Universitat Autònoma de Barcelona

TECHNICAL NOTE 94.41

USER REQUIREMENTS FOR C1 FILTRATION UNIT OPTIMIZATION

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List of acronyms

CI : compartment I

CII : compartment II

CIII : Compartment III

CIVa : Compartment IVa

CIVb : compartment IVb

CV : Compartment V

HPC: Higher Plant Chamber

MELiSSA: Micro-Ecological Life Support System Alternative

UAB: Universitat Autònoma de Barcelona

UPS: Uninterrupted Power Supply

NTU: Nephelometric turbidity units

CFU: colony-forming units

COD: Chemical oxygen demand

DM: Dry matter

1. Context: the MELiSSA Project and the MELiSSA concept

1.1. The MELiSSA Project

Over the last 15 years several Space Agencies (i.e. NASA, JAXA, RSA, CSA, ESA) have been studying the regenerative life support systems needed to sustain long-term manned space missions.

Space exploration constraints dictate that the primary objective of the studies is to reduce the launched mass of metabolic consumables (i.e. water, oxygen, food) by increasing their recycling rates up to, ideally, closure of the gas, liquid and solid loops.

Within Europe, the main part of the work has been performed within the MELiSSA (Micro-Ecological Life Support System Alternative) project by a highly comprehensive European and Canadian scientific and technical network, coordinated by the European Space Agency (specifically the European Space Research and Technology Centre ESTEC).

Within MELiSSA, it is proposed to follow a global approach of Life Support requirements by addressing jointly the main Life Support functions, i.e.:

- Air revitalization,
- Water production,
- Waste management,
- Food production and preparation
- Quality Control and Safety issues
- Ergonomics and Habitability

With regards to the challenge of sustaining Human Life during long-term manned space missions, a stepwise engineering approach is followed in MELiSSA, starting from basic research and development studies, including preliminary flight experiments, up to a comprehensive ground demonstration of the technologies developed.

1.2. The MELiSSA concept

The MELiSSA concept is based on the duplication of the functions of the earth without benefiting from earth's large resources (i.e. oceans, atmosphere..) and from terrestrial comfort.

The goals of the MELiSSA loop are the recovery of food, water and oxygen from wastes, i.e. CO_2 and organic wastes, using light as a source of energy.

From the observation of a lake ecosystem (i.e. the identification of the elementary consumption, degradation and production functions composing this ecosystem), the MELiSSA loop is conceived as a closed regenerative system, based on five compartments duplicating the lake ecosystem's elementary functions (see below Figure 1, further information is available at http://www.estec.esa.int/ecls).

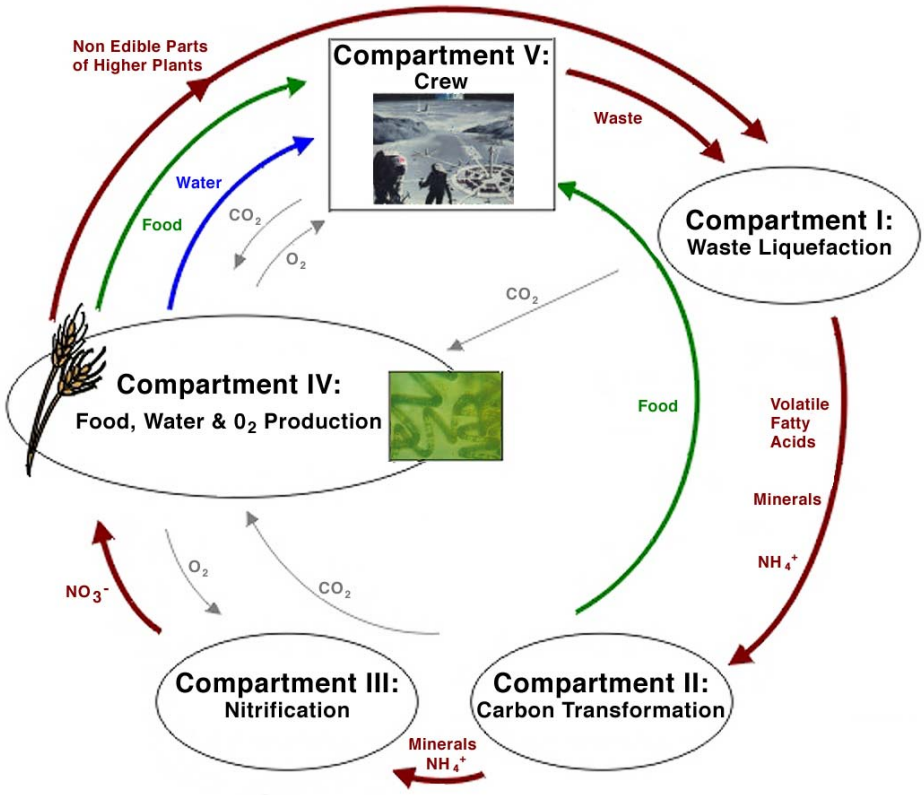

Figure 1: MELiSSA Advanced Loop Concept

Each compartment has a given objective within the complete biotransformation and connections with other compartments.

The basics are the followings:

- In Compartment I, the different waste sources are degraded in an anaerobic thermophilic bioreactor. The wastes include non edible material from plants, excess bacterial material from other compartments, fecal material, etc. The degradation yields a range of volatile fatty acids (VFA) that are transferred in Compartment II.

- Compartment II is photobioreactor where the VFA produced by Compartment I are further converted, basically to CO_2, by the photoheterotrophic growth of the bacteria *Rhodospirillum Rubrum*.
- Compartment III is responsible for the bioconversion of the nitrogen source, i.e. from ammonium NH_4^+, as produced in CI, into nitrate NO_3^-. Compartment III is a fixed-bed bioreactor, with a co-culture of *Nitrosomonas* and *Nitrobacter* bacteria immobilized onto a solid support (beads).
- The production compartments are Compartment IVa and IVb:
    - Compartment IVa is devoted to the culture of the photoautotrophic cyano-bacteria *Arthrospira platensis* (*a.k.a. Spirulina platensis*), and is used mainly for the production of oxygen from CO_2,
    - Compartment IVb is devoted to the culture of a number of selected higher plants (i.e. wheat, lettuce and beet), for the production of food and oxygen.
    - These compartments are the closing steps for the loop, since they provide with the functions of atmospheric regeneration (converting the CO_2 generated by the crew and other bacterial compartments into O_2) and edible material generation. In addition, higher plants can also provide a way to biologically regenerate potable water through transpiration.
- Compartment V corresponds to the crew (i.e. consumer) compartment. For the first demonstration of the MELiSSA loop, it has been decided to work with laboratory animals.

The development of each individual compartment follows the same engineering logic:

- Technologies characterization in batch and continuous modes,
- Stoichiometry studies,
- Hydrodynamic characterization,
- Static Modeling,
- Dynamic Modeling,
- Control Model (for predictive control),
- Safety issues (chemical and microbiological),
- Maintenance and Dependability.

At the upper level of the complete loop (i.e. closed loop of interconnected compartments), a system approach is mandatory to achieve mass balance closure, a relevant safety of the complete system and its reliability for long term operation. This

system approach is supported by a knowledge-based control leading to the development of a predictive control based management of the overall MELiSSA loop.

2. The MELiSSA Pilot Plant

2.1. Overall presentation

As expressed previously, the challenge of sustaining human life in frame of long-term missions is such that an extensive demonstration of MELiSSA on ground is a mandatory step in the process of its adaptation to space.

Owing to the state of the art at laboratory scale, the five MELiSSA compartments are progressively developed up to a pilot scale, according to a sizing scenario defined by the MELiSSA Consortium as representative of a full scale manned mission **(i.e. production of 1 eq-man oxygen, production of 20% of 1 eq-man daily diet)**.

The European Space Agency (ESA) has entrusted the implementation of the MELiSSA Pilot Plant to the Universitat Autonoma de Barcelona (UAB), with **the challenge to make it the primary European Facility for Life Support Ground-Demonstration.**

The MELiSSA pilot compartments will be integrated (i.e. connection of the gas, solid and liquid phases) within the MELiSSA Pilot Plant, with **the ultimate objective of a long-term demonstration (i.e. around 3 years of continuous operation) of the MELiSSA loop (i.e. 5 compartments interconnected).**

A new MELiSSA Pilot Plant facility has been built by the Universitat Autònoma de Barcelona., in the Departament d'Enginyeria Química, Escola Tècnica Superior d'Enginyeria (ETSE). This new facility of 214 m^2 will be devoted to the location of:

- compartments I, II, III and IVa, three Higher Plants Chambers composing CIVb, the animal compartment (i.e. CV),
- a human waste collection unit,
- a control room,
- Auxiliary equipments.

2.2. MELiSSA Pilot Plant: integration strategy

The main goal of the MELiSSA Pilot Plant described in the previous section will be achieved once all the different compartments will be operated at is final scale, in continuous mode, fully connected, under the control system, for a long operation mode. To achieve it, an step-wise integration strategy will be defined.

The closure of the MELiSSA loop is envisaged using animals as a mock-up of the crew compartment. Indeed, this is a more realistic scenario to demonstrate and study the first closure of the loop, including the effect of perturbations. The number and type of animals to use will be defined in the corresponding study. Using animals instead of humans for this demonstration step also reduces in a great extent the feasibility of the experiments in terms of economical cost and associated safety measures.

In such scenario, the closure will be completed mainly at the level of the gas phase and water. The animal faeces and urine will not be used, that is, they will not be introduced as feed in any of the Compartments of the loop. In turn, and in order to obtain more realistic data for the MELiSSA loop operation, human faeces and urine will be collected from a group of donors, and will be used as part of the feed material to the MELiSSA loop. In this way, the closure scenario proposed will be highly realistic, and the data obtained will enable to design future closure scenarios with humans.

The integration strategy within the MELiSSA Pilot Plant will follow a **step-wise approach**:

- The first steps will focus on the continuous operation of the pilot scale compartments individually. These steps will be the opportunity of additional characterization and validation activities that cannot be performed at laboratory scale, due to the level of instrumentation or the size of the hardware. The knowledge gained will potentially engender future optimization both in terms of hardware, of mathematical models and of control.
- In parallel, studies will be performed to develop the interfaces that will be necessary between the compartments. (e.g. a waste collector to collect urine and faeces, a waste preparation unit, biomass harvesting systems…)
- Then, a progressive connection of the compartments will be performed up to the ultimate closure. This progressive connection concerns all three, i.e. solid, liquid, and gas phases. Delicate issues will have to be addressed, such as, among others:
    - Prevention of any contamination of the compartments working under axenic conditions (i.e. pure mono- or multi- bacterial culture),
    - Low range of flows to be carried from one compartment to another,
    - flexibility of the design, to follow the evolution of the integration requirements and specifications

- operator safety and high quality control.

2.3. Detailed description

The MELiSSA Pilot Plant is divided into different rooms, as described hereafter on Figure 2 and Table 1. Basically, it consists of one area (9A, 9B, 9C and 9E) devoted to the bioreactors (i.e. compartments I, II, III and IVa), the waste collection unit and the animal compartment, one area (9 D) for the Higher Plants Chambers, and a central area for offices/meeting room and the control room.

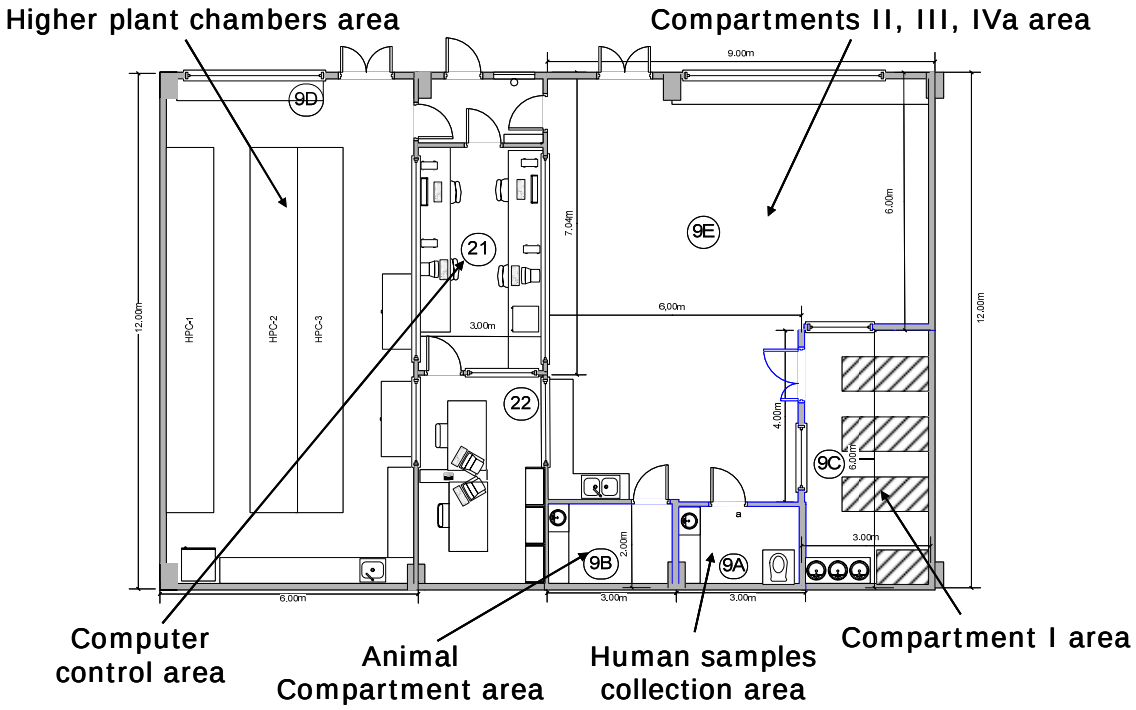

Figure 2. Basic layout of the MELiSSA Pilot Plant laboratory.

Room	Description
9E	Bioreactors area (includes compartments II, III and IVa)
9A	Human waste collection room
9B	Animal Compartment
9C	Compartment I area
9D	Higher Plant Chambers (Compartment IVb)

21	Control Room
22	Office

Table 1. Basic description regarding the distribution of the MELiSSA Pilot Plant

The document *MELiSSA Pilot Plant General Resources, Interfaces and Environment* (TEC-MCT/2006/3493/lnBLA), describes in detail all aspects of the MELiSSA Pilot Plant :

- access and design: covering sizes, maximum loads, surfaces characteristics…
- general utilities and facilities such as air filtration and ventilation, storage capacities, freezers…
- services provided by central systems, distributed over the MELiSSA Pilot Plant: steam, gas, power, cooling water..
- interfaces: with these provided services (connection types and their exact location), with additional networks (drains, gas exhausts..)..
- monitoring, alarms and safety issues.

As examples, Figure 3 provides the specific sizes of the MELiSSA Pilot Plant, and Figure 4 indicates the distribution of the different lines for power supply.

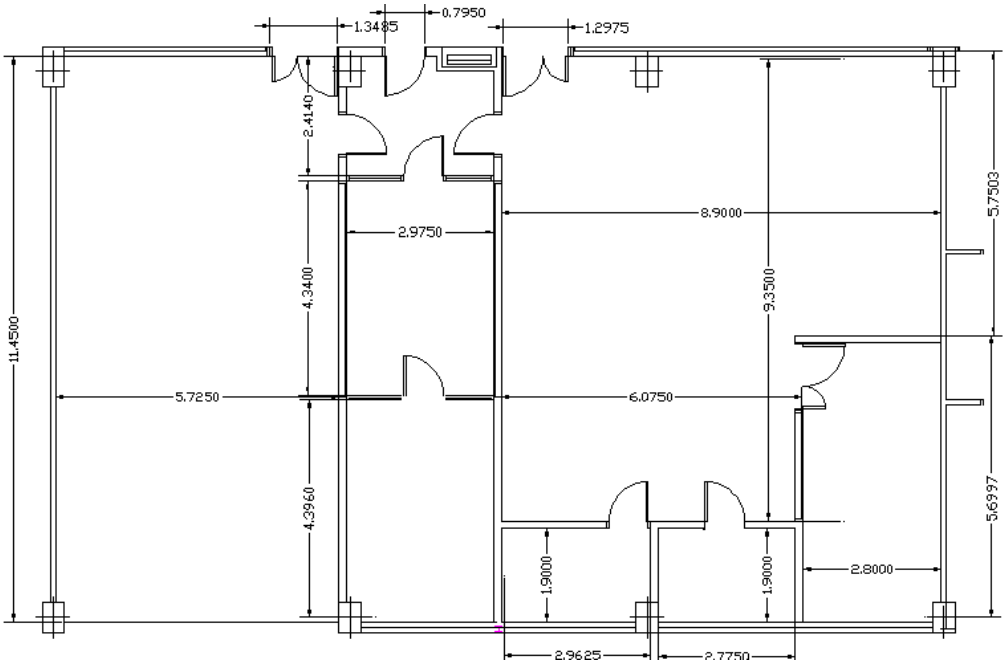

Figure 3: Sizes of the different areas in the MELiSSA Pilot Plant

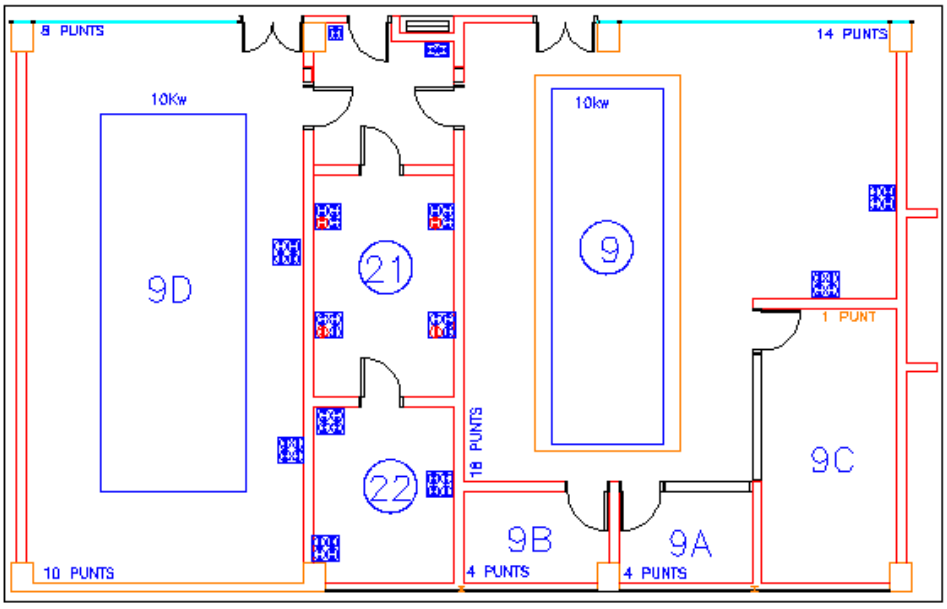

Figure 4: Distribution of the power lines in the MELiSSA Pilot Plant.

2.4. Additional technical information over the MELiSSA compartments

A brief description of each compartment in the MELiSSA loop is presented in the next paragraphs.

2.4.1.Compartment I

Compartment I, as illustrated on Figure 5, is composed of a membrane bioreactor connected to an influent feed tank and an effluent (i.e.filtrate) collection tank. The bioreactor has an approximate volume of 100 L

For the preparation of the influent, a waste preparation unit will be installed. During the integration phase, the waste preparation unit will probably be connected to the liquid phase of CIVb

Besides C-I equipment, room 9C is equipped with:

- Inert gas line to establish anaerobiosis (Helium).
- Air cooling/venting system.
- Steam line.
- Cool liquid line for temperature control and gas condensation system.
- Demineralized water.
- Tap water
- Compressed air (use of pneumatic devices).

2.4.2.Compartment II

Compartment II bioreactor will be located in room 9E. Bioreactor volume is about 50 L. A description of the reactor is given on Figure 6.

The output of C-II bioreactor, collected in an effluent collection tank, contains biomass to be further separated from the liquid output by a biomass harvesting system (today under study). The connection from the influent tank to the biomass harvesting system shall be foreseen.

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

- Demineralized water,
- Tap water,
- Inert gas line to establish anaerobiosis (Helium),
- He and H_2 lines for gas chromatography,
- Air cooling/venting system,
- Liquid cooling supply system,
- Steam line,
- Compressed air (use of pneumatic devices).

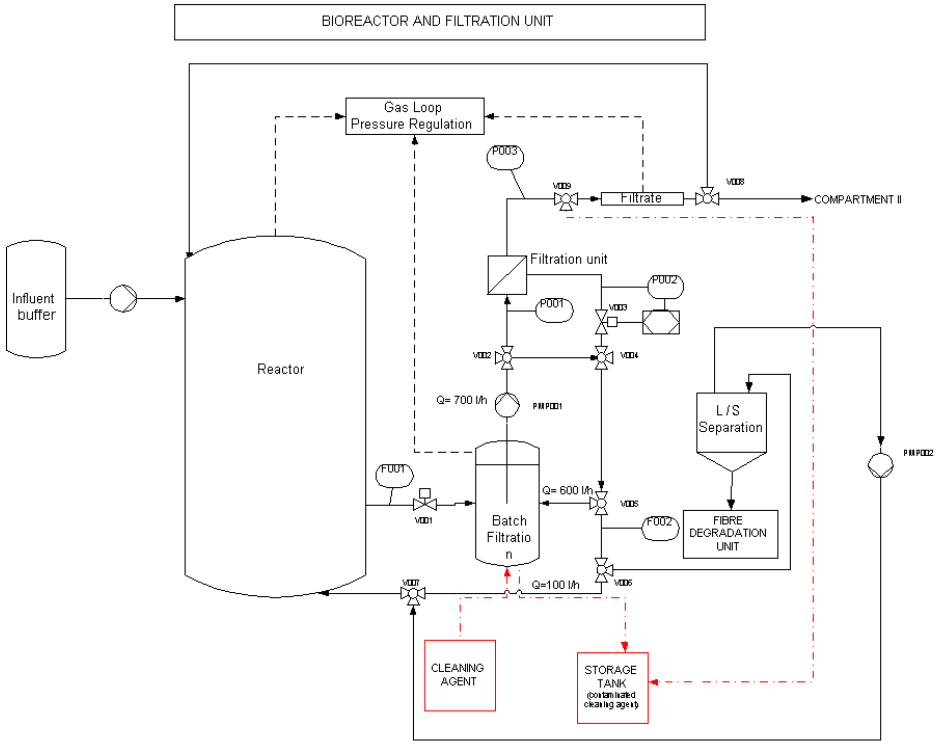

Figure 5: Schematic design of compartment I and its filtration unit.

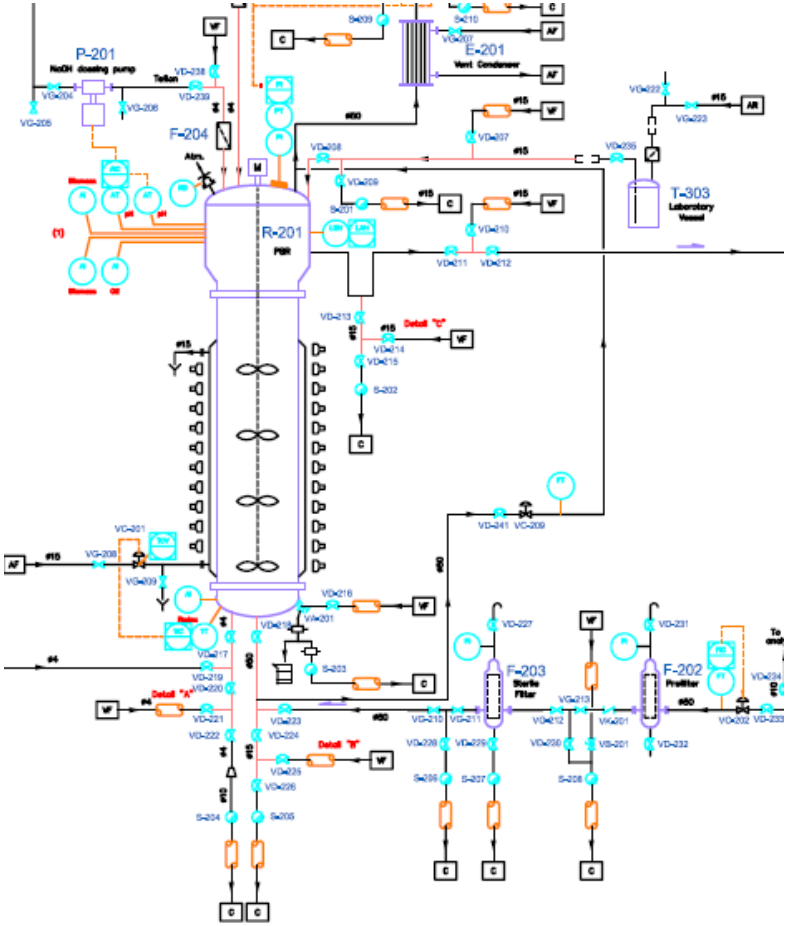

Figure 6: Configuration scheme of compartment C-II.

2.4.3.Compartment III

Compartment III bioreactor will be located in room 9E. The volume of the bioreactor is 8 L.

The present bioreactor (see Figure 7 for a schematic overview and associated picture), will be now up-graded, and the work presented here is indeed related to this up-grade.

Compartment III 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

2.4.4.Compartment IVa

Compartment IVa bioreactor will be located in room 9E. The volume of the bioreactor is 77 L. A schematic overview of this compartment and the equipment involved is provided on Figure 8 and associated picture.

Compartment IVa 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

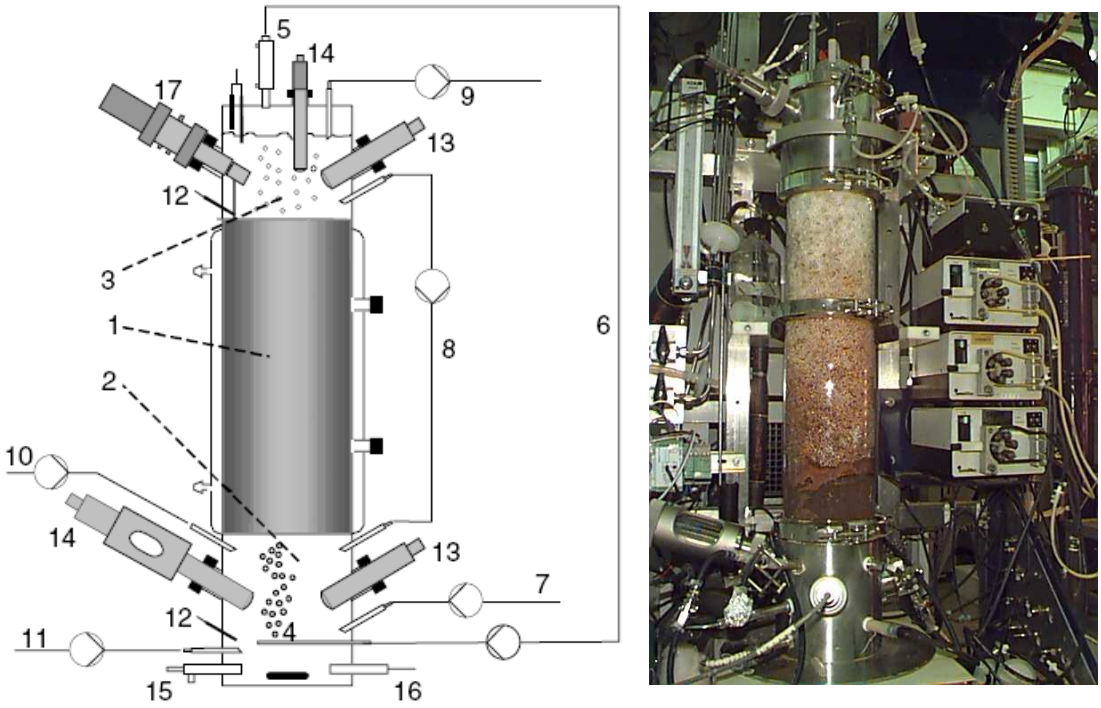

Figure 7: : Schematic overview of compartment III.

General schematic (left) and picture (right) of the nitrifying pilot bioreactor. (1) Packed-bed section with immobilized culture, (2) bottom section for aeration, liquid distribution and instrumentation, (3) top section for gas disengagement, (4) gas sparger, (5) gas exit condenser, (6) gas loop, connected to oxygen/nitrogen regulated supply to control dissolved oxygen, (7) liquid feed, (8) liquid recirculation, (9) liquid outlet, (10) acid addition, (11) base addition, (12) temperature probes, (13) dissolved oxygen probes, (14) pH probes, (15) cooling system, (16) heating system, (17) sampling device.

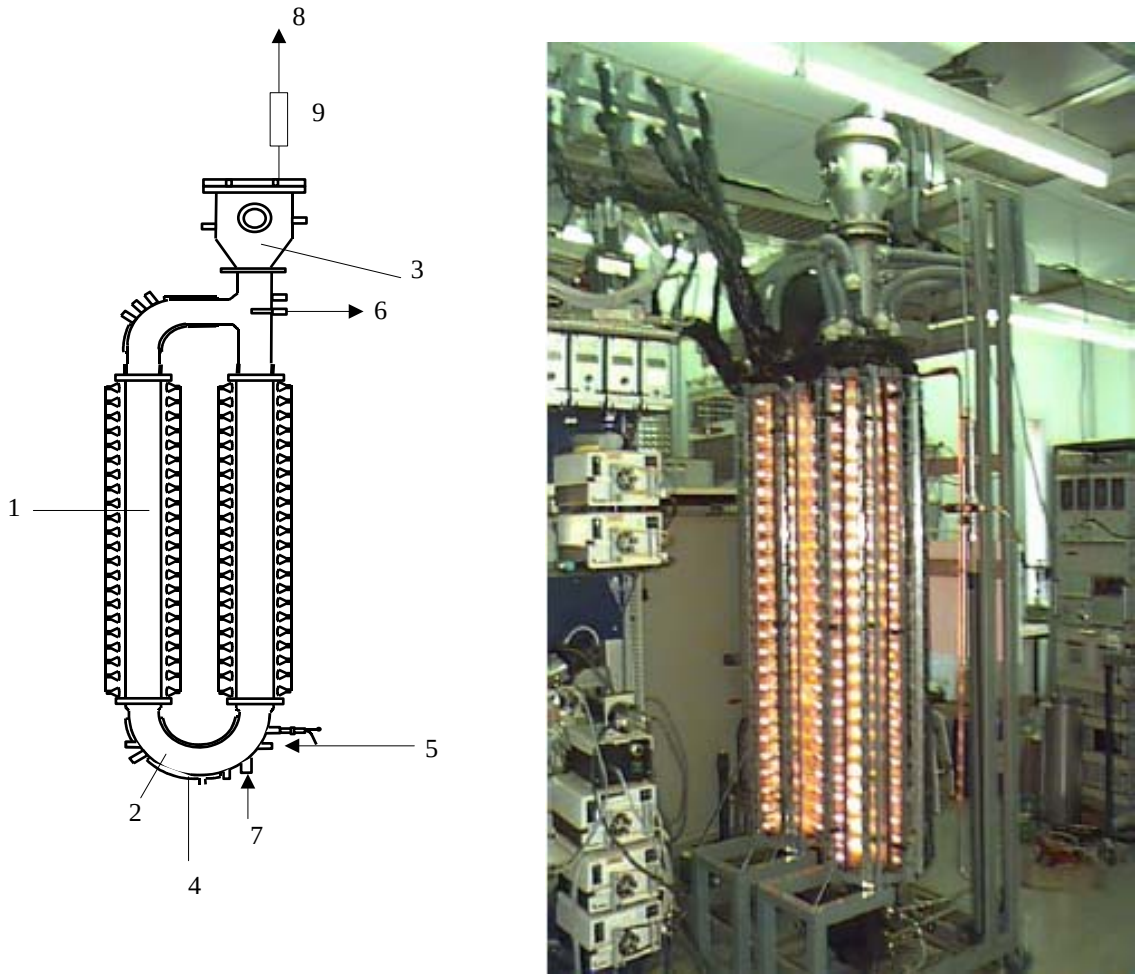

Figure 8: Schematic view of compartment IVa.

General scheme of the 77 litres photobioreactor designed for the culture of Spirulina cells. 1, transparent cylindrical parts (illuminated section) : riser (right column and downcomer (left column), 2, stainless steel connection parts , 3, gas-liquid separator, 4, external cooling jackets, 5, liquid medium inlet, 6, liquid outlet, 7, gas inlet through sparger, 8, gas outlet, 9, condenser, 10, halogen lamps.

2.4.5.Compartment IVb

The higher plant compartment C-IVb will be installed in room 9D. It will be composed of 3 Higher Plants Chambers. A schematic overview of the compartment is shown in Figure 9.

CIVb 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 evapo-transpiration condensation depending on chamber design (green solid line in figure 15).

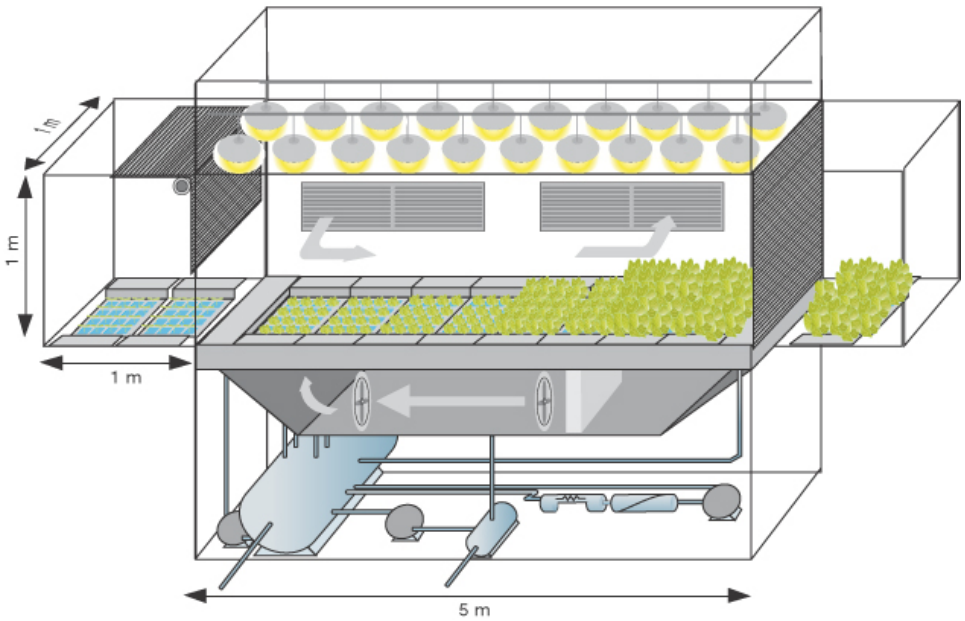

Figure 9: schematic view of the design concept for the Higher Plant chamber.

2.4.6.Compartment V

The animal compartment will be installed in room 9B. This compartment is currently under design. In principle, it will consist in an air tight cage where animals are going to live.

The animal compartment (CV) 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 for humidity of breath air condensation.

3. Frame of the work: Compartment I additional characterisation phase

Compartment I was developed during many years by the MELiSSA partner EPAS (Eco Process Assistance), a company located in Ghent (Belgium). EPAS constructed the hardware corresponding to Compartment I of the MPP, and completed with the final delivery to the MPP of C-I compartment hardware, on September 2007. The proposed study on this Compartment will allow the further characterisation of the Pilot Compartment I at the MPP site in UAB. The pilot reactor was installed in the MPP and connected to all utilities, and will be completed in terms of hardware/software for its monitoring and control, and tested for approximately eighteen months. During this period, it will be operated in order to collect data for process, model and control development. The Filtration Unit will be optimised and an up-scaling of the waste preparation system will be performed.

According to this description, the objectives of this work are the installation and integration of Compartment I in the MELiSSA Pilot Plant, the performance of a long series of experiments with the proper analyses to fully characterize its operation and to provide data for mathematical model and control algorithms development, the improvement and optimization of the unit to prepare the feed to the reactor, and the optimization of the membrane unit of Compartment I. At the finalisation of the work,

Compartment I should be completely operational in the MPP at the corresponding quality standards and ready to be connected to other MPP compartments.

The optimization of the membrane unit of Compartment I in the MELiSSA Pilot Plant (mainly membranes with/without corresponding modules) involves a number of steps:

1. Definition of the User's Requirements for the selection of the new membrane
2. Assessment of the current membrane versus the user's requirements, with regards to the membrane material choice and membrane cut-off selection
3. Assessment of the filtration unit operation protocol and of cleaning procedures
4. Trade off and selection of a better suited membrane via off-site tests in CI representative conditions, to validate alternative membrane material and membrane cut-off
5. hardware procurement
6. Delivery and installation of the hardware in the MELiSSA Pilot Plant.
7. Demonstration and validation of the optimized filtration unit in the MELiSSA Pilot Plant.

The present document defines the User's Requirements document for optimization of the membrane unit of Compartment I, as the step 1 of the previous list , being the basis for the following tasks.

4. Scope of the study: User's Requirements for Compartment I Filtration Unit Optimization

The aim of the present document is to define the User´s Requirements for the Filtration Unit optimization regarding the UF membrane. The main conditions considered to be critical for the new membrane to be selected are:, filtrate sterility, no retention of VFA, no retention of ammonium, reduction of clogging and fouling, reduction of cleaning needs.

The User's Requirements for the Filtration Unit optimization have been split in three main categories:

4.1 Requirements regarding retention properties

4.2 Process requirements

4.3 Requirements regarding long-term operation

4.1. Requirements regarding retention properties

1. The filtration unit shall retain 100% of all solid particles. To be more precise, it shall retain all compounds with a size bigger than 0,45 µm, what can be quantified as 0,1 NTU (nephelometric turbidity units). As a consequence, some indicative features can be proposed:

- filtrate side turbidity : < 0,1 NTU

- COD total and soluble should be similar in the filtrate. In another words, the proportion of particular COD (COD_{Part} = COD_{Total} - $COD_{Soluble}$) retained in the bioreactor must be average > 99%.

2. The filtration unit shall protect CII axenicity. As a consequence, it should retain 100% of all microorganisms, i.e. the filtration unit shall provide sterile filtrate to the following compartment, the criterion for sterility being lower than 100 CFU/100 mL.

Derived requirements from this:

3. The filtration unit shall be steam sterilizable in place.

4. The filtration unit shall resist to chemical disinfection.

5. The filtration unit shall prevent contamination of the filtrate side This contamination could come from:

- Working with high trans-membrane pressure (2 bar and more) that can generate a deformation of the biofilm and a penetration of cells throughout the membrane thickness.

- Working with membrane presenting structure default or too large pores (> 400 nm)

- Working with an opened permeate side that allows a contamination by ambient air

So it is determining to (i) chose a membrane conforming to the retention of germs (MF) or virus (UF), (ii) check the quality of the membrane structure and (iii) avoid any external contamination on the permeate side. It is also recommended to work under low TMP.

For information, the presence of a biofilm on the retentate side has a direct negative effect on permeate flux because it induces an additional hydraulic resistance to cross the porous media, but this biofilm generally presents a positive effect (when working with low TMP) on germ retention and decontamination.

Moreover, according to the temperature closed or higher than 50°C, the disparition of pathogens inside the bioreactor could be very important according to temperature and high sludge retention time in the bioreactor.

6. The filtration unit shall not selectively retain any of the other product compounds (i.e VFA, ammonium, minerals…) that should be further used in the MELiSSA loop.

Derived requirements from this:

7. With the assumption that there is no volumetric concentration factor of CI bioreactor content, then the concentration of VFA in the bioreactor shall be equal to the concentration of VFA in the filtrate.

8. The concentration of ammonium in the bioreactor shall be equal to the conc. of ammonium in the filtrate.

9. Idem for other relevant mineral compounds: $PO4^{3-}$, $SO4^{2-}$, Cl^{-}, Na^{+}, Mg^{2+}, K^{+}, Ca^{2+}, etc.

4.2. Process requirements

10. The filtration unit and process shall not damage CI consortium micro-organisms.

11. Process parameters nominal set points/ranges are:

a) Feed

- particle size: vegetables, up to 2 mm; straw, up to 0,2 mm

b) Bioreactor broth

- temp: 55 ºC
- pH: 4.5 to 6,5
- Viscosity: 10 to 20 cP*
- dry matter: 40 g/L

c) Filtrate

- filtrate flow: 10 l/d up to 15l/d for a 100L reactor

* Viscosity measurement procedure to be confirmed.

4.3. Requirements regarding long-term operation

12. The filtration shall be performed in continuous mode.

13. Redundancy of membrane modules shall be implemented

14. Filtrate flow shall be kept regular: the critical ratio TMP/flow should be checked with water and with broth, providing different profiles depending on the velocity. At normal velocities (1-2 m/s) , flux should be in the range 30-60 $L/m^2/h$

Derived requirement of this:

15. Mechanical fouling shall be limited.

16. Chemical fouling shall be limited: membrane characteristics should be adequate to minimise interactions with compounds in suspension and facilitate membrane regeneration.

17. Hydrodynamics conditions should be optimal to minimise the fouling rates.

18. Presence of exogenous compounds in the filtrate shall be avoided.

19. Cleaning shall be optimised; the ability of the membrane to recover its permeability after use will be evidenced by means of a water permeability test. In particular:

- In coherence with Req. 18, the membrane should not need chemical agents for its cleaning, or should need them in a limited amount.
- Frequency of backwashing and cleaning should be reduced as much as possible.

20. Safety of the operators shall be guaranteed.

21. Filtration process shall be fully automated for all operation modes.

22. Energy consumption of the FU for long operation periods shall be minimized.

5. References

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6. Comments

TN 94.41
User Requirements for C1 Filtration Unit optimization

Comments

Detailed comments

Page/paragraph	Comment
1/Reference	*"CCN7 to contract 13292/98/NL/MV"* This one is completed and closed already OK, updated to present Frame Contract
23/Req. 1	*"- COD total and soluble should be similar in the filtrate.* *- Proportion of particular COD (COD_{Part} = COD_{Total} - $COD_{Soluble}$) retained in the bioreactor must be average > 99%."* These two requirements are actually only one, aren't they? Yes, we´ve rephrased to join them in the same sentence: *"COD total and soluble should be similar in the filtrate. In another words, the proportion of particular COD (CODPart = CODTotal - CODSoluble) retained in the bioreactor must be average > 99%."*
23/Section 4.1, Req. 1	*"The DM of permeate should be constant in the time and lower than the one of the reactor content, which should increase in the time".* This statement is not correct; if the DM linked to solutes is increasing in the reactor, then it will increase in the permeate, as we don't want any solutes retention. If we change intentionally the operating conditions of CI, then the DM of the permeate will change as well. The statement comes from TN 71.9.1, maybe is not updated, but it compares the dry matter in the permeate with the DM in the reactor (I understand both solutes and particles), so not solutes in both sections. Anyway the phrase "which should increase in time" I suppose it is true until it´s drained, so I´m not sure that is well described
23/ Section 4.1, Req. 2	*"The filtration unit shall retain 100% of all microbiological compounds, i.e. the filtration unit shall provide sterile filtrate to the following compartment, the criterion for sterility being lower than 100 CFU/100 mL."* The overall requirement is that the filtration unit shall protect the axenicity of CII. Derived from this statement, we can say that ideally, the membrane shall retain 100% of microbiological compounds; however, in the real life we do not target to retain plasmids for

	example. Therefore, either we derived again the requirement in 100% of bacteria or we say directly that the filtrate shall be sterile with indeed the indication of a sterility criterion Amended in the text: *"The filtration unit shall protect CII axenicity. As a consequence, it should retain 100% of all microorganisms, i.e. the filtration unit shall provide sterile filtrate to the following compartment, the criterion for sterility being lower than 100 CFU/100 mL."*
23/ Section 4.1, Req. 2	*"…the criterion for sterility being lower than 100 CFU/100 mL."* This criterion, proposed by A.Gransmick, needs to be agrred with ESA ESA agrees with this value.
24/ Section 4.1, Req. 5	*"The filtration unit shall prevent the biofilm contamination of the filtrate side, i.e. the biofilm on the retentate side has to be managed to be sufficient for promoting permeation but not strong enough to let bacteria pass through the membrane."* This requirement is not understood: biofilm contamination of the filtrate side? Biofilm on the rententate side promoting permeation? First question: rephrased cancelling the word "biofilm" respect to the filtrate side. Second question: this requirement was rephrased already based on the discussion with A.Grasmick in TECHNOMEMBRANES (see MPP-MOM-08-1009(0)-AF-20080630, page 6, "Req. 2.3"), maybe is good to double check with him, we don´t have enough expertise on this matter to know if retentate side biofilm can help in the permeation.
21/ Section 4.1, Req. 5	"*Derived requirements from this:* *3. The filtration unit shall be steam sterilizable in place.* *4. The filtration unit shall resist to chemical disinfection.* *5. The filtration unit shall prevent the biofilm contamination of the filtrate side, i.e. the biofilm on the retentate side has to be managed to be sufficient for promoting permeation but not strong enough to let bacteria pass through the membrane."* Those requirements are linked to the top level one "protection of CII axenicity OK, included in req. 2: *"The filtration unit shall protect CII axenicity…."*
24/ Section 4.1, Req. 9	*"9. Idem for phosphate and other mineral compounds still to be defined."* To be confirmed by Technomembranes and A. Grasmick: SO42-, Cl-, Na,Mg, K, Ca, others?

25/ Section 4.2, Req. 10	*"10. Microorganisms present in CI consortium shall not be damaged by the filtration process."* We would prefer: the filtration unit and process shall not damage CI consortium microorganisms. We don´t see the difference, anyway the wording is updated: *"The filtration unit and process shall not damage CI consortium microorganisms."*
25/ Section 4.2, Req. 11	11. Process parameters nominal set points/ranges are: filtrate flow: 10 l/d up to 15l/d for a 100L reactor viscosity: 10 to 20 cP pH: 4.5 to 7 particle size: up to 2 mm temp: 55 ºC in nominal operation mode Dry matter (bioreactor content): 40 g/l in nominal operation mode Please specify for viscosity, pH…if you refer to bioreactor content, feed of the bioreactor? OK, rephrased: 11. Process parameters nominal set points/ranges are: a) Feed - particle size: vegetables, up to 2 mm; straw, up to 0,2 mm b) Bioreactor broth - temp: 55 ºC - pH: 4.5 to 6,5 - Viscosity: 10 to 20 cP - dry matter: 40 g/L c) Filtrate - filtrate flow: 10 l/d up to 15l/d for a 100L reactor
25/ Section 4.3, Req. 13	*"13. Filtrate flow shall be kept regular: the critical ratio TMP/flow should be checked with water and with broth, providing different lines depending on the velocity."* Different lines? What is the meaning of this? Reworded: *"Filtrate flow shall be kept regular: the critical ratio TMP/flow should be checked with water and with broth, providing different profiles depending on the velocity."*
25/ Section 4.3, Req. 13	*"At normal velocities , flux should be in the range 10-60 L/m^2/h."* Please insert figures about normal velocities Data included, after discussion with J.Christophe lasserre: *"At normal velocities (1-2 m/s) , flux should be in the range 30-60 L/m^2/h"*
26/Section 4.3,	"Cleaning conditions should be optimised."

Req. 19	What does this mean? On which criteria? Rephrased and completed: *"Cleaning shall be optimised; the ability of the membrane to recover its permeability after use will be evidenced by means of a water permeability test. In particular:* - *In coherence with Req. 18, the membrane should not need chemical agents for its cleaning, or should need them in a limited amount.* - *Frequency of backwashing and cleaning should be reduced as much as possible."*
26/Section 4.3, Req. 18	*"18. Redundancy of membrane modules shall be implemented."* This requirement should be placed after req 12 OK, done.
26/Section 4.3, Req. 17	*"17. Presence of exogeneous compounds in the filtrate shall be avoided; cleaning conditions should be optimised accordingly."* Please recheck the numbering of the requirements; this specific one should be linked to the previous req 17. OK, organised differently.

Second set of comments

Detailed comments

Page/paragraph	Comment
23/Section 4.1, Req. 1	*"The DM of permeate should be constant in the time and lower than the one of the reactor content, which should increase in the time".* I have checked TN 71.9.1 and located indeed this statement. This statement has been written in a test plan /protocol to validate the performances of the membrane filtration. It is valid as evaluation tool (although potentially to be rediscussed or precised) but not as an overall requirement for the filtration unit. I would suggest to remove it. OK, understood; phrase removed
24/Section 4.5, Req. 5	*"The filtration unit shall prevent contamination of the filtrate side. This contamination could come from:* *- Working with high trans-membrane pressure (2 bar and more) that can generate a deformation of the biofilm and a penetration of cells throughout the membrane thickness.* *- Working with membrane presenting structure default or too large pores (> 400 nm)* *- Working with an opened permeate side that allows a contamination by ambient air* *So it is determining to (i) chose a membrane conforming to the retention of germs (MF) or virus (UF), (ii) check the quality of the membrane structure and (iii) avoid any external contamination on the permeate side. It is also recommended to work under low TMP.* *For information, the presence of a biofilm on the retentate side has a direct negative effect on permeate flux because it induces an additional hydraulic resistance to cross the porous media, but this biofilm generally presents a positive effect (when working with low TMP) on germ retention and decontamination.* *Moreover, according to the temperature closed or higher than 50°C, the disparition of pathogens inside the bioreactor could be*

	very important according to temperature and high sludge retention time in the bioreactor." This new more complete description of Req. 5 has been proposed by TECHNOMEMBRANES and A. Grasmick. It could be maintained as it is or summarise it; anyway, the answer to previous ESA comment is now more clear: the biofilm doesn't promote permeation but helps in bacteria retention Indeed there is a bit more than a strict requirement wording however ,as it is given for information, I do not mind keeping it in this TN
23/Section 4.1, Req. 5	Previous text: *"The filtration unit shall prevent the biofilm contamination of the filtrate side, i.e. the biofilm on the retentate side has to be managed to be sufficient for promoting permeation but not strong enough to let bacteria pass through the membrane."* New text: *"The filtration unit shall prevent contamination of the filtrate side"* I anticipate maybe a wording issue linked to the word "biofilm"; physically speaking what is TM talking about? Please check with them. Once checked again with TECHNOMEMBRANES, we think we misunderstood the explanation of the biofilm influence in the previous discussion with them: the comment was that the biofilm presents a positive effect on germ retention and a negative one on permeate flux (see following paragraphs).
24/ Section 4.1, Req. 9	*"9. Idem for phosphate and other mineral compounds still to be defined."* To be confirmed by Technomembranes and A. Grasmick: SO42-, Cl-, Na,Mg, K, Ca, others? Please finalize OK, rephrased after checking with Technomembranes: *"Idem for other relevant mineral compounds: $PO4^{3-}$, $SO4^{2-}$, Cl^{-}, Na^{+}, Mg^{2+}, K^{+}, Ca^{2+}, etc."*
25/ Section 4.2, Req. 11	*"- Viscosity: 10 to 20 cP"* Conditions for this measurement are not clear in EPAS info Please include a note in the TN text like measurement procedure

	TBC OK, done: *"* Viscosity measurement procedure to be confirmed."*
27/ Section 5	*"5. References"* Any reference to be included? Please include relevant TN numbers e.g. 71.9.1 and maybe some reference documents on membrane filtration Included some habook references provided by TECHNOMEMBRANES