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MELiSSA – Adaptation for Space

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TECHNICAL NOTE 72.10

Proposed future work

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1. Introduction

In the MSA contract, a breadboard for *Arthrospira* harvesting was designed, constructed and tested. It consisted of an ultrasonic separation system, followed by an ultrafiltration unit. In this technical note, suggestions for future work are formulated as well as suggested improvements for the breadboard and its adaptation for space.

To give a general idea about the constructed breadboard, an overview of the conceptual scheme is shown in Figure 1.

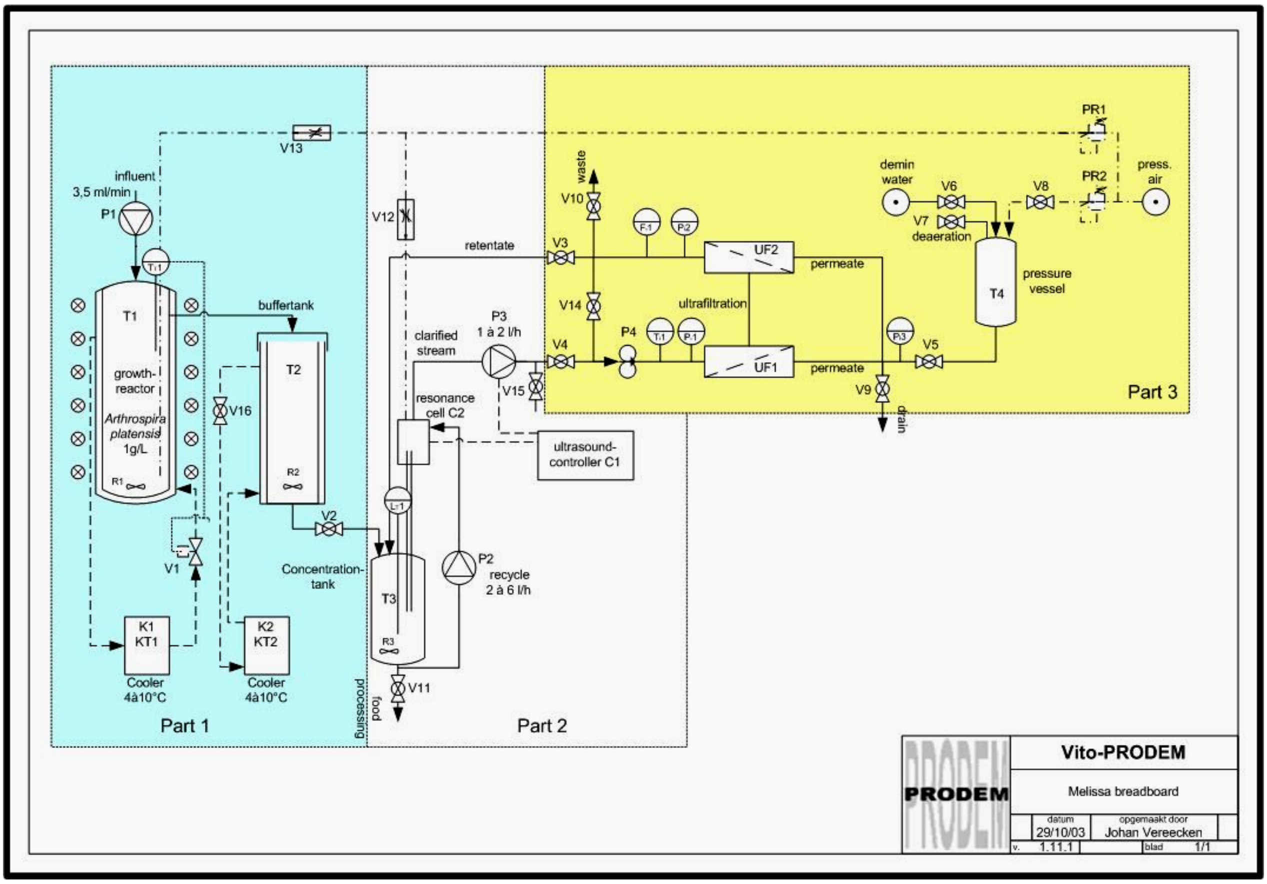

Figure 1. Overview scheme of the breadboard for *Arthrospira* harvesting.

2. Suggestions for future work

2.1 Critical items

2.1.1 Photobioreactor

The photobioreactor was not considered as a sub-system in the harvesting system of *Arthrospira platensis.* It was constructed to ensure a daily algal production of 5 L to be processed by the harvesting system. It is not in the scope of this study to focus on the adaptations that should be made on the photobioreactor since, it is planned in framework of other projects like ; Biorat. However, some suggestions, deduced from our experiments on the photobioreactor could be helpful for the other projects.

The first major changes will concern the light intensity. During the testing of the breadboard, a focus was made on the growth rate. Light calibrations were fixed upon stable daily algal production. A 5 litres of algal suspension with a concentration of around 1 g/L was produced per day. It was not in the scope of the study to demonstrate light influence and adaptation in function of the required oxygen production. It would therefore, be interesting to study this parameters, and by this mean to construct a breadboard where the photobioreactor (compartment IVa in the MELISSA-loop) is aimed to produce and process biomass (harvesting and desalination) and to produce the necessary oxygen or partly of the oxygen needed for the crew.

2.1.2 Automation of the breadboard

In the first test experiments, the harvesting system was not automated. Despite the fact that the algal production was operated in a continuous mode, the harvesting and desalination operations modes were discontinuous, meaning that the concentration and desalination were manually initiated and stopped. The option to automate the breadboard should be studied and optimized for each sub-system (ultrasound unit and ultrafiltration unit) and compared with the efficiencies obtained with the non-automated system.

2.1.3 Sterility of the system

The sterility of the breadboard is still an open question. How sterile the system has to be? Do we need a complete sterilization of the loop including the growth reactor and harvesting/desalination system? Which sterility techniques could be used ?

2.1.4 Chemical consumption

The consumption of chemicals is mainly an item for the operation of the membrane filtration unit. In the constructed breadboard, no need for membrane cleaning occurred, in spite of the fact that the initial tests were performed at much higher algae concentrations than it was designed for. Membrane fouling was probably reduced by sufficiently high cross-flow velocities and by regular backwashes with clean water.. It remains to be seen whether a backwash with spent culture medium (after desalination by electrodialysis) will be as efficient for this purpose.

Membrane fouling can not be avoided in any case. Long term system running will end up with clogging of the ceramic membrane despite their robustness and long life. Therefore, techniques related to cleaning procedures (chemicals, high temperatures,…) should be tested and evaluated. It was experimentally observed that the degree of irreversible algae binding to ceramic membranes was lower than for polymeric ones. Selecting the ceramic ones will therefore reduce the number of cleanings and the amount of chemicals required. Choosing for ceramic membranes also has the advantage that they can withstand higher temperatures than polymeric membranes. So it is possible to use a heat treatment for membrane cleaning. The disadvantages are however that the weight of ceramic membranes is much higher and that more energy is consumed when heat treatment would be used for membrane cleaning. Additional tests should be performed on membrane cleaning to determine what is the optimal cleaning procedure and frequency.

In the breadboard, a continuous supply of chemicals is needed to prepare fresh Zarrouk medium. The overall harvest concept however included an electrodialysis step which would split the spent medium into a clean water flow which can be used in the next washing step of the algae suspension and a concentrated salt solution which can be used to prepare fresh medium to the photoreactor. This would significantly reduce salt consumption, although some chemicals would be needed for cleaning of the electrodialysis membranes and electrodes.

It is doubtful that growth of *Arthrospira* on full strength Zarrouk medium will occur in the final MELiSSA concept. A reduction in the salinity of the feed to compartment IV will of course be beneficial in terms of chemical consumption but can only be applied when the growth pattern of *Arthrospira* is not disturbed.

2.2 Influent supply and waste recovery

During the demonstration tests, the feed medium was Zarrouk medium. the possibility to recycle the filtrated liquid stream wasted out of the system to the photobioreactor was not investigated. By including an electrodialysis sub-system, it could be possible to separate the desalinated diluate (free of salts) from the concentrated liquid stream (with high salt contents). Using this technology, the clarified stream will serve to wash the alga, minimizing thus the need of high volumes of demineralised water, and the concentrated stream will be recycled to the photobioreactor for the growth of the alga. This option could give a positive insight to the future flight possibilities by minimizing the use of chemicals in the feed medium of *Arthrospira platensis*. Influence of low salts concentrations on growth rates, on the shape of the alga , exoplysaccharides production and light intensities should be considered in the study.

2.3 Ultrasound separation system

The purpose of this unit is to harvest the algal biomass by concentrating it to a 10 to 20 folds. In our investigations, a maximum of 10 folds biomass concentration factor was obtained. The possibility to increase this factor was studied during the operation phase of the breadboard but not successful. The option of increasing the concentration efficiency up to a factor of 20 or even more could be studied in the future by adding an extra pumping system capable of pushing the aggregates accumulated in the resonance cell down to the concentration tank giving by this means an additional force to the recirculation pump (P2) aimed to recycle the biomass accumulated in the resonance cell to the concentration tank.

2.4 Energy requirements

If the breadboard will be automated and additional sub-systems included like the electrodialysis, additional on-line sensors, actuators will have to be considered. This suggest that additional power must be added to the supply line. Thus, energy requirements of the system will be somewhere higher than the one needed for the non-automated system.

3. Breadboard improvements

Overall, the breadboard test results demonstrated that the proposed concept of ultrasound and ultrafiltration works and that it performed according to our expectations. For further evaluation, potential upscaling and/or coupling to the Pilot Plant, improvements may be required.

3.1 Critical items

. The limiting factors in the design of the breadboard were the following:

3.1.1 growth of the algae

As mentioned already, the focus in this study is not directed towards the performances of the photobioreactor. However, a good quality algal suspension is related to the growth conditions in the photobioreactor and other parameters. In the photobioreactor and the collection of the overflow of cells is continuous. The concentration steps by ultrasonic separation and the washing steps occur batchwise because the amount of water needed to achieve a certain degree of desalination is much lower compared to continuous operation. Furthermore, the last step in the harvesting necessarily needs to be a batchwise concentration of cells. Otherwise, one would never obtain a concentrated cell suspension required for food processing.

3.2 Harvesting/washing cycles

the overflow of the *Arthrospira* growth reactor had to be harvested and washed during one working day. Because harvest and washing occur sequentially, the time available per cycle was limited to about 2 h.

3.3 Ultrafiltration unit

In the ultrafiltration unit (UF), the clarified stream had to be concentrated 20 times to a final volume of 200 ml. The total dead volume of the ultrafiltration unit therefore needed to be approximately 200 ml.

3.4 Upscaling of the breadboard

The following constraints have to be considered for upscaling:

1. A batchwise approach for harvest and concentrating steps is still advisable in view of a reduced water need and the final concentrated cell suspension.

2. For practical reasons, it is preferable to finish the concentration and washing cycles in one working day. When *Arthrospira* is cultivated in Zarrouk medium, at least two washing cycles will be required to meet nutritional demands. In other words, each concentration cycle will have to be finished in 2 hours. Since the largest ultrasound system available has a capacity of 200 l/d or 8.4 l/h, 3 systems will have to be operated in parallel to process a 70-l harvest per day. However, it is improbable that compartment IVa will be fed Zarrouk medium in the coupled MELiSSA loop. In that case, the number of washing cycles can be reduced, the duration of a washing cycle can be extended and the capacity of the ultrasonic system can be reduced.

3. In the tested breadboard, it was decided to further concentrate the ultrasound effluent in a batchwise mode in the ultrafiltration loop. This implies that all the biomass coming from the

ultrasonic system is recirculated for 2 h in the loop. Then, it is recovered by returning it to the concentrated cell suspension for washing. As a result of the stress, biomass quality may deteriorate. Alternative approaches are the following:

a. it may be advisable to waste the biomass going to the ultrafiltration unit rather than recycle it. The UF remains important though in the total concept. In the overall concept, the permeate of the ultrafiltration step is further desalinated by electrodialysis, generating two streams: a desalinated diluate which can be used to wash the concentrated cell suspension and a concentrate enriched in salts which can be recycled to the photobioreactor in which *Arthrospira* is grown. The electrodialysis step was not included in the breadboard and washing steps were performed with distilled water. When electrodialysis is included, pretreatment of the water by ultrafiltration is advisable.

b. The cell suspension could be continuously recycled to the concentrated cell suspension. However, this would continuously dilute the cell suspension Even when larger volumes of algae cells are to be processed, the duration of cell recycle will still be high and lead to deterioration of nutritional quality.

The breadboard was not designed to operate in axenic conditions. Adaptations would require:

- closure of all tanks and vessels
- construction in sterilizable materials
- provisions for axenic sampling
- ..

3.5 Automation

The breadboard was not automated. Automation will require a different choice of valves and pumps, the incorporation of transmitters and fieldpoint modules, the presence of on-line sensors for measurement of algae breakthrough, conductivity etc. In principle, the UF can easily be automated. This has been demonstrated in many other applications. For the operation of the ultrasound system, it is not clear at present how the controller can be extended or taken over externally.

3.6 Desalination of the spent culture medium

The breadboard did not yet contain a technology for desalination of the spent culture medium and the washing solutions. From experiments at laboratory-scale, electrodialysis was preferred to reverse osmosis because of its flexibility and better adaptation to space requirements. Inclusion of an electrodialysis step requires the following investigations:

- performance on the real feed of spent culture medium and washing waters
- effect of the generated diluate on the washing processes
- effect of the concentrate on fresh medium preparation and composition

Applicability of the presented breadboard to the harvesting of other organisms in the MEliSSA loop is low because ultrasound is not applicable to bacterial cells.

4. Adaptation for space

Some of the aspects have already been discussed in TN 72.8. The space adaptations which should be applied to the photobioreactor, the cooling systems, electronics,…etc. are studied in other projects. Here, we focused on the adaptations that should be made on the harvesting system for flight experiments.

4.1 Compatibility with microgravity

In the tested breadboard, two processes were gravity dependent i.e. cell transfer between tanks (from growth reactor to buffer tank and from buffer tank to concentration tank) and the ultrasonic separation. The dependency of cell transfer on gravity can be eliminated by using pumps.

For the ultrasonic separation, part of the separation process consists of a settling of aggregates. A first pump circulates the cell suspension from a reactor through a resonance chamber back to the reactor. A second pump drags clarified water (= harvest) out of the chamber at the top. Acoustic forces retain the cells in nodal planes where they form loose clumps. As long as the ultrasonic field is switched on, the clumps are held stationary against the fluid drag in the chamber. However, to prevent clogging of the chamber with cells, the field needs to be switched off at regular time intervals. During that period, the pump in the harvest line is switched off and the aggregates settle due to gravitational forces.

To eliminate the dependency of ultrasonic separation on gravity, two approaches can be envisaged:

- a suction could be applied on the recirculation line to drag the aggregates back into the reactor when the ultrasonic field is switched off. Even at normal gravity, this may aid the settling process.
- a prime rate reverse pump can be used in the harvest line. It has the advantage that it automatically reverses the flow direction when the ultrasonic field is switched off. However, attention has to be paid to the fact that the resonance chamber may be completely empty of the cell suspension and part of the clear filtrate in the harvest tube may return into the chamber. Therefore, stop times should be sufficiently short.

4.1.1 Effect of reduced gravity on the physiology of *Arthrospira platensis*

No very much is known about the behaviour of *Arthrospira platensis* under microgravity conditions. The possibility to form gas vacuoles and to float during separation, their settling velocity have so far not been specifically studied. This could be done in the framework of space adaptation of the breadboard.

4.2 Equivalent system mass considerations

As yet, the design of the harvesting system has not been optimised with respect to volume requirements. Aspects to be addressed are:

- the number of tanks needed
- the possibility to combine different units in one
- the time frame in which the harvesting and washing has to be finished, etc.

These will depend to a large extent on the medium composition for *Arthrospira* cultivation that will be decided upon.

Operation of the breadboard is quite labour intensive. To reduce manpower requirement, the harvesting system will need to be automated to the highest possible degree. The collection of the growth reactor overflow in the buffer tank, the transfer of the harvest of one day to the concentration tank, the sequence of concentrating and washing steps, temperature control and the operation, backwashing and cleaning of the ultrafiltration (UF) unit can easily be automated. When an electrodialysis unit is included in the final design for desalination of the UF permeate, automated control can be included for the electrodialysis process. As mentioned in 3, the ultrasonic separation system will present more challenges for external control and automation.

Power consumption of the breadboard is high and will even increase when an electrodialysis unit is included in the harvest system, when analysers and controllers have to be added, etc. In a worst case scenario, currents were estimated to amount to 32 A at 380 V for the constructed breadboard.

4.2.1 Volume and mass restrictions

For space adaptations, some constraints exist. In the previous study, the opportunity to compact the breadboard down to defined volume was not of major importance. For flight experiments, in general, some limitations in volume and mass are required according to rack standards. It is therefore important to study these criteria when designing a space adapted breadboard.

4.3 Lighting system

Light emitting diodes (LEDs) and fluorescent lamps can be used to supply photosynthetic active radiation (PAR). From a constructive point of view, the LEDs offer the most benefits for use in space environment (Summary report Biorat accommodation study). The major benefits are:

- The wavelength can be chosen
- Long life
- Robust and not sensitive to vibrations or chocks and no risks for explosion or fractures
- Fewer heat losses compared to fluorescent lamps
- Possible to control the light intensity.

4.4 Safety considerations

4.4.1 Axenicity

To avoid contamination of gaseous and aqueous environments, all tanks and reactors will have to be constructed as air-tight closed and sterilizable vessels. This will in turn require pressure control throughout the system, both in the liquid and the air loop. Special attention will have to be given to the control of pressure differences and the accumulation of gases in the ultrafiltration unit.

4.4.2 Safety

The choice between an electrodialysis and reverse osmosis unit as desalination technology was among others made based on safety aspects. Reverse osmosis operates at high pressures and therefore requires a high pressure pump and pressure-resistant materials in the filtration loop. The high pressure pump causes vibrations which is undesirable for space applications.

The ultrafiltration unit in the breadboard operates at much lower pressures and does not pose this type of safety risk.