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NTE

MELiSSA – Adaptation for Space

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Requirements and performances of the Liquid-Solid Separation Systems

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1. Introduction

The work performed in the MELISSA Pilot Plant during the last ten years has been focused on different main lines of work:

- Characterisation of the main relevant physical characteristics of the 75 litres bioreactor for compartment IVa, and first operation under carbon limitation conditions.
- Operation of the different packed-bed reactors used for compartment III, focusing mainly on the effect of liquid flow, oxygen gas flow, and ammonium input concentration and recirculation flow rate.
- Initial experiments under illuminated anaerobic conditions in the airlift bioreactor of compartment II, focusing on the effects of light intensity and carbon limitation.
- Bench scale basic studies of cell growth at different illumination conditions for the cells used in compartment II, *Rhodospirillum rubru*m.
- Operational tests for the harvesting procedure to separate the cells produced in compartments II and IVa, foreseen to be used as crew food ingredients.
- Bench scale physical connection of bioreactors from compartments II, III and IV.

Liquid-solid separation is an important aspect of the MELISSA loop since the different five compartment of the loop contain their specific micro-organisms. A liquid-solid separation system has two major functions: first to prevent that organisms are transferred from one compartment to another and second to permit the fermentation "wanted" product to be transferred from one compartment to another.
The compartments that need a separation system are:

Compartment I: Liquefying compartment → compartment II *(Rhodospirillum rubrum)*
Compartment II: Rhodospirillum→ Compartment III (Nitrifying reactor)
Compartment III: Nitrifying → Compartment IVa (Arthrospira)
Compartment IVa: *Arthrospira platensis* → Compartment I (consumer)

The traditional methods of separating cells from culture broth have been conventional sedimentation, centrifugation and filtration. However, methods of liquid–solid separation based on sedimentation are generally problematic and inefficient since they do not produce clean liquid as desired in all the cases in the MELISSA loop.
Some measurements parameters are important to make a decision for the selection of the most appropriate liquid-solid separation technology for specific requirements , mainly:
particle size, sedimentation velocity, solids concentration, liquid vapour pressure, solid and liquid flows rate, batch or continuous mode and the expected percentage separation without damaging the active biomass.

2. Definition of LSSS requirements

Most liquid –solid separation systems, required in the MELISSA loop, aim to the recovery and further proceeding of the biomass for substantial use as food. The only reactors, which do not comply with this law, are the waste compartment I and the nitrifying compartment III, where the harvesting and proceeding of the biomass is not necessary. However, maintaining

the amount of suspended solids in these compartments is important to guaranty stable quality of the effluents.
The separation technology used for one compartment is not the same as the one used in the other compartment since each of them is inoculated with one specific type organism, possessing its own morphological and physico-chemical characteristics. It is therefore, necessary to define, for each reactor, the appropriate liquid-solid separation technology.

Liquid-Solid Separation is a major unit operation that exists in almost every flow scheme related to the chemical process industries, ore beneficiation, pharmaceutics, food or water and waste treatment. The separation techniques are very diverse and depending on the purpose of the separation:

- Solids recovery
- Liquid recovery
- Both solid and liquid recovery
- Preventing water pollution

2.1 Physical Liquid-Solid Separation systems

These LSSS usually involve settling by gravity, screening, or centrifugation. The processes rely on: The density, The size, or shape of the individual particles.

A basic requirement for an efficient physical separation is the continuous agitation of the liquid that is to be processed. Otherwise, the relatively fast sedimentation processes that occur during storage leads to reduced efficiencies.

Different separation systems are actually being used mostly for:

- Filtration by vacuum and pressure filters.
- Centrifugation by filtering and sedimenting centrifuges.
- Sedimentation by conventional, storage and high-rate thickeners.
- Clarification by conventional, solids-contact and sludge-blanket clarifiers.
- Polishing by vacuum precoat filters and pressure filters
- Upward separation by dissolved-air flotation.
- screening, hydrocycloning and froth flotation

Other related subjects that have to be considered in the LSSS in general are:

- Coagulation and flocculation by bridging polymers.
- Moisture reduction by wetting agents.
- Particle analysis and its influence on performance.
- Defining a Relative Filtration Index during the research phase.
- Establishing diminishing returns on wash efficiencies and drying.
- Filtering medium and its selection.
- Evaluating long-term effects on separation rates.

It is not in the scope of this study to point out all the above-cited techniques but some of them, which, could be relevant in the future technology of the separation techniques in MELISSA loop are lighted out but not definitely selected.

2.2 Limitations of the LSSS in MELISSA loop

2.2.1 Chemicals addition

Because the addition of chemicals for effective separation is not considered, especially in the *Arthrospira* and *Rhodospirillum* compartments, most attention will be pushed towards mechanical and physical Liquid-Solid Separation techniques.

Because of the nature of solid and liquid quality that is wanted, the addition of flocculants, coagulants or any chemical to enhance separation is not possible to prevent chemical contamination. Moreover, the use of chemical induces mass accumulation.

2.2.2 Continuous or batch mode

Finally, the choice of continuous or batch mode usually depends on the nature of the upstream and downstream process. If the upstream and downstream process is continuous, a continuous separation device reduces the hold-up and storage of slurry and solids to keep these processes going. A batch unit stream can feed a batch separation with little or no immediate hold-up.

In the MELISSA loop, the processes are mainly in continuous mode. Thus, the solid-liquid separation equipment has to be capable to operate in continuous mode, but, in some cases, due to specific requirements or characteristics, batch mode should be considered as well. The latter could be helpful in the following cases:

- Flow –rate variations
- Low process dynamics
- Contamination prevention

3. Requirements and performances of the LSSS

Liquid-solid separation is required in compartments I to IV as mentioned above. For compartment I, the task for the definition of the liquid separation requirements is in the scope of the contract “Engineering of the Waste Compartment”. In the three other compartments, different separation processes should be taken into account since each compartment is colonized with one microbial community completely different from the one colonizing in the next compartment (Table 1).

The most difficult compartment in terms of liquid-solid separation is the *Arthrospira* compartment IVa. This photosynthetic compartment will have to ensure the major tasks of the ecosystem since it will have to regenerate the atmosphere and to produce consumable biomass. The photosynthetic filamentous Cyanobacteria, *Arthrospira* are good candidates for this compartment on account of their high photosynthetic efficiency, their reasonable biomass production and ease of harvesting by simple separation techniques.

Table 1. Liquid-Solid Separation Systems in MELISSA-loop

Compartment	**Liquid-Solid Separation**	**Reference**
Waste compartment (I)	Engineering of the waste compartment – Contract	EWC-study
Photoheterotrophic compartment (II)	Centrifugation	Vernerey, A., Montesinos, J.L., Godia, F. (TN 37.30)
Nitrifying compartment (III)	Fixed bed reactor	Perez, J.; Montesinos, J.L.; Godia, F. (TN 37.510)
Photosynthetic compartment (IVa)	Melissa Space Adaptation – Contract	In the loop of this study

3.1 General considerations for liquid-solid separation systems

At each level of the MELISSA as well as at outputs and inputs of each compartment, some parameters and variables should be controlled to ensure efficient connection between the compartments and thus efficient solid-liquid separation. The major parameter is the flow rate. The latter is important since synchronization in the liquid transfer between the different compartments is generally based on continuous circulation mode of the liquid stream, as suggested in the proposal of MELISSA project. However, because cell concentrations, which should be harvested for use (mainly from compartment II and IVa), are rarely high enough to ensure satisfying separation efficiencies through centrifugation or filtration and thus, require separation techniques in batch mode to fulfil the requested percentage separation. The latter in most cases should be as close as possible to 100%. Control variables for each compartment are presented in Table 2.

Table 2. Control and disturbance variables in MELISSA-loop.

Compartment	Control variables	Disturbance variables
Compartment I	Temperature Total pressure pH dissolved oxygen Volume Dilution rate NH_4^+ and VFA/CO_2	
Compartment II	Temperature Total pressure pH Urea VFA Biomass	Liquid out put of CI (Flow rate, composition)

Compartment III	Temperature Total pressure pH Volume Dilution rate Dissolved O_2 NH_4^+, NO_3^-, NO_2^-	Liquid out put of CII (VFA, NH_4^+, urea)
Compartment IVA	Temperature Total pressure pH Volume Dilution rate Biomass Dissolved O_2	Liquid out put of CIII (flow rate and NO_3^-)

3.2 Relevant features for the selection of the separation system

For effective solid-liquid separation in all MELISSA-compartments, some considerations and features should be included in the choice of the separation technology:

- Minimum and maximum capacity of the separation technique
- Pressure required
- Biomass concentration attained
- Biomass purity
- Salts retention
- Liquid and solid percentage recuperation (yield)
- Separation efficiency
- Life time of the membranes….

Other variables have also to be taken into account such as accumulation of poly-hydroxy-butyric acid (PHB) in the photoheterotrophic compartment (compartment II), which can affect the performance of the separation unit and exopolysaccharides (EPS), which may decrease the separation efficiency as well in the phototrophic compartment (compartment IVa).

3.2.1 Compartment I

The liquefying compartment, or compartment I, is responsible for the biodegradation of human faecal material and other waste generated by the crew like non edible parts of the plant material, toilet paper,…. The volatile acids, ammonia, gases and soluble components produced during the fermentation are fed into the second compartment of *Rhodospirillum rubrum* species. Previous to entering the second compartment, the fermentation products need to be separated from the remaining non-soluble fraction of the MELISSA substrate and the anaerobic bacteria escaped from the liquefying reactor. For this purpose different strategies have been proposed to obtain rich-salts, VFA, ammonium-sterile filtrate with the aim of feeding the second compartment (TN 51.4). Up to now, the volatile fatty acids and the ammonium are separated from the liquefying reactor content by centrifugation at a speed of

3000 rpm. The supernatant, separated from the cake, contains 96% of the total volatile fatty acids and 79% of the total ammonia, but also 40% of total dry weight is present in the supernatant. This must be avoided, therefore better separation techniques need to be found. MELISSA partner laboratories have also tested the efficiency of membrane filtration in compartment I. The separation techniques at this level are not in the scope of this project and are included in the project of Engineering of the Waste Compartment.

3.2.2 Compartment II

The anoxygenic phototrophic compartment II metabolises some of the compounds of the liquid loop, mostly the carbon sources likely to be found at the exit of Compartment I of MELISSA and which, have been identified as the following volatile fatty acids : acetic, propionic, butyric, isovaleric and isobutyric.), with edible biomass generation. To achieve this goal *Rhodospirillum rubrum* is cultured in an anaerobic environment, either in photoheterotrophic or in photoautotrophic conditions.
Rhodospirillum is a genus of photosynthetic bacteria of the family Rhodospirillaceae. This bacteria falls under the Alpha subdivision of the kingdom Proteobacteria. Their cells are generally spiral-shaped, polarly flagellated and contain vesicular, lamellar of stacked photosynthetic membranes (Singleton and Sainbury). They range from 3 to 10 µm in length and 1/2 to 1 micrometers in width. Cells divide by binary fission. One of the type species of this genus is *Rhodospirillum rubrum*, a purple nonsulfur bacteria inoculated in compartment II. Purple non-sulphur bacteria like *Rhodospirillum* are capable of photoheterotrophic growth on an array of organic carbon sources and some have also been reported as being able to grow photoautotrophically with H_2 as an electron donor and CO_2 reduction (Yoch, 1978). *Rhodospirillum* as well as *Arthrospira platensis* can be harvested by centrifugation, and many studies demonstrate that the same method can be carried out for both microorganisms (Becker 1981, Borowitzka 1988, Ripley and Fox 1996, Grizeau et al. 1996).

The photobioreactor is known as a continuous stirred reactor and yields an effluent from which the biomass must be recovered and used as human feed since it high content in amino acids. The final effluent should be exempt from bacteria because it flows to the third nitrifying compartment. The latter being very sensitive to extern contamination. Thus, the aims of separation process could be resumed as follows:

- Cells concentration
- Recovery of intact cells without any changes in taste and quality
- Obtain clear and clean effluent at the outlet of compartment II

The selection of the best separation technique is not in the scope of this note. However, since some work has already been performed in the photoheterotrophic compartment as well as in the phototrophic compartment by MELISSA partners in Barcelona (TN 37.30), some data are reported in the note, essentially with two main solid-liquid separation techniques: centrifugation and filtration. Both compartments are indeed aimed to feed the space crew in the MELISSA loop and the separation technique, which could be applied for one, has may be its applications on the other one.

3.2.2.1 Measured variables

In compartment II, some variables should be measured to allow proper selection of the solid-liquid separation technology.

Biomass concentration in the reactor

Cell concentration in the output of the photoheterotrophic compartment is low, in the order of 0.5-3 g/L with a steady state achieved at 1.4 g DW/L (at light flux F_R = 136 W/m^2 , Dilution rate D= 0.04/h (HRT = 24 h) and Carbon concentration C= 1 g/L) as reported in technical note 37.30. Separation of *R.rubrum* at these concentrations does not seem to be critical as shown by the good results obtained after separation through centrifugation in MELISSA loop. The final configuration of the biomass harvester included two separation steps: the first separation unit was a disc stack centrifuge working at flow rates of 1 to 4 L/h and high rotation speed of 10000 rpm. The second system consisted of a microfiltration step which, had as major task to clarify the liquid stream. The overall system could ensure a water yield of 75-80%, a solid yield of more than 95% with a total separation efficiency higher than 95%. The energy requirements of such a combined systems was estimated of about 55 Kwh.

Concentrating in a continuous mode has the disadvantage that the supernatant is not completely free of cells and that these cells may be damaged or altered during their thickening. In most of the tests performed in Barcelona, the cell concentration in the clarified stream after centrifugation was always lower than 0.1 g/l. This is not satisfactory since the stream which, is aimed to flow to the nitrifying compartment should be exempt of cells at least with cell concentrations lower than 0.01 g/l. The technique, however, is being successfully used in the pilot plant to harvest *Rhodospirillum rubrum* in Barcelona despite its disadvantages.

Sedimentation velocity

It can be considered that the efficiency of centrifugation step is directly dependent on the sedimentation velocity of the particle to be removed at fixed operation conditions. Sedimentation velocity depends on the solid density, the solid size (effective or mean diameter) as well as on liquid viscosity. *Rhodospirillum rubrum* is a small bacteria. There size range from 3 to 10 µm in length and 1/2 to 1 micrometers in width (http://distans.livstek.lth.se:2080/rhodospi.htm), thus having high biomass density if compared with *Arthrospira (*2 µm in width and form spirals or straight filaments with length up to 100µm) . As a result, its sedimentation velocity is higher than *Arthrospira* and so, in terms of liquid -solid separation and clean water recuperation, is higher.

Separation efficiency and flow rates

Different possibilities to carry out this function were analysed in TN 37.30, and a combination of two techniques, centrifugation for cell recovery, and membrane filtration of the liquid stream before connection to the next compartment were selected as the most appropriate processes for the pursued objective. The first is a continuous disc stack centrifuge working at moderate flow rates (1.0-4.0 l/h) and high rotation speed (1000rpm). The performance of this system in term of separation efficiency is very high (> 95%). The second separation unit is a tangential filtration unit having the ultimate role of clarification of the liquid stream. It operates at high permeate/retentate ratio (around 5) and a feed flow rate of 2 l/h in order to recover the liquid as much as possible and with a minimal cell concentration (< 0.01g/l). The separation efficiency of this system is > 95%. The water yield is always kept above 75 % reaching at steady state, after approximately 60 min, a value close to 100% being higher than 95% of recovery.

Osmotic changes

Rhodospirillum can be more sensible to osmotic changes than *Arthrospira,* but on the other hand its culture medium pH is very close to neutrality and should not present any problem from a practical point of view. However, one should take into account that the separation techniques in use have to ensure 100% separation efficiency or a closer percentage. If the latter is lower than 100%, contamination of compartment III may occur. Knowing that the *Rhodospirillum* needs a minimum of light to grow at near neutral pH, and that the nitrifiers from compartment III are very sensitive and have slow growth rate, it is very important to obtain 100% separation from compartment II to avoid loss in the nitrification efficiency of compartment III.

3.2.2.2 Estimated variables

Some variables should be calculated from the given data mainly, the shape of the cells, which should be taken into account when the separation technique has to be selected. One should be aware that the structure of the cells should stay intact after separation. Moreover, some component, could be formed in excess, after separation due to cell breakage, in the liquid like poly-hydroxy-butyrate (PHB). This may negatively influence the performances of the separation technique. PHB is considered as a normal constituent in the body of *Rhodospirillum* present at low concentrations (intracellular level < 3%) (Vincenzini et al., 1990) as reserve of carbon and energy. Excess of PHB presence in the liquid stream may be due to two reasons: Leakage of the biomass and therefore release of body inclusions or excess PHB production due to the stress of the biomass after separation.

Separation efficiency and damage of the cells

The integrity and viability of the cells is higher for the membrane processes than for the centrifugation ones. In general, it can be estimated that the cell disruption, for one single centrifugation cycle, is lower than 5-10% of the total of cells treated.

PHB accumulation

The presence of important quantities of poly-hydroxy-butyrate (PHB) can decrease the efficiency of the separation system and more specifically of the centrifugation step. Since PHB is an important molecule on cytoplasm and cell walls, biomass centrifugation may promote the creation of aggregates due to the polymeric structure of PHB that link bacteria to each other. It was shown that nitrogen limitation induces accumulation of PHB and glycogen at the expense of protein. While the quantity (i.e. mass percentage in the biomass) is under the control of the C-source excess, the choice of the storage material (glycogen or PHB), depends on growth conditions (Poughon, 1995), and is probably under the control of an energetic balance (ATP/reduced power).It can then be assumed that the more the assimilation of a substrate leads to the synthesis of reduced cofactors, the more important will be the synthesis of PHB versus the synthesis of glycogen. On the other hand, if the substrate leads to ATP synthesis, rather than reduced cofactors synthesis, the carbon storage will be oriented to a glycogen synthesis rather than a PHB synthesis. The storage material (PHB, glycogen) appears to be under the control of two factors (which can themselves depend on the different growth condition): the type of substrate and the internal energetic balance of the cell. This balance depends on the type of the substrate, but depends too on the growth conditions.
Preliminary kinetic experimental results obtained on batch cultures of *Rhodospirillum rubrum* in rectangular photobioreactors (PBR) showed that it was necessary to consider an

intermediate zone in the photosynthetic bioreactor, with a particular relaxation metabolism, which could be responsible in the high level of intracellular PHB accumulation (Cornet *et a*l., 1999; Cornet and Albiol, 2000).

Membranes fouling and clogging

If one consider tangential filtration step as possible technique of solid-liquid separation in compartment II, problems concerning clogging and fouling of the membranes are not expected to be important since the small diameter of *Rhodospirillum* (width of ½ to 1 µm).

3.2.2.3 Connection of compartment II to compartment III in bench-scale

The volumes managed during the continuous runs of the photosynthetic reactor at bench scale, were too small to use a continuous centrifuge as reported in TN 43.8. .For this reason a batch centrifuge (10.000 rpm, 4°C 20 min) was proposed. As the centrifugation operation was a discontinuous process, two buffer tanks, one for the outlet of compartment II and the other for the inlet of compartment III were required. This allowed collecting the liquid effluent for centrifugation and storing the biomass free medium after the centrifugation step. This centrifugation step was done daily as the quality of *Rhodospirillum rubrum* decreases significantly when it is stored during a longer period.
To avoid contamination of the centrifuged media for compartment III, sterilisation technique before introducing it to the input storage tank for the third compartment is required. To this purpose, attention should be paid to select the most indicated sterilization technique.

3.2.3 Compartment III

The objective of compartment III (nitrifying compartment) is to transform the ammonium ions present in the exit stream from compartment II into nitrate, the most appropriate nitrogen source assimilated by the cells cultured in compartment IVa. It consists of a packed-bed reactor with cells of two bacterial strains *(Nitrosomonas europaea* and *Nitrobacter winogradskyi)* immobilized. The support material was selected in previous studies (Forler, 1994). and consists of polystyrene beads (Biostyr).

3.2.3.1 Measured variables

Specific growth rate

Due to the very low maximum specific growth rate of both species of nitrifying bacteria (Hunik et al., 1993), a washout of the microorganisms could be a main drawback in continuous cultures. It can be overcome by biomass retention as applied in biofilm reactors. In addition, the lack of interest in generating cells that cannot be used as food did that the nitrifying process was conceived as an immobilized cells bioreactor.. Most of the bacteria are supposed to grow, forming a biofilm on the packed material, but one can not exclude the fact that the effluent still contains free cells from the reactor as well as detached flocs of biofilm due to die-off or fall-off. The effluent originated from compartment III should be exempt from bacteria and remains as axenic as possible to be further used as influent for compartment IVa.

3.2.3.2 Estimated variables

Separation efficiency

It is not necessary to recover dewatered biomass since the latter will not be used as edible, consumable biomass. The separation in this case is based on the recovery of clean liquid, where no cells are suited to be present. The choice of the solid-liquid separation technique was focused on the cleanliness of the liquid flow and not on the quality of the cells after separation. This have made the selection of the method easier than in the previous compartment. However, one should not forget to take into account that the nitrifying bacteria are difficult to sediment and to centrifuge and that addition of flocculants or coagulants is not considered in the running of MELISSA loop.

3.2.3.3 Connection of compartment III to compartment IVa in bench-scale

Some experiments were performed in bench-scale by MELISSA partners in Barcelona (TN 43.8). In their investigations, they found that the outlet of compartment III contained, in some cases, escaped biomass. Two filtering steps using membrane (ultra)-filtration were, based on their results, necessary included to retain the nitrifying biomass from entering the phototrophic compartment and to avoid wash out. The first one in the output of compartment III and the second one at the input of compartment IVa. In this way, the two compartments were isolated allowing disconnection in case of malfunction in one of them. It is thus very primordial that the flow from the nitrifying reactor should be completely free of cells before entering the photosynthetic compartment. Moreover, the separation technique should not influence the nutritional quality of the liquid, in other terms, the amount of salts and more specifically of nitrate produced should not be retained or decrease because of the separation technique used in order to satisfy the demand of the phototrophic compartment.

3.2.3.4 Quality control of the liquid stream from compartment III

The bioreactor outlet and the input media could be checked for bacterial contamination by accurate microbiological methods, such as direct observation using microscopic techniques and new molecular techniques like DGGE. The collected nitrifying biofilm is not supposed to be reused and can thus be removed from the system as dry as possible.

The separation efficiency should be close to 100% and if not, the nitrifying bacteria may enter the phototrophic compartment. However, if the separation efficiency is lower than 100%, the nitrifying bacteria will not develop in the phototrophic compartment since the high pH and temperature. At a temperature of 35°C, only the ammonium oxidizers may grow over the nitrite oxidizers and thus ammonium oxidation will certainly stop at nitrite rather than at nitrate state which, is not suited since the poisonous effect of nitrite on plants, algae and human. Moreover, it is obvious that at such high pH of 10-11, bacteria are not supposed to grow and certainly not the nitrifiers

3.3 Requirements and performances of the LSSS in compartment Iva

The objectives and requirements of the LSSS in the MELISSA project aim to concentrate the cells to a certain extend depending on the final processing and uses of the biomass. The concentration of the latter is of major importance in:

1. Pilot plant where MELISSA Space Adaptation is situated: Aims to harvest, concentrate and further processing (MELISSA phase 1).
2. Biorat contract: Aims to concentrate. (MELISSA phase 2)
3. Future flight and Mars based concept.(MELISSA phase 3)

The work presented in this note deals with phase 1 of MELISSA project and thus one will focus on the requirements and objectives related to photosynthetic compartment (CIVa) in this cadre.
The general concept of MELiSSA includes the use of the biomass generated in the two photosynthetic reactors, *Arthrospira* in compartment IVa and *Rhodospirillum* in compartment II, as food supply. The main task of the photosynthetic compartment (CIVa) within the MELISSA loop is the fixation of CO_2, concomitant with the generation of edible biomass, and the regeneration of O_2 for crew respiration by means of the growth of the micro-algae *Arthrospira platensi*s.

Previous studies conducted by Soeder 1986; Borowitzka and Borowitzka 1988; Borowitzka 1992; Sirenko and Pulz 2000; Tsoglin and Gabel 2000 have shown that both microorganisms can be used as supplement in the food diet of men. On the other hand, *Arthrospira* has been used as an important source of proteins in children suffering from malnutrition and different types of food and pills based on *Arthrospira* are commercialised widely. Also, it has been used as human food for centuries, and forms part of the diet of tribes of Lake Chad and was used as food by aztecs in Mexico. This cyanobacteria presents a high nutritional value and contains all the essential aminoacids, besides cysteine, in the adequate concentrations according to the FAO proposed standards. The production system is currently implemented in airlift reactors where *Arthrospira platensis* is cultivated.

The bioreactor requires light supply, and the cells use nitrate as nitrogen source. The work developed, up to now, in the MELISSA project on this compartment includes two main aspects. First, the physical characterisation of the external loop airlift photobioreactor already installed in the pilot plant in Barcelona, which, allows the identification of the most appropriate operational conditions, as well as an improved interpretation of experimental data. Second, the continuous operation under carbon limiting conditions allows to adapt the mathematical models, previously developed by partner laboratories, to the bioreactor and operational conditions of the pilot plant.

3.4 Selection criteria for the LSSS: Biomass concentration

To be able to make a satisfactory trade-off of the different Liquid-Solid Separation technologies in use for the photosynthetic compartment , some selection criteria should be taken into account. The possible separation techniques, which were selected, to be tested in the photosynthetic compartment, are presented in Table 3. The major important selection criteria, their weight (importance) and classification are lighted out.

3.4.1 Biomass concentration and cell dilution

One of the most important criteria for the selection of the Liquid-Solid Separation Technique is the separation efficiency. The envisaged separation has to recover the cells, producing a paste with a high percentage of liquid elimination (at least between 75% - 90%). The objective is to provide a complete clear liquid stream to be pumped to the next compartment. As mentioned in TN 37.30, if centrifugation is selected as the most promising separation

technique, the continuous centrifugation uses liquid to discharge the solid paste retained in it, that is the cells, there is a certain final degree of dilution of the cells (even minimizing this amount) which, typically are discharged from the centrifuge at a concentration of about 10-20 g/L. It is clear that if the final use of the cells as food additive requires a lower percentage of water (for example, if they are required completely dried, or freeze-dried), then an additional step has to be incorporated for the elaboration the final product.
Additional experiments allowing minimization of the phenomenon of cells dilution during discharge, have been carried out during the period 1998 by MELISSA partner-laboratories. The concentration factor, that is to say, paste concentration/feed concentration, have been increased to values up to 20-30, that implies a net recovery of water higher than 95 %. This factor is fixed in ideal conditions by the ratio between solids discharge volume and feed volume. As the solids discharge volume is rather constant independently of the operational conditions, the most important variable to take into account is the feed volume.

3.4.2 Breakthrough of the cells

The passive passage of the cells in the liquid stream may occur if the Liquid-Solid Separation technique does not ensure a complete separation of the liquid stream from the concentrated biomass. As proposed above, the liquid stream should be clear to be re-pumped to the photobioreactor or eventually to another compartment. However, in case the liquid stream is pumped back to the photobioreactor, the breakthrough of the cells does not represent any danger to the system.

3.4.3 Energy requirements

Each LSSS requires a certain energy for its functioning on the desired capacity. The energy requirements are directly related to the heat production. High energy uses means a lot of heat production which might be recovered, reused or dissipated . For each technique the energy requirements expressed as Kwh/m^3.harvested biomass will have to be determined.

3.4.4 Biomass integrity

Individual cells of *Arthrospira* are around 2 µm large and form spirals or straight filaments with length up to 100µm. On one hand, the separation technique, which would be selected has to ensure a complete end product without disturbances in the form and as complete integrity as possible with conservation of the nutritional quality of the alga. On the other hand, the same separation technique should be able to separate, cell debris, dead cells from the intact cells without breakage of the latter. Cell breakage and shear stress, which may happen during the separation, can occasionally, lead to extra exopolysaccharides (EPS) production in the liquid flow. EPS, outside the cells, are recognized to increase the viscosity of the liquid and cell density, and thus to decrease the separation efficiency (data of GEPEA-Nantes University, actually under investigations).
Whatever will be the separation technique, the latter should ensure the integrity of the cells in the concentrated product.

3.4.5 Water recovery

This criterion is important in the sense that the liquid stream collected has to be forwarded back to the photobioreactor or sent to the next compartment. In each of this cases, the quality of the recovered water is different. In case the liquid stream has to be recycled to the photobioreactor as recycle feed or to wash the biomass, water recovery is important and may

reach percentages of 95% to 99%. If the liquid stream is aimed to be stocked in a tank and not introduced in the MELISSA loop, the water recovery is of less importance. Finally, if the liquid stream has to enter the next compartment (CIVb), the water recovery should be optimal, at least 95% to 99% recovery.

3.4.6 Salts recovery

The separation of *Arthrospira* is more complex than the liquid-solid separation in the other compartments since the medium contains high salts concentration, among which carbonates and thus needs to be separated from the biomass and, if needed, to be recycled back to the photobioreactor. Moreover, the pilot photobioreactor, when connected to the other compartments of the MELISSA loop will probably be in limiting conditions which, supposes that the salts will always be limiting, reason why it would be interesting to recycle the salts-rich liquid stream to the photobioreactor. In addition, the salty taste of the concentrated paste could be diminished if the salts are recovered in the liquid stream.

3.4.7 Consumables

It is important to mention for each techniques in use, the frequency of cleaning, the type of cleaning agents used and the life time of the elements constituting the system. Some separation techniques need maintenance regularly and some others none.

3.4.8 Mass

The weight of the system is an important criterion in the sense that the system is aimed to be up scaled to space use. The mass of each system with its accessories has thus to be defined and compared with the other systems.

3.4.9 Safety issues

Pressure, high velocities and temperature and space safety requirements are additional parameters which, should be considered for the selection of the LSSS. Safety issues are one of the most important criteria for the selection of the most promising separation technique. By defining these parameters as one of the main selection criteria, one can show the difference in the LSSS from this point of view.

3.4.10 Potential improvement for space

Not all the LSSS used on earth could be upscaled or improved to be used for space applications. If centrifugation may have a significant weight (importance) among the other techniques, it should however, have a potential improvement for space; which is obviously not the case because of the high speed applied and the centrifugal forces. It is also of main importance to determine the potential of improvement for each separation technique for space.

Table 3, represent the selection criteria for the used LSSS. The importance of each criterion is represented by a weight. The latter is specific for the actual application (MELISSA SPACE ADAPTATION).

Table 3. Selection criteria for the LSSS for a concentration 10 - 20 g/L

				TECHNIQUES			
	CRITERIA	**UNITS**	**WEIGHT**	**A**	**B**	**C**	**D**
1	Separation efficiency	%	100				
2	Breakthough of cells		50				
3	Energy requirements	KWh/m^3	50				
4	Biomass integrity	%	100				
5	Biomass recovery	%	100				
6	Water recovery	%	50				
7	Salts recovery	%	50				
8	Consumables		50				
9	Mass	Kg/m^3	100				
10	Safety issues		100				
11	Potential of improvement		100				
Total							

A = Ultrasonic separation **B** = Ultrafiltration **C** = A + B **D** = Centrifugation

1	**2**	**3 (to be adapted)**	**4**	**5**	**6**	**7**	**8**	**9 (to be adapted)**	**10**
0% = 0	YES = 0	1000 kWh/m^3= 1	NO = 0	NO = 0	90% = 1	NO = 0	YES = 0	> 500 kg/m^3 = 0	Not adapted = 0
~50% = 1	NO = 1	500 kWh/m^3 = 2	~ 50%= 1	~ 50%= 1	95% = 2	~ 50%= 1	NO = 1	100 kg/m^3 = 1	Adapted = 1
~95% = 2		100 kWh/m^3 = 3	~ 90% = 2	~ 90% = 2		~ 90% = 2		50 kg/m^3 = 2	
100% = 3		50 kWh/m^3= 4	100% = 3	100% = 3		100% = 3		10 kg/m^3 = 3	

3.5 Secondary criteria

3.5.1 Flow rate regulation during separation

From the fact that the feed volume is the product of the discharge time and the flow-rate, best results will be obtained for both high discharge time and flow-rate. However, high discharge times has the disadvantage of producing excessive breakage of the cells and consequently a decrease on the product quality. As a consequence, from a global point of view the best strategy is to use high flow-rates with moderate discharge times (0.5-2 h). From the moment that the flow-rate recommended is about 10 L/h this implies that a strictly continuous operation is not possible. For instance *Arthrospira* bioreactor works with an outlet flow-rate ranging from 0.5 to 2.5 L/h. The possibility to re-circulate the filtrate obtained from the centrifuge allows to increase feed flow-rate and then to reach high-concentration factors (always related to entrance/exit of the separation technique of use). However, the fresh feed solution is diluted by the re-circulation and then, the overall efficiency of the system is kept low (solids concentration). Thus, an operation in batch or at least semi-continuous mode is recommended for this centrifugation step.

Problems induced by membrane filtration if the latter is selected:

- Changes in the size and morphology. Recent studies performed by MELISSA partners showed sealing of the filaments of Arthrospira and changes in the shape of the alga due to circulation in the filtration unit. However, these changes did not damage the alga itself but may complicate the efficiency of harvesting.
- The circulation of the alga in the filtration unit may induce more exopolysaccharides (EPS) presence outside the filaments, this is due to the shear stress (data reported by GEPEA-Nantes University in the cadre of the actual contract). This can affect the harvesting system significantly. When the EPS concentration increases, inducing variations in the physical properties such as density, viscosity, size, a risk of fouling, concentration polarization and clogging of the membranes may occur.
- Further experiments conducted by partner-laboratories of MELISSA project will focus on the changes, which, may occur when different solid-liquid separation techniques are used.

3.5.2 Washing and conditioning of the biomass

There are as well other factors that should be considered in order to define more precisely the process of preparation of the biomass as food. These are, at least the washing of the cells, in order to eliminate their excess of salts and the treatment of the food to get it free of microbiological activity and with the required degree of water content. Clearly, these steps can be combined, as some operations can enable to reach simultaneously two of the objectives. Their selection and application depends also on the definition of the conditions envisaged in the final product.

3.6 Selection criteria for the LSSS: Biomass desalinisation

The removal and recycling of salts is an important aspect for the separation system of *Arthrospira* algae. The NaCl concentration for example in the Zarrouk medium is quite high (1g/L) and may have high influence on the final quality of the consumable *Arthrospira.* Therefore, it is important to find a strategy to separate the salt from the edible biomass. Salts, in general, can be removed by washing several times the algae. However, this operation requires additional washing water. Two possible alternatives to prevent a high use of washing water are the use of electrodialysis or inverse osmosis to concentrate the salts. It is not in the scope of this technical note to select the most promising technology for salts removal and cells concentration. It seems to us however, necessary to present the technological aspects of some methods, which are actually used for desalination.

Membrane technology

In nature, membranes play an important role in the separation of salts. This includes both the processes of dialysis and osmosis that occur in the body. Membranes are used in two commercially important desalting processes: electrodialysis and reversed osmosis (RO). Each process uses the ability of the membranes to differentiate and selectively separate salts and water. However, membranes are used differently in each of these processes. Electrodialysis uses an electrical potential to move salts selectively through a membrane, leaving fresh water behind as product water. In the (RO) process, pressure is used for separation by allowing fresh water to move through a membrane, leaving the salts behind (Figure 1).

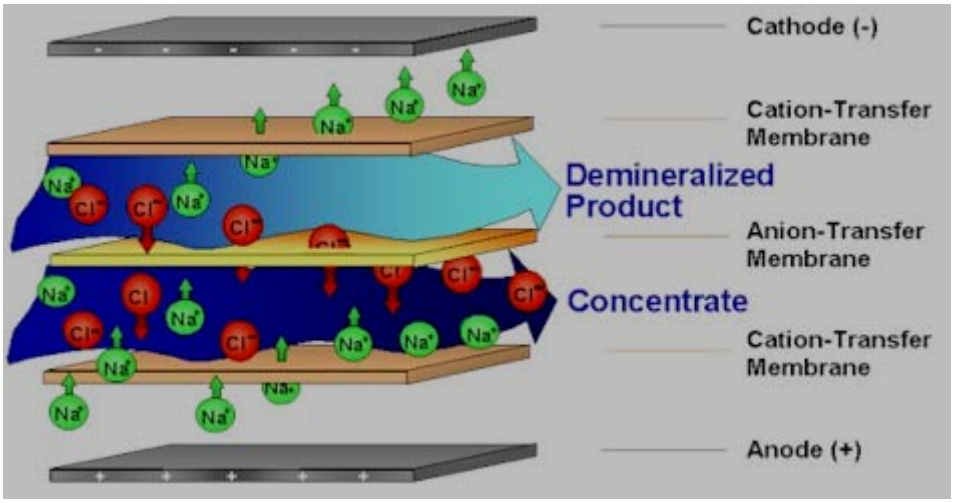

Figure 1. Schematic representation of electrodialysis process (http://www.aquacool.com/toolbox/edr.htm)

It is very important to select the most appropriate desalination method for *Arthrospira platenis* to guaranty the highest nutritional quality of the alga. The main disadvantage encountered in most of the industrially cultured farms was the taste of the alga during

consumption may be due to the fragmentation of the filaments during processing by electrodialysis at high current intensities.

4. Conclusions

The most important variables, which should be considered when selecting the solid-liquid separation technique in the MELISSA loop are almost the same in most of the compartments (e.g. biomass concentration, % separation, liquid and solid flow rates, aspect of the cells…). However, the purpose of the solid-liquid separation should be also taken into account as in the case of the photoheterotrophic compartment (CII) and the photoautotrophic compartment (CIVa). In these two cases especially, the separation technique should guaranty a 100% (or as closer as possible) separation efficiency and the recovery of high quality biomass (retentate), the latter aimed to be used as edible biomass by the crew of shuttle space. Moreover, the potential toxicity and pathogenicity of the recovered biomass should be eliminated to ensure high quality biomass use as food.

5. References

Becker E.W. (1981). Algae mass cultivation-Production and Utilisation. *Process Biochemistry,* p. 1 O-27. Aug/Sep

Borowitzka, W.A. and Borowitzka, L.J. (eds) (1988). Micro-algal biotechnology. Cambridge University press

Borowitzka, MA. (1992) Algal biotechnology products and processes: matching science and economics. *J Appl Phycol.* 4: 267–279

Soeder, C.J. (1986) An historical outline of applied algology. In: Richmond A (ed) Handbook of microalgal mass culture. CRC, Boca Raton, Fla., p 25

Ciferri O. (1983). Arthrospira, the edible microorganism. *Microbiological reviews.* p.551-578.

Colon, W.J. 1990. Membrane processes. In : Pontius, F.W. (ed.) ,Water Quality and Treatment. A handbook of Community Water Supplies, American Water Works Association, McGraw-Hill, 4th edition, 709-746

Cornet, J.-F., Dussap C.G. and Leclercq J.-J. 1999. Simulation and model based predictive control of photobioreactors. In: *Proceedings of the ninth European Congress in Biotechnology (ECB9*), July 1999, Bruxelles.

Cornet J.-F. and Albiol J. 2000. Modelling photoheterotrophic growth kinetics of *Rhodospirillum rubrum* in rectangular photobioreactors. *Biotech. Pro*g. In press.

Crew, N.; Albiol, J. and Godia, F. 1999. Preliminary connection between 3 compartments. ESTEC/CONTRACT11549/95/NL/FG

Demey, D.; Van Meenen, P. and Hermans, V. Contract :ESA/ESTEC PO 163031

Forler, C. (1994), MELISSA : development of a fixed bed pilot reactor for a continuous axenic coculture of Nitrosomonas europae and Nitrobacter *winogrudskyi*. YGT ESA/YCL, X-997, 1994.

Hermans, V. and Demey, D. 2000. Filtration : a literature review. T.N.51.4. ESA contract 12922/98/NL/MV

Hunik, J.H., Bos, C.G., Van Den Hoogen, M.P., DE Gooijer, C.D. and Tramper, J ; (1994). Co-Immobilized *Nitrosomonas europea* and *Nitrobacter ugilis* cells : validation of a dynamic model for simultaneous substrate conversion and growth in K-carrageenan gel beads. *Biotechnol. Bioeng.*, 43, 1153- 1163.

Grizeau, D; Dupre, C; Guary, J.C and Bernard, S. (1996). Production of freeze-dried biomass of *Rhodospirillum rubrum.* Furniture and analytical results. (T.N. 29.2). Equipe Biotechnologies Marines. Conservatoire National des Arts el Metiers. Institut National des sciences et Techniques de la mer. Cherbourg. Delivery to ESA_ESTEC

Lembi, C0., Wharton, R., Smernoff, D. and Averner, M. (1988). Algae and human affairs.. Algae in space. Cambrige University press. p.485-509.

Lenguaza, B., Albiol, J. and Godia , F. (1998). Growth on different C sources. Part II. T. N: 37.82

Poughon. L. (1995). Modelling of the MELiSSA artificial ecosystem. (T.N. 23.3) ESTEC/CONTRACT ECT/FG/CB/95.205 PRF 141315.

Ripley, L. and Fox, J.M. (1996). Arthrospira, production and potential. Edisud Aix-en-Provence.

Sirenko, L. and Pulz, O. (2000) Influence of algae on physiological and biochemical processes in plants. Abstracts of the 4th European workshop on biotechnology of microalgae. Bergholz-Rehbrücke, Germany

Tsoglin. L. and Gabel, B. (2000) The technology of production of biomass labeled with stable isotopes. Abstracts of the 4th European workshop on biotechnology of microalgae. Bergholz-Rehbrücke, Germany.

Vernerey. A., Monterinos. J.L. and Godia, F. (1998). "Biomass Harvester" ESTEC/CONTRACT I1549/95/NL/FG (T.N.: 37.30)

Vincenzini. M., Sili. C., De Philippis. R., Ena. A. and Materassi. R. (1999). Occurrence of Poly-β-hydroxybutyrate in *Spirulina* species. Journal of Bacteriology, pp. 2791 – 2792.

Yoch, D. (1978). Nitrogen fixation and hydrogen metabolism by photosynthetic bacteria. In: Clayton R. and Sistron W. (Eds.) The photosynthetic bacteria, Plenum press, New York, pp. 657-676.