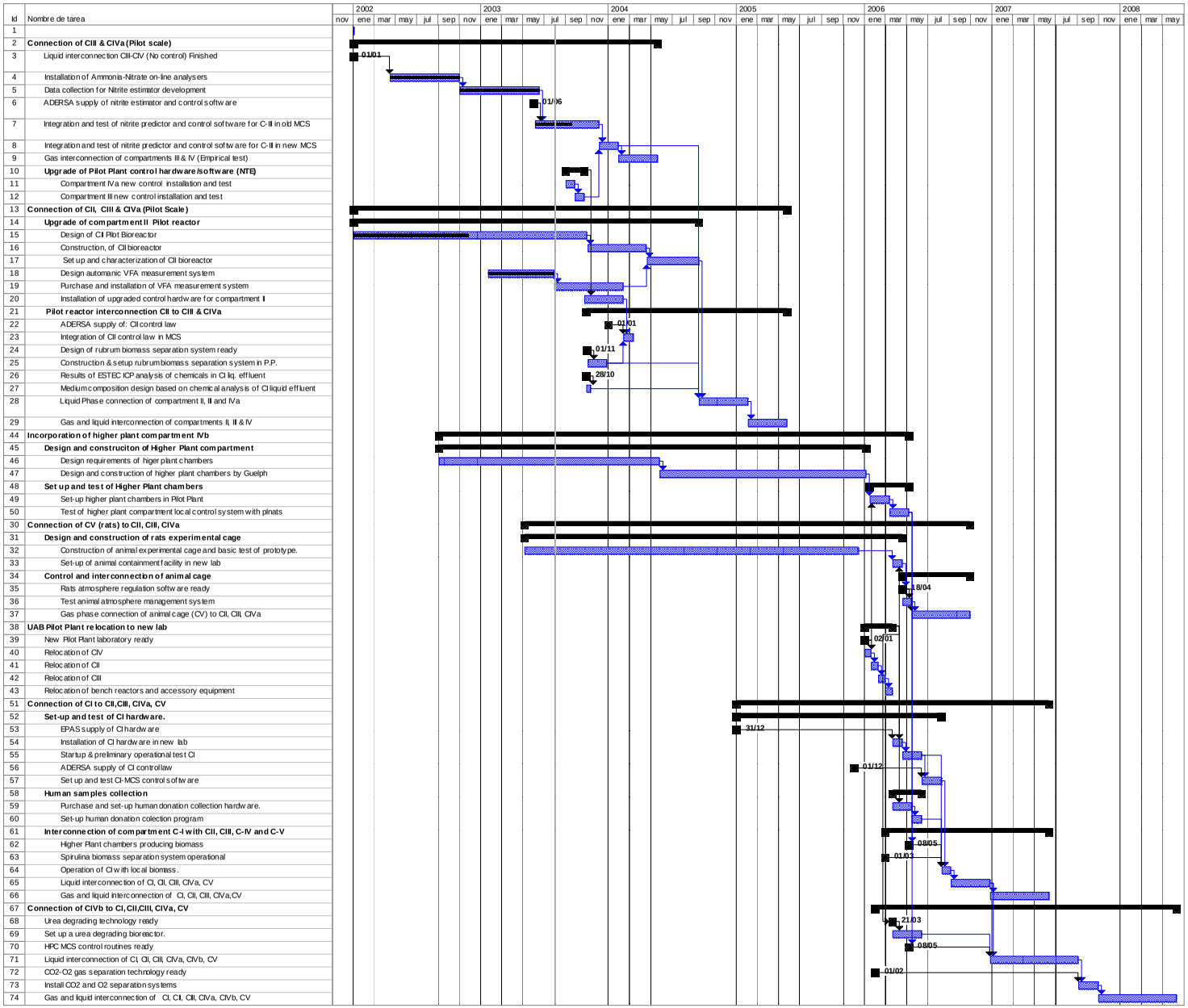
As the pH of the liquid components of each compartment is different, a global pH maintenance strategy should be considered. This approach may attempt to find different alternatives so as to drive the system to complete auto sufficiency. Those might be for example to operate the bioreactors in such a way that the addition of external chemicals for pH regulation is minimized or avoided or to regenerate such chemicals from the internally generated materials. To this purpose a special study should be engaged.

11.4 Gas (CO_2 and O_2) separation technology

Operation of the higher plant compartments and crew compartments at the same atmosphere composition is in principle possible. However this does not allow to take advantage of operating the higher plant compartments at different conditions such as for example elevated CO_2 partial pressures. On the other hand safety reasons would advise to maintain a stricter control on crew atmosphere composition and allow to isolate this one from the rest of the chambers, using stored gasses in case of emergency (p.e. atmosphere contamination, pressure loss,…). In both cases the crew quarters should be atmosphere controlled independently of the rest of the system which requires at least partial isolation of the atmospheres with possibility of automatic complete isolation.

In order to maintain independent the gas composition between the crew compartment and the oxygen generating and carbon dioxide consuming compartments, and also to simplify safety measures for the crew, the convenience of separating the oxygen and the carbon dioxide produced in different compartments should be considered. They could be stored in buffer tanks and distributed in the corresponding compartments as necessary. In case of emergency the crew would survive, during some time, using the storage tanks.

To this purpose it is necessary to have a technology for an efficient separation of those gasses. Therefore it is proposed to engage a study dealing with the convenience to maintain those gases separated in front of the alternative of modifying the operational conditions of the MELISSA compartments in order to always maintain the human habitat to the required composition. If gas separation is considered the best option, a study for the availability and incorporation of the required techniques in the MELISA Pilot Plant should also be engaged.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1
2 Connection of CIII & CIVa (Pilot scale)
3 Liquid interconnection CIII-CIV (No control) Finished
4 Installation of Ammonia-Nitrate on-line analysers
5 Data collection for Nitrite estimator development
6 ADERSA supply of nitrite estimator and control software
7 Integration and test of nitrite predictor and control software for C-II in old MCS
8 Integration and test of nitrite predictor and control software for C-II in new MCS
9 Gas interconnection of compartments III & IV (Empirical test)
10 Upgrade of Pilot Plant control hardware/software (NTE)
11 Compartment IVa new control installation and test
12 Compartment III new control installation and test
13 Connection of CII, CIII & CIVa (Pilot Scale)
14 Upgrade of compartment II Pilot reactor
15 Design of CI Pilot Bioreactor
16 Construction, of CII bioreactor
17 Set up and characterization of CII bioreactor
18 Design automanic VFA measurement system
19 Purchase and installation of VFA measurement system
20 Installation of upgraded control hardware for compartment I
21 Pilot reactor interconnection CII to CIII & CIVa
22 ADERSA supply of: CII control law
23 Integration of CII control law in MCS
24 Design of rubrum biomass separation system ready
25 Construction & setup rubrum biomass separation system in P.P.
26 Results of ESTEC ICP analysis of chemicals in CI liq. effluent
27 Medium composition design based on chemical analysis of CI liquid effluent
28 Liquid Phase connection of compartment II, II and IVa
29 Gas and liquid interconnection of compartments II, II & IV
44 Incorporation of higher plant compartment IVb
45 Design and construciton of Higher Plant compartment
46 Design requirements of higer plant chambers
47 Design and construction of higher plant chambers by Guelph
48 Set up and test of Higher Plant chambers
49 Set-up higher plant chambers in Pilot Plant
50 Test of higher plant compartment local control system with plnats
30 Connection of CV (rats) to CII, CIII, CIVa
31 Design and construction of rats experimental cage
32 Construction of animal experimental cage and basic test of prototype.
33 Set-up of animal containment facility in new lab
34 Control and interconnection of animal cage
35 Rats atmosphere regulation software ready
36 Test animal atmosphere management system
37 Gas phase connection of animal cage (CV) to CII, CIII, CIVa
38 UAB Pilot Plant relocation to new lab
39 New Pilot Plant laboratory ready
40 Relocation of CIV
41 Relocation of CII
42 Relocation of CIII
43 Relocation of bench reactors and accessory equipment
51 Connection of CI to CII,CIII, CIVa, CV
52 Set-up and test of CI hardware.
53 EPAS supply of CI hardware
54 Installation of CI hardware in new lab
55 Startup & preliminary operational test CI
56 ADERSA supply of CI controllaw
57 Set up and test CI-MCS control software
58 Human samples collection
59 Purchase and set-up human donation collection hardware.
60 Set-up human donation colection program
61 Interconnection of compartment C-I with CII, CIII, C-IV and C-V
62 Higher Plant chambers producing biomass
63 Spirulina biomass separation system operational
64 Operation of CI with local biomass.
65 Liquid interconnection of CI, CII, CIII, CIVa, CV
66 Gas and liquid interconnection of CI, CII, CIII, CIVa,CV
67 Connection of CIVb to CI, CII,CIII, CIVa, CV
68 Urea degrading technology ready
69 Set up a urea degrading bioreactor.
70 HPC MCS control routines ready
71 Liquid interconnection of CI, CII, CIII, CIVa, CIVb, CV
72 CO2-O2 gas separation technology ready
73 Install CO2 and O2 separation systems
74 Gas and liquid interconnection of CI, CII, CIII, CIVa, CIVb, CV
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