MELiSSA

TECHNICAL NOTE

Departament d'Enginyeria Química
Escola Tècnica Superior d'Enginyeries
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TECHNICAL NOTE 66.4

CRITICAL INSTRUMENTATION

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1. 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.

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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).

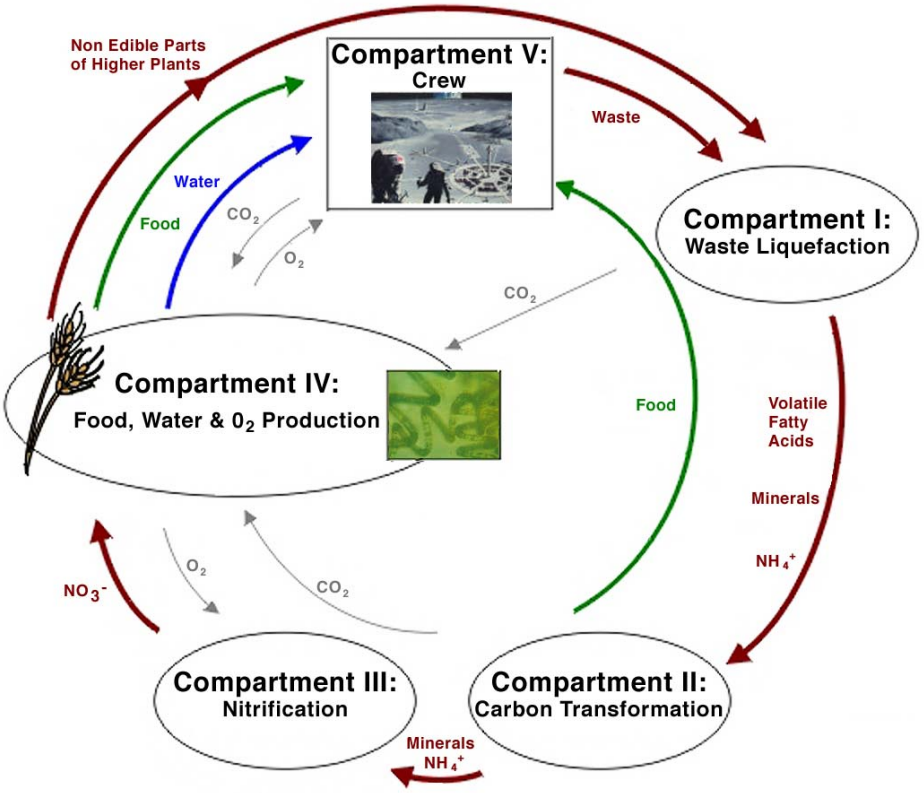

Figure 1: MELiSSA Advanced Loop Concept

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 (Biostyr beads).

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- 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

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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 an 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

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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.

3. Scope of the study: Critical instrumentation and materials selection

In the context described in the previous sections, all the aspects related to ensure the continuous operation of the different compartments of the MELiSSA Pilot Plant under proper monitoring and control are of high relevance. Among them it is very important:

a) the selection of the most reliable **instrumentation**, especially for those variables considered critical
b) the selection of **materials** that can guarantee their specifications and the axenicity of the process for a long-term continuous operation of the pilot plant.

The scope of this technical note is to specify the requirements concerning the specific design items, and the materials selections (instruments, piping, equipments, ..) **for compartments III and IV of the Melissa Pilot Plant**, taken here as a first study

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case, then to be generalized to the rest of the MELiSSA Pilot Plant. Due to the high degree of control required in the operation of these compartments, the selection of the materials of the plant, and the main instrumentation, are considered as critical in order to guarantee the axenicity of the bioreactors.

In addition, one related interest of the current study is that it will provide inputs for the generation of a list of harmonized hardware for the MELiSSA Pilot Plant. Indeed, the study is proposing a limited number of selected instrumentation for a given measurement, with the aim that the MELiSSA Pilot Plant will take those selected instruments as a reference in other studies. In this way, it is the intention that the MPP will have a more efficient operation in terms of functioning, service, maintenance and supply of the most important instrumentation. It should be mentioned here that the specific selected suppliers identified in this work are potential suppliers, recommended in first option as compiling with the required performance. A reduced number of suppliers is selected for each measurement, but certainly other possibilities could also be explored in case that the final technological and economical offers from the suppliers were not fully satisfactory for the optimal deployment of the MPP. The suppliers selection has been made on the basis of existing equipment and instrumentation and the experience provided by its use in several applications in the field of industrial fermentation processes, with high requirements of axenicity and long term operation.

4. Description of Compartments III and IVa and proposed instrumentation priority

The level of criticity of a given measurement is indeed related to the conditions required for the operation of the process at its optimal conditions. For this, the main characteristics of Compartment III and Compartment Iva are provided bellow:

Compartment III, as mentioned in the introduction, is devoted to the nitrification step within the loop. Nitrifying bacteria are required in a life support system to carry out the oxidation of ammonium to nitrate. In the MELISSA loop, nitrification is carried out in an up flow co current packed bed reactor where the two selected strains, *Nitrosomonas europaea* (ATCC 19718) and *Nitrobacter winogradskyi* (ATCC 25391), are immobilized on polymeric (expanded polystyrene substratum (Biostyr®) which has an average diameter of 4.1mm. In order to avoid inhibition by light, the fixed bed is protected with thin foil. The scheme of Compartment III and a picture during its operation can be observed in Figure 1, and further details on the design of this reactor can be found in Pérez *et al.* 2004.

The operating conditions in the reactor were as follows: pH 8.1, magnetic stirring at the bottom at 400 rpm and temperature controlled at 28.0 ± 0.1 ºC. The gas phase is introduced from the reactor bottom, as mixture of oxygen, nitrogen and CO_2 controlled by the control system to maintain a dissolved oxygen set point of 80%. Dissolved oxygen in the culture medium, pH and temperature were measured by

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means of two on-line probes located at the top and at the bottom of the reactor, whose measurements were weighed by the control system. Dissolved oxygen concentration was controlled by adding pure oxygen or nitrogen to the input gas, a solution of Na_2CO_3 was used to increase pH when necessary, and CO_2 was added when pH needed to be decreased.

Compartment IVa, as described in the introduction, is one of the photosynthetic elements of the MELiSSA Pilot Plant, devoted to the culture of *Arthrospira platensis* (*a.k.a. Spirulina platensis*). This cyanobacteria regenerates CO_2 into O_2 within the loop, and additionally it will partially be used for food provision, due to its edible nature. The pilot scale reactor for compartment IVa is a continuous external loop gas-lift photobioreactor (Bioengineering AG, Wald, Switzerland), with external illumination that can be regulated in intensity through the control system. The illuminated parts of the reactor consist of two cylindrical 15 cm diameter sections and 1.5 m height, serving as riser and downcomer for the liquid circulation in the gas-lift reactor. For safety aspects, the columns are manufactured on polyamide foil. These columns are connected in the upper and lower parts by curved stainless-steel parts, supporting the instrumentation and external jackets for water circulation for temperature control. Illumination is provided by a total of 350 externally mounted halogen lamps (Sylvania, BAB 12V 20 W, Belgium). The reactor has a total volume of 77 l, and an illuminated volume of 55 litres, therefore the illuminated to total volume ratio of this reactor is 0.71. pH is controlled at 9.6 by means of CO_2 addition. Temperature is regulated at 36 °C by means of temperature- controlled water circulation in the external reactor jackets.

Figure 2 and Figure 3 bellow provide more details one the characteristics of the hardware for Compartments III and Iva in the MELiSSA Pilot Plant

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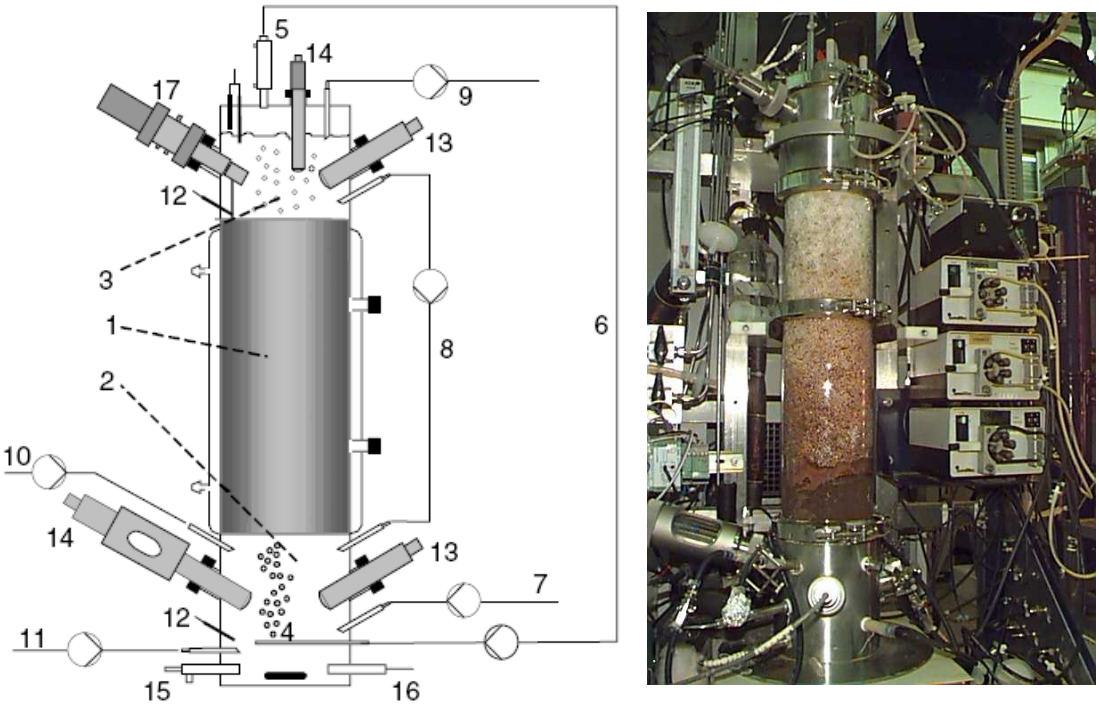

Figure 2: : Schematic view of compartment III. **General scheme of the nitrifying bioreactor (left) and picture (right). (1) Packed-bed section with immobilized culture, (2) bottom section for aeration, liquid distribution and instrumentation, (3) top section for gas disengagement, (4) gas sparger, (5) 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.**

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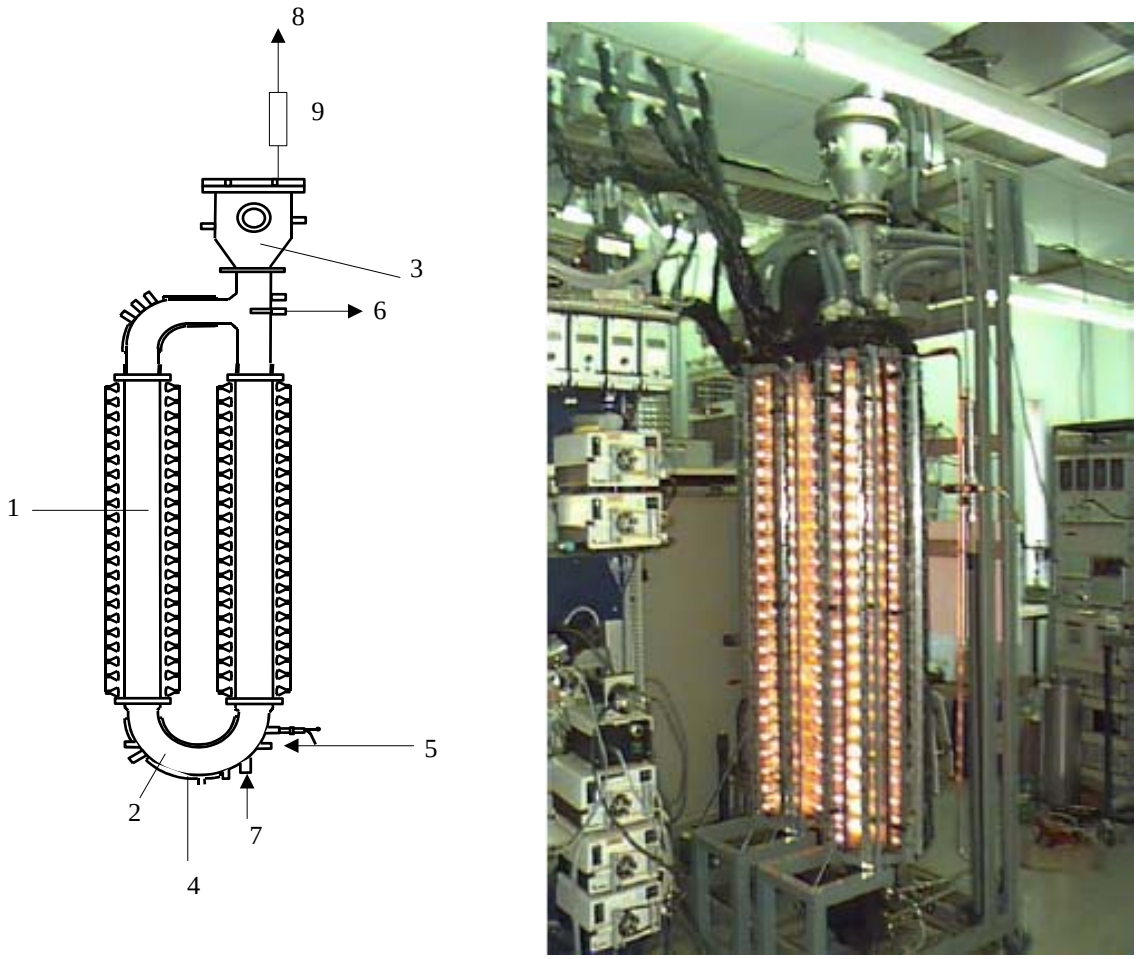

Figure 3: Schematic view of compartment IVa. General scheme of Compartment Iva (left) and picture (right). 1, transparent cylindrical parts (illuminated section) : riser (right column and downcomer (left column), 2, stainless steel connection parts , 3, gas-liquid separator, 4, external cooling jackets, 5, liquid medium inlet, 6, liquid outlet, 7, gas inlet through sparger, 8, gas outlet, 9, condenser, 10, halogen lamps.

Priority of measurements should be then established taking into account the described characteristics, and the experience already gained in the previous period of operation of those compartments in the MELiSSA Pilot Plant. In this study, the following logic has been applied:

Compartment III priority criteria:

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pH and Oxygen concentration, in this order, are key variables to maintain, in order to obtain the desired nitrification. Due to the high sensitive of cell metabolism to these two variables, they have received the highest level of priority. If pH and Oxygen level are not measured correctly, this will affect directly cell viability, will cause partial nitrification, and will have other adverse consequences, leading to the collapse of the Compartment.

Level, gas flow-rate and pressure are the second block of measurements in priority, this being related to the maintenance of proper hydrodynamic condition in the reactor, ensuring the required liquid and gas flow-rates. Disturbances in these measurements will cause undesired changes in the efficiency of the bioreactor operation, lower conversion rates, potential clogging effects, etc. In addition, the bioreactor hydrodynamics is directly linked to the gas-liquid transport capacity, and therefore to the aeration capacity of the reactor. This means that a poor aeration rate would ultimately affect the Oxygen concentration, that is critical, as previously mentioned.

Finally, a third block with low priority is given to those measurements that are either very robust (Temperature) or that are basically for on-line measurement of variables, but not directly operational process conditions of the reactor, such as the Ammonium and Nitrate content. Although very important to know how the reactor is operating, the low priority in the scope of this study is given on the criteria that a failure in those measurements does not collapse directly the bioreactor operation. It should also be mentioned here that a nitrite on-line measurement system is also under study, to be incorporated to Compartment III operation.

Table 1: Compartment III variables priority

Variable	Priority	(control/indication)
pH	10	Control
Oxygen	8	Control
Level	5	Control
Gas flow-rate	5	Indication
Pressure	4	Control
Temperature	2	Control
Nitrate content	1	Indication
Ammonium content	1	Indication

Compartment IVa priority criteria:

Reactor pressure has in this case received the highest score in terms of priority, due to the nature of the Bioreactor material. Indeed, the transparent cylindrical columns of Compartment IVa hardware were built on polyamide foil, which is a flexible material. This was considered an important design criterion by the manufacturer, in order to avoid potential breaking of other more typical materials, such as glass, during the sterilisation process. However, this selection has as a drawback than if the bioreactor pressure is increased, the flexible foil material will first expand to certain extend, and

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finally break, with the irreversible collapse of the operation. For this reason, reactor pressure is scored at the highest priority level, as it has to be very fine-tune controlled.

A second group of variables has received also a high priority score: pH, CO_2 gas flow, total gas flow-rate, light intensity and biomass concentration. All of them are basic parameters that need to be very well controlled to maintain the bioreactor operation at a desired condition. pH is relevant due to the high pH value need for *Arthrospira platensis*. CO_2 gas flow is critical, since it will adjust the feeding requirements for the cells. Total gas flow-rate is very important because in a gas-lift reactor it dictates a high percentage of the reactor hydrodynamics, including mixing and gas-liquid transfer rates, that in tern dictate how CO_2 is transported from the gas to the liquid phase, and vice versa for O_2 . Light intensity control is critical in any photobioreactor, since this is the main source of energy for the photosynthetic metabolism of the cells. Finally, Biomass determination has also been considered within this group, since the control of the operation of the reactor is based on this measurement, an error in it will affect the optimal operation of the compartment, and introduce instability. After fixing the error, the normal operation at optimal conditions should be restored

A third group of variables has received a medium priority score. Still important variables, it is considered that a failure in their measurement would not directly have an irreversible effect on the bioreactor operation, or they are considered robust measurements. Among them are liquid flow-rate and liquid level in the reactor. In the case of temperature, it is indeed important to be maintained at the bioreactor set point, but certainly its measurement is one of the most robust ones, so has been considered within this intermediate priority level. Foam detection is also important to follow-up in this reactor, mainly because excessive foaming in the headspace area would contribute to a potential clogging of the gas outlet filters and therefore increase the pressure in the bioreactor.

Finally, a fourth group of variables is scored with the lowest priority level: dissolved O_2 concentration, and % of CO_2 and O_2 in the gas phase. In the same approximation followed for compartment III, it is considered that this measurements are important to follow-up bioreactor operation, but they are not critical in the sense that their failure would have immediate consequences and affect bioreactor operation.

Table 2: Compartment IVa variables priority

Variable	Priority	(control/indication)
Reactor pressure	10	Control
pH	8	Control
CO_2- gas-flow	8	Control
Total gas flow-rate	8	Control
Light intensity	8	Control
Biomass	5	Control
Liquid flow-rate	5	Control
Temperature	5	Control

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Level	5	Control
Foam detection	5	Indication
Dissolved O_2	2	Indication
CO_2-%	2	Indication
O_2-%	2	Indication

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5. MATERIAL COMPARTMENT ANALYSIS

5.1. Instrumentation

5.1.1. Biomass measurement

• Measuring principle

On-line monitoring of viable cells concentrations can be made, mainly, following two different technologies:

- optical density
- capacitance

Optical density technology: Depending of the optical path, two different categories can be established:

- Fixed optical path length where biomass concentration is measured as a function of near infrared absorption.

- Backscattered light where biomass concentration is measured as a function of near infrared reflectance.

Capacitance technology: Cells with intact plasma membranes behave as tiny capacitors under the influence of an electric field. The resulting capacitance, can be measured and accurately correlated to biomass concentration.

• Process requirements (Compartment IVa: priority 8)

Biomass concentration instruments must fulfil the following process requirements:

- Sensors must be installed in retractable housings, when possible
- Sensors must be sterilizable without process interruption
- Process connection must be biosafe in terms of protecting the axenicity of the process, and also in terms of avoiding the direct contact of the operator with the biological material of the sample.

Due to process priority we consider biomass concentration measurement should be redundant.

• Selected suppliers

(1) METLER TOLEDO: Sensor InPro 8000 series, Housing InTrac 799e, Transmitter Trb 8300

- Technology: optical, backscattered light
- Sterilizable retractable housing
- Sensor suitable for CIP / SIP
- Wide linear measuring range
- Compact design due to use of fibre optic technology
- FDA conformity
- Recommended process connection: weld-in socket DN25

(2) OPTEK: Sensors ASD19-N, ASD25-BT-N, AS16-N

- Technology: optical, NIR absorption probe
- Sensor suitable for CIP / SIP
- Direct measurement
- Sapphire optical window with no seals, gaps or crevices
- Path length optimized for cells and extremely dense bacterial cultures
- Hybrid LED light source provided superior stability without replacement
- Wetted parts with surface electropolished Ra<0.4
- 3-5 year lamp-life, replaced without removing probe from fermentor
- FDA conformity
- No retractable housing.

(3) ABER INSTRUMENTS: 12 mm probe, biomass monitor 220

- Technology: capacitance
- Suitable for CIP / SIP
- Flush electrodes with no sharp or protruding surfaces
- Measures capacitance in all conductivity ranges
- System is insensitive to cells with leaky membranes, gas bubbles and cell debris
- High biomass concentrations can be measured
- FDA conformity
- No retractable housing

5.1.2. pH measurement

• **Process requirements** (Compartment III: priority 10 / compartment IVa: priority 8)

pH instruments must fulfil the following process requirements:

- Sensors must be installed in retractable housings
- Sensors must be sterilizable without process interruption

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- Process connection must be biosafe in terms of protecting the axenicity of the process

Due to process priority we consider pH measurement should be redundant in both compartments.

• **Selected suppliers**

(1) METLER TOLEDO: Sensor InPro 3250 series, Housing InTrac 797e, Transmitter M700S

- Sterilizable retractable housing
- Low maintenance electrodes
- Pre-pressurized liquid electrolyte
- Electrodes suitable for CIP / SIP
- Built-in temperature sensor
- Biocompatibility and EHEDG certificate
- Constant self-cleaning action at the diaphragm
- Sensors with ISM technology (Intelligent sensor management)
- Double channel transmitter (pH/pH, DO/DO, pH/DO)
- Recommended process connection: weld-in socket DN25

(2) ENDRESS HAUSER: Sensor Orbisint CPS71D, Housing InFit H CPA475, Liquisys M CPM253

- Sterilizable retractable housing
- Long-term stable electrode with double junction reference system
- Pressurized reference system specially design for fermentation process
- Integrated bridge electrolyte
- Electrodes suitable for CIP / SIP
- Built-in temperature sensor
- Biocompatibility certificate
- Sensors with Memosens technology (Digital data transmission, predictive maintenance,…)
- Contact less inductive signal transmission (Memosens technology)
- Recommended process connection: weld-in socket DN25

5.1.3. DO (dissolved oxygen) measurement

• **Process requirements** (Compartment III: priority 8 / compartment IVa: priority 2)

DO instruments must fulfil the following process requirements:

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- Sensors must be installed in retractable housings
- Sensors must be sterilizable without process interruption
- Process connection must be hygienic

Due to process priority we consider DO measurement should be redundant in compartment III.

• **Selected suppliers**

(1) METLER TOLEDO: Sensor InPro 6800/6900, Housing InTrac 797e, Transmitter M700S

- Sterilizable retractable housing
- Compatible with pH assemblies
- Extremely low detection limit (1 ppb)
- Durable and rugged sensor
- Sensor suitable for CIP / SIP
- Built-in temperature sensor
- EHEDG certificate and 3A compliant
- Quick disconnect system, easy-to-replace membrane body
- Double channel transmitter (pH/pH, DO/DO, pH/DO)
- Recommended process connection: weld-in socket DN25

(2) ENDRESS HAUSER: Sensor Oxymax H COS21, Housing InFit H CPA475, Liquisys M CPM253

- Sterilizable retractable housing
- Long-term stable sensor
- Short response time
- Wide measuring range (0.01…20mg/l)
- Electrodes suitable for CIP / SIP
- Built-in temperature sensor
- Compatible with all pH assemblies
- Recommended process connection: weld-in socket DN25

5.1.4. Level measurement

Regarding the level measurement we should distinguish between detection of level and continuous measurement of level. For continuous level measurement several instruments following different technologies (hydrostatics, radiometrics, ultrasonics, buoyancy, capacitance,…) can be found on the market, but we consider hydrostatics technology is the more suitable one, since it fits process and installation requirements

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for this application. In pressurized vessels, to calculate hydrostatic pressure, it would be necessary to have either two pressure transmitters (hydrostatic and head pressure) or one differential pressure transmitter with two remote capillary connections.

For level detection we also can find several products following different technologies. In this case we selected different technologies depending on the product to be measured:

- Liquids: vibration limit switch
- Foams: capacitance or impedance limit switch

• **Process requirements** (Compartment III: priority 5 / Compartment IVa: priority 5)

Level instruments must fulfil the following process requirements:

- Process connection must be hygienic
- Sensors must be suitable for cleaning in place (CIP) and sterilization in place (SIP)
- Measuring range suitable for the application

• **Selected supplier for continuous level**

(1) ENDRESS HAUSER: Deltapilot DB50 S

- Technology: hydrostatic pressure
- Measured variable: continuous level
- Watertight, condensation-free with long-term stability
- Continuous temperature compensation of fill liquid ensure accurate measurement under changing process conditions and reduce downtime after SIP/CIP cleaning processes
- Suitable for SIP/CIP
- Flush-mounted process connections acc. 3A sanitary standards
- EHEDG certification
- Measuring cell ranges suitable for the application (100mbar or 400mbar)
- Recommended process connection:
    - Flush-mounted: universal mounting adapter with welded spud (1 ½")
    - Others: Sanitary dairy coupling DIN 11851 (DN40)

(2) ENDRESS HAUSER: DELTABAR S FMD78

- High reference accuracy: up to 0.075% of measured range
- Turndown 100:1
- Suitable process ranges: 100 mbar, 500 mbar
- Recommended process connection:

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- Remote flush-mounted diaphragm: sanitary dairy coupling DIN11851 DN-50.

• **Selected supplier for liquid level detection**

(1) ENDRESS HAUSER: Liquiphant M FTL 50H

- Technology: vibrating fork
- Measured variable: level detection
- No mechanical moving parts, no calibration
- Polished tuning fork and hygienic process connections: standard acc 3A
- EHEDG certification
- Recommended process connection:
    - Flush-mounted: welding neck (1")
    - Others: Sanitary dairy coupling DIN 11851 (DN32, DN40)

• **Selected suppliers for foam detection**

(2) CHARIS TECHNOLOGY: sensor FP103, transmitter FCW2

- Technology: Impedance measurement
- Application: level detection
- Specifically design to measure foam
- Suitable for SIP/CIP
- Controllers with IMA Sensing technology (Intelligent Multi-Action sensing)
- Hygienic process connections
- Immune to fouling
- Recommended process connection: weld-in socket INGOLD DN25

(3) ENDRESS HAUSER: Multicap DC 11TES , electronic FEC22

- Technology: capacitance measurement
- Measured variable: level detection
- Probes with universal use
- Suitable for SIP/CIP
- Hygienic process connections
- Active built-up compensation for limit detection, accurate switching point even with contamination on the probe
- Recommended process connection:
    - Flush-mounted: sanitary dairy coupling DIN11851 (DN50)

5.1.5. Gas / liquid flow measurement

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For low-flow control and measurement the instrument, which best meet the requirements is a mass flow controller.

• **Process requirements** (Compartment III: priority 5, compartment IVa: priority 8)

Mass flow instruments should fulfil the following process requirements:

- Process connection must be hygienic
- Sensors must be suitable for CIP and SIP
- Measuring range must be suitable for application

• **Selected suppliers**

(1) BROOKS: QUANTIM (liquid & gas)

- Combines sensor, transmitter, control valve and PID electronics in one compact, integrated package
- Accurate low-flow measurement
- Multivariable output: mass flow, volumetric flow, density and temperature
- Globally approved for a variety of service areas

(2) MKS (gas)

- Combines sensor, transmitter, control valve and PID electronics in one compact, integrated package
- Accurate low-flow measurement
- Widely use in a variety of gas delivery applications

5.1.6. Pressure measurement

• **Process requirements** (Compartment III: priority 4 / compartment IVa: priority 10)

Pressure instruments must fulfil the following process requirements:

- Process connection must be hygienic
- Sensors must be suitable for CIP and SIP

• **Selected supplier**

(1) ENDRESS HAUSER: CERABAR S

- High reference accuracy: up to 0.075% of measured range
- Turndown 100:1

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- Suitable process ranges: 100 mbar, 250 mbar

Two different types of measuring diaphragm materials may be used for pressure measurement:

Ceramic measuring diaphragm (PMC): The ceramic sensor is a dry sensor, the process pressure acts directly on the robust ceramic diaphragm and deflects it. A pressure-dependent change in capacitance is measured at the electrodes of the ceramic carrier and the diaphragm. The measuring range is determined by the thickness of the ceramic diaphragm.

Advantages:
- Guaranteed overload resistance up to 40 times the nominal pressure
- Thanks to highly-pure 99.9% ceramic:
    - extremely high resistance compared to Alloy
    - less relaxation
    - high mechanical stability
- Suitable for vacuums

Metallic measuring diaphragm (PMC): The operating pressure deflects the separating diaphragm and a fill fluid transfers the pressure to a resistance measuring bridge (semi-conductor technology). The pressure-dependent change of the bridge output voltage is measured and processed further.

Advantages:
- Can be used with process pressures up to 700 bar
- High process temperature without diaphragm seal up to 280°C/536°F
- High long-term stability
- Guaranteed overload resistance up to 4 times the nominal pressure
- Recommended process connection:
    - Flush-mounted diaphragm: thread 1 ½" G or NPT (PMC and PMP)
    - Others: sanitary dairy coupling DIN11851 DN-50

5.1.7. Temperature measurement

In temperature measurement we should distinguish two cases, depending on the measurement target:
- if the unique objective is to know the temperature, then, insertion type sensors would be the ones, i.e. which perform with better accuracy and fast response,
- if there is an additional objective to assure sterilization conditions in all parts of system, then it would be better to use surface temperature sensors strategically installed.

• **Process requirements** (Compartment III: priority 2 / compartment IVa: priority 5)

Insertion temperature instruments must fulfil the following process requirements:

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- Process connection must be hygienic
- Sensors must be suitable for CIP and SIP
- Continuous operation

• **Selected suppliers for insertion sensors**

(1) ENDRESS HAUSER: OMNIGRAD M TR47 / TR45

- Resistance thermometers specially designed for hygienic applications
- Replaceable mineral insulated inset
- Fast response time
- Surface finishing down Ra<0.4 micron
- Double Pt-100 for redundancy or validation purposes
- Sensing element with class A accuracy (DIN EN 60751)
- Welded or threaded thermowell, suitable for CIP /SIP process
- 3A certification
- Recommended process connection:
    - Welded thermowell (TR47)
    - Others: sanitary dairy coupling DIN 11851 DN-25, Ingold port DN25. (TR45)

• **Selected supplier for surface sensors**

(1) DESIN INSTRUMENTS: ST-FHH sensors (thermocouple or Pt-100)

- Thermocouple or resistance thermometer
- Thin and flexible, conform tightly to sensed surface, leaving no air gaps to block heat transfer
- Fast response, must be insulated from surrounded air
- Easy installation, in all positions, specially recommended when minimal distances must be kept.

(2) MINCO: thermal ribbons

- Thermocouple or resistance thermometer
- Thin and flexible, conform tightly to sensed surface, leaving no air gaps to block heat transfer
- Fast response, must be insulated from surrounded air
- Easy installation, in all positions, specially recommended when minimal distances must be kept.

5.1.8. Biomass cell-free sampling

• **Process requirements**

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- Process connection must be biosafe in terms of protecting the axenicity of the process
- The equipment must be suitable for CIP and SIP

In principle, a system is proposed here for “in situ” extraction of biomass-free samples. However, other possibilities may be also considered, depending on the reactor needs. Particularly, it could be interesting in some cases where the reactor has a recirculation line (i.e., high liquid flow) to install an external tangential filtration device, from which the retentate would be recycled into the reactor, and the permeate would allow for biomass-free sampling

• **Selected supplier**

(1) TRACE: ESIP filtration sampling probe

- Designed to withdraw sterile cell-free filtrate from bioreactors and fermentors
- Flow rates up to 1.5ml/min.
- Sterile filtration, no solids > 0.2 micron
- Increased safety by using a solid membrane
- Configured in either 12, 19 or 25 mm diameter
- Recommended process connection: weld-in socket INGOLD DN25

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5.2. Piping material

5.2.1. Pipe

- Material: AISI 316L
- Surface finish: Ra < 0.64 μm
- Unions: Welded or Sanitary Unions

5.2.2. Valves

- Type: Diaphragm valve; Saunders or similar
- Connections: butt welding ends
- Surface finish: Inside Ra 0.6 μm +electropolished
- Materials: Body = AISI 316L, Diaphragm= EPDM-PTFE
- Installation: The valve should be positioned at about 20º from the horizontal line in order to allow the fully draining of the line during the steam sterilization.

Note: plug and ball valves are not suitable for application in lines with a risk of infection, because product residues may be deposited between the moving surfaces and the space behind the plug or ball which cannot be removed without dismounting but may come into contact with the medium during operation.

5.2.3 Check valves

- This type of valve will have to be mounted to the greatest possible extent in a part of the pipe, which causes no risk of infection yet.
- Type: disc type check valve
- Material: AISI 316L
- Surface finish: Ra 0.6 μm +electropolished
- Connections: butt welding ends

5.2.4. Steam traps

- For removing air and condensates during steam sterilization. The design of the steam trap should be cover the following requirements:
  - For sterilization to be effective the system should be de-aerated prior to the steam sterilization phase.
  - At the start of and during sterilization the condensation water formed should be removed from the units concerned.
  - Contamination by (small quantities of) product residues must not obstruct drainage of condensation water.
- Type: membrane steam traps; Spirax-Sarco MST21 or similar
- The operation principle of these steam traps is a balance between the temperature and the current pressure. The steam trap is automatically opened or

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closed by means of a membrane. The condensate is drained at a temperature a few degrees below the steam saturation temperature.
- The recommendation is to install beyond the infection sensitive part to the greatest possible extent.

5.2.5. Safety valve

- Spring-loaded safety valves and vacuum valves should preferably not be applied in sterile parts. If a safety device is required, rupture disc is to be used.

5.2.6. Sampling valves

- Requirements:
    - For sterile sampling
    - Possibility to sterilize the valve before and after every sample
- Type: membrane sample valve; Keofitt M4 or similar
- Material:
    - Body : AISI 316
    - Diaphragm : Silicone

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5.3. Equipments

5.3.1. Agitators

- Magnetic drives seal is recommended to prevent any foreign body or microbiological contamination.

5.3.2. Pumping

- Due to the small-required capacity, the best option is the selection of a peristaltic pump for aseptic purpose. The main infection risk for this pump, when working during long periods of time, is the risk to break the hose, stopping consequently the process and losing the sterility of the installation.

Another inconvenience for the peristaltic pump is the control of the flow-rate due to the fluctuations produced by the pump.

- As alternative to the peristaltic pump it is proposed the transfer by means of gas pressure. The feeding vessel should be pressurized with a gas at a pressure around 1 bar above the discharge vessel. The liquid flow-rate will be regulated by means of a mass flow controller.

- When a flow-rate control is not needed (for example the liquid transfer from the reactor to the discharge vessel) it is recommended to adapt the layout to allow the transfer by gravity. In this case the installation of a siphon in the line, will be necessary to avoid the pass of the gas. For this configuration it is necessary to guarantee a similar pressure in both vessels (in the reactor and in the discharge vessel)

5.3.3. Gas compressor

- The compressor should be located, when possible, in the not sterile part.
- Oil free and gastight type is required to avoid process contamination and lost of the gas.
- Diaphragm type as KNF or similar it is recommended.

5.3.4. Sterile filters

- Filtration degree: 0.2 μm
- Steam sterilizable
- Membrane type filter; Sartorious or similar

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6. References

Pérez, J.; Montesinos, J.L.; Albiol, J. and Gòdia, F. (2004) Nitrification by immobilized cells in a micro-ecological life support system using packed-bed bioreactors. Journal of Chemical Technology and Biotechnology 79: 742-754

7. Annex

In electronic format all the supplier information for the instrumentation proposed in this technical note is annexed in a CD, containing:

- Biomass
- Biomass cell-free sampling
- Flow
- Oxygen
- pH
- pH-O2 transmitters
- Pressure
- Retractable sensor housing
- Temperature

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8. Comments

Page/paragraph	Comment
overall	It should be clarified that the "selected" suppliers mentioned are potential suppliers, recommended for our purpose. A new sentence has been added regarding this points, in page 9 of the revised document, at the end of section 3: *"It should be mentioned here that the specific selected suppliers identified in this work are potential suppliers, recommended in first option as compiling with the required performance. A reduced number of suppliers is selected for each measurement, but certainly other possibilities could also be explored in case that the final technological and economical offers from the suppliers were not fully satisfactory for the optimal deployment of the MPP. The suppliers selection has been made on the basis of existing equipment and instrumentation and the experience provided by its use in several applications in the field of industrial fermentation processes, with high requirements of axenicity and long term operation"*
overall	When possible and relevant, it would be interesting to motivate the suppliers list proposal": is it based on a paper assessment from suppliers'documentation, on experimental feed-back, on experimental feed-back from similar application to ours? E.g. with regards to the use of the biomass-free sampling probe, do we have any idea about the suitability of the probe design to our reactors? Any information on clogging of the membrane? Does the sampling flow meet our requirement? The same sentence of the previous point is consideing these general aspects as well
25/5.1.8	Rk: you recommend a biomass-free sampling probe; however, in the TN on CIII redesign, you ask the potential supplier to propose /design technologies. Any comment? This is because in here we concentrated in sensors that could be used "in-situ" in a bioreactor. Then, when discussing with Applikon in regards to the sampling port for ammonium, etc., the proposal of am external tangencial filter came in, that in case it is also interesting as part of the recirculation loop. A sentence has been added to mention this other potential solution. A new comment on this direction has been added in point 5.1.8: *"In principle, asystem is proposed here for "in situ" extraction of biomass-free samples. However, other possibilities may be also considered, depending on the reactor needs. Paticularly, it could be interesting in some cases where the reactor has a recirculation line (i.e., high liquid flow) to install an external tangencial filtration device, from which the retentate would be recycled into the reactor,*

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	and the permeate would allow for biomass-free sampling"
26/5.2	What is the rationale of the Ra value you give with regards to our application? This is a typical value for pharmaceutical industry and axenic processes Any numbering issue for check valves? OK, this is fixed now, with the number 5.2.3

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