**Departament d'Enginyeria Química**
Escola Tècnica Superior d'Enginyeries
Universitat Autònoma de Barcelona
Tel.: 93.581.10.18 Fax: 93.581.20.13
08193 Bellaterra Spain

# MELISSA

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## Pilot design of the MELISSA solid loop

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**CREUS, N.; MONTRÀS, A.; MASOT ,S.; PÉREZ, J.; ALBIOL, J.;**

**GÒDIA, F.**

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# 1 INTRODUCTION

The MELISSA project (Microbiological Ecological Life Support System Alternative) of the European Space Agency (ESA) is a tool for the development of a biological life support system to be used during Manned Space Missions. In order to achieve this purpose the project proposes the connection between five compartments, four of which contain microbial organisms and one higher plants. These different compartments of the loop have been developed during previous phases.

The liquefying compartment, or compartment I, is responsible for the biodegradation of human fecal material and other wastes generated by the crew. The volatile fatty acids, ammonium, gases and soluble components produced during the fermentation are fed into the second compartment.

The anoxygenic phototrophic compartment II metabolizes some of the compounds produced in the anaerobic liquefying compartment, with edible biomass generation. To achieve this goal *Rhodospirillum rubrum* and *Rhodobacter capsulata* are cultured in an anaerobic environment, either in photoheterotrophic or in photoautotrophic conditions.

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 IV. It consists in a packed-bed reactor with cells of two bacterial strains (*Nitrosomonas europaea* and *Nitrobacter winogradskyi*) immobilized onto polystyrene beads (Biostyr).

Compartment IVa has as its main task the carbon dioxide removal and supply of oxygen for the crew respiration generating at the same time edible biomass as food supply. This compartment is currently implemented in airlift reactors where *Spirulina platensis* is cultivated. 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 higher plants compartment, or compartment IVb, is the basic food supplier for the crew.

The research done until now has been mainly focused on compartments II, III and IVa, thus the interconnected operation of these compartments at two different levels, bench scale and pilot scale, has been also studied during a long term operation either under different perturbations of the system or at optimal conditions.

However, in order to demonstrate the validity of MELISSA as a model system for biological ALSs, the closure of the complete loop in the Pilot Plant has to be achieved. This closure, also includes the crew compartment, which, in the Pilot Plant , will be inhabited by rats; and the faecal material and urine collecting chamber.

The achievement of this goal is a very complex task, requiring a careful design, combining all the information and conclusions generated during the previous years of research, including the different MELISSA brainstorming sessions and design meetings, together with a thorough preparation, scheduling and meticulous implementation.

A preliminary review of the Pilot Plant integration loop, to be used as a starting point, was previously done (Gòdia, F. *et al.*, 2001).

In this technical note the preliminary engineering design of the MELISSA solid loop to be installed in the MELISSA Pilot Plant laboratory is described. This technical note is complemented by technical notes, 62.3 and 62.4 which describe, correspondingly, the engineering design of the MELISSA liquid loop and the MELISSA gas loop components.

This description is the base line for further discussion and will finally lead to its physical implementation in the upgraded MELISSA laboratory.

# 2 COMPARTMENT SET UP DESCRIPTION

## 2.1.- Crew compartment

As previously established, rats will inhabit the crew compartment in the MELISSA Pilot Plant. The rats caring will be done manually.

Neither the urine nor the faecal material of the rats will be recycled in the MELISSA loop, as its purpose is to recycle human wastes. Thus, these rats' wastes will be taken out from the loop.

Rats will be initially fed using external water and food; however, the use of loop water and loop plants in further rats diet can be studied.

Thus, this compartment will only be joined to the MELISSA loop through the gas phase. The $CO_2$ produced by the rats will be converted to $O_2$ by means of compartments IVa and IVb.

## 2.2.- Collecting compartment

It was previously agreed that “no-mix-toilets” will be used. Thus, urine and the faecal material will be collected and stored separately. These toilets already exist (Braun, U; 2001 and Galler, L; 2001).

However, different aspects have to be decided.

Taking into account its future use in the space, the toilets used in this compartment should be similar to the ones used in Space missions.

As water cannot be used in the weightlessness of space to pull the waste into the canister below the closet, suctioned air can be used for this purpose. This will also allow

storing the human faeces without extra water, decreasing its volume, weight and facilitating further uses.

Suctioning the wastes to the treatment canister below is also the solution actually used in space shuttles and stations by NASA and ESA (Halvorson, T., 2001). This system is a little different depending on whether the astronaut urinates or defecates in the toilets used by the Japanese Space Agency (NASDA) (NASDA, 2001). When urinating, the waste is sucked away in a hose. But when defecating, the toilet is used the same way as a normal toilet and the astronaut pulls a lever to have the waste sucked away. Then the waste is vacuum-dried.

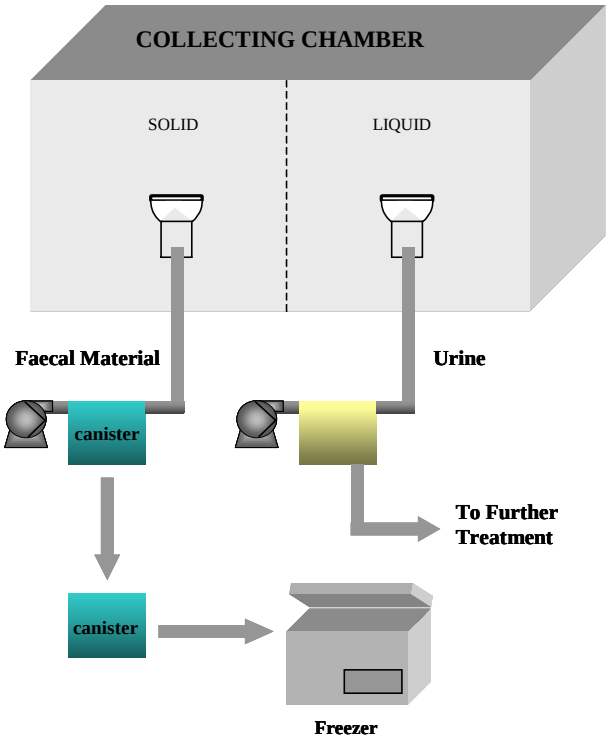


**Figure 1.-** Scheme of the collecting chamber

Thus, by sucking, the faecal material can be collected in a canister and then it can be either dried or frozen, as it is done now (Hermas, V. and Demey, D., 2001a). This canister can be a single-use canister that can be manually changed every time after using the closet in order to avoid the degradation of the faecal material. The faecal material can be stored frozen in the same canister until use in order to make this process as user-friendlier as it can be, or it can be vacuum-dried. However, in order to facilitate

the process it seems easier to directly freeze the canister than having to dry the faecal material. A scheme of the collecting chamber is found in figure 1.

The urine, as said, will be stored separately. The conversion of the containing urea to ammonium can be easily done by means of urease (Behrendt, J., *et al.*, 2001). It is not agreed where the urine will be added but it is evident that, as one of the main objectives of the MELISSA loop is to demonstrate the nitrogen closure, and as the main part of nitrogen coming from human wastes is found in the urine, urine has to be used in the loop.

## 2.3.- Compartment I

This compartment has as its main task to decompose or liquefy the biomass components and macromolecules generated in the other compartments to, basically, volatile fatty acids and ammonium.

The main solid out coming from this compartment is the non-degradable cake that is obtained in the membrane modules of this compartment (Hermas, V. and Demey, D., 2001b and Albiol, J. *et al.*, 2001). This cake is of no use in the actual MELISSA loop. However, new studies are being developed in order to investigate its possible utilization. As in the MELISSA Pilot Plant different solid treatment process will be available, they can be applied to this cake in case it is needed.

Preparation of the input of this compartment will require, as a first step, to collect the biomass from different sources and its proper storage to avoid degradation. The different collection methods and treatments are described in this technical note.

Higher plant biomass, faecal material and microbial biomass will be available frozen, knowing its amount of residual water. Thus, periodically, dry weight measurements of each input will be required.

Preparation of this mixture is expected to be done in batches of around 50 litres in volume. The preparation procedure will consist in the addition of biomass and further addition of water to the tank until the desired weight is reached.

Depending on the decided storage strategy, the appropriate dilution of the biomass will be performed. The mixture is proposed to be prepared in an anaerobic tank and the contains of this tank will be feed to compartment I in a semicontinuous mode. It was agreed that sterilization is not needed in this tank.

## 2.4.- Compartment II

The anoxygenic phototrophic compartment II metabolizes some of the compounds produced in the anaerobic liquefying compartment, with edible biomass generation. To achieve this goal *Rhodospirillum rubrum* and *Rhodobacter capsulata* are cultured in an anaerobic environment, either in photoheterotrophic or in photoautotrophic conditions.

These cells have to be removed from the outlet media of this compartment. Thus, a separation unit is required. Centrifugation is a desirable technique that has been used until now in order to do this task. The biomass separation could be done once a day as these cells will be stored at low temperatures in the outlet buffer tank of compartment II, and at low temperatures cells are not degraded until 48 hours of storage (Morist, A., 2000).

Once separated from the media, cells will be recycled to the first compartment being previously treated in order to avoid their degradation. This treatment can be done in different ways.

*R.rubrum* and *R. capsulata* cells can be dried and then vacuum stored. This same dryer can be also used to dry the non-edible part of the plants and S. *platensis* cells. After having dried the cells, they would be vacuum stored in bags containing a determined dry weight. This possibility would require using extra energy, as biomass, which will be further re-dissolved, has to be previously dried.

A more automatized way to treat the cells could be to pasteurize them directly and continuously after the separation procedure and to store them after pasteurization in a refrigerate tank. This process is more automatic but requires extra equipment and as the daily biomass flow, which will have to be treated, is rather small it is probably no worth.

Another possibility is to daily freeze the cells in different containers after the separation procedure, having previously taken out a sample in order to know its water content. This possibility seems to be the more accurate within the whole concept of the loop. A scheme of this process is presented in figure 2.

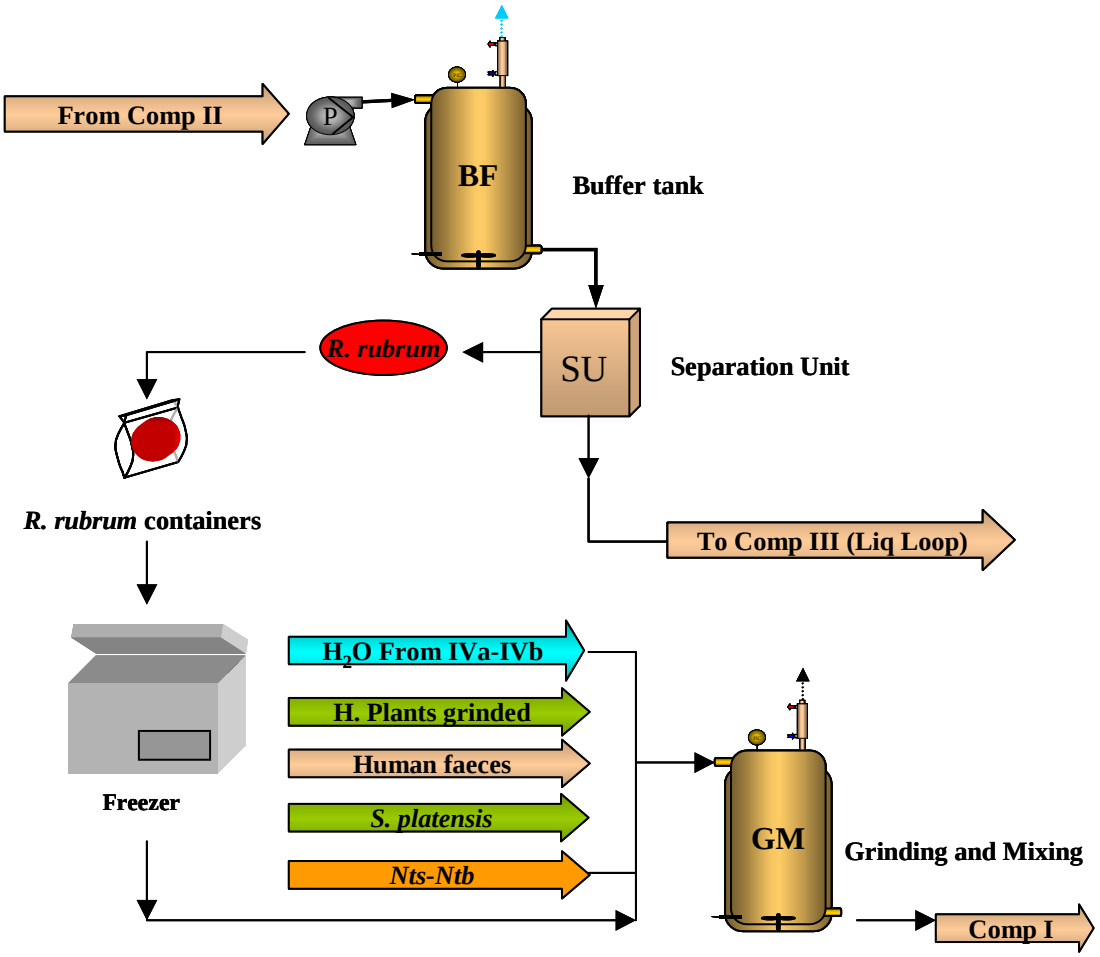


**Figure 2.-** Treatment of the cells coming from compartment II

## 2.5.- 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 and completely necessary in order to grow plants in compartment IVb. It consists in a packed-bed reactor with cells of two bacterial strains (*Nitrosomonas europaea* and *Nitrobacter winogradskyi*) immobilized onto polystyrene beads (Biostyr).

In stationary operational conditions almost no solids are produced in this compartment. Taking into account that this compartment is a fixed bed reactor, all the biomass is trapped in the bed and few biomass is found in its outlet. Biomass, which will be taken out from the outlet media through a separation step will be treated as the biomass outcoming from compartment II (figure 3). Thus, it will be periodically frozen having previously determined its water content.

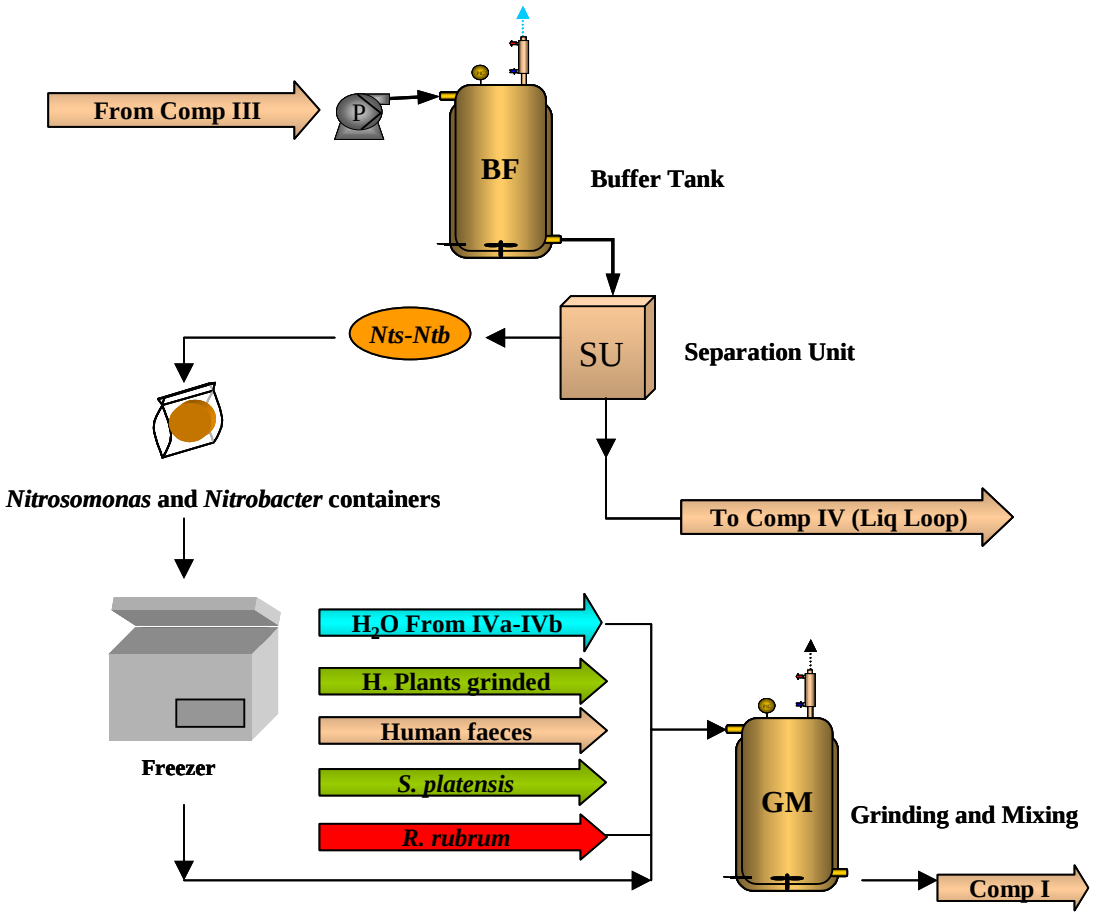


**Figure 3.-** Treatment of the cells coming from compartment III

## 2.6.- Compartment Iva

Compartment IVa has as its main task the carbon dioxide removal and supply of oxygen for the crew respiration, generating at the same time edible biomass as food supply. This compartment is currently implemented in airlift reactors where *Spirulina platensis* is cultivated.

*Spirulina platensis* cells have to be removed from the outlet of this compartment. Centrifugation is not a desirable technique when wanting to do this removal with fresh *S. platensis* as they have vacuoles and they float. Thus, other separation techniques such as membrane modules, will have to be used. The separation process can be done once a day as *S. platensis* cells will be stored at low temperatures in the outlet buffer tank, and

at low temperatures *S. platensis* cells are not degraded till 48 hours of storage (Morist. A., 2000).

Once separated from the media, part of the *S. platensis* cells will be treated to make them edible, work is being done in this side, and, the rest that will be recycled to the first compartment will be treated in order to avoid its degradation. The same treatments done to *R. rubrum and R. capsulata* and *N. europaea* and *N. winogradskyi* presented in section 2.4 can be done to *S. platensis*.

A scheme of this process is presented in figure 4

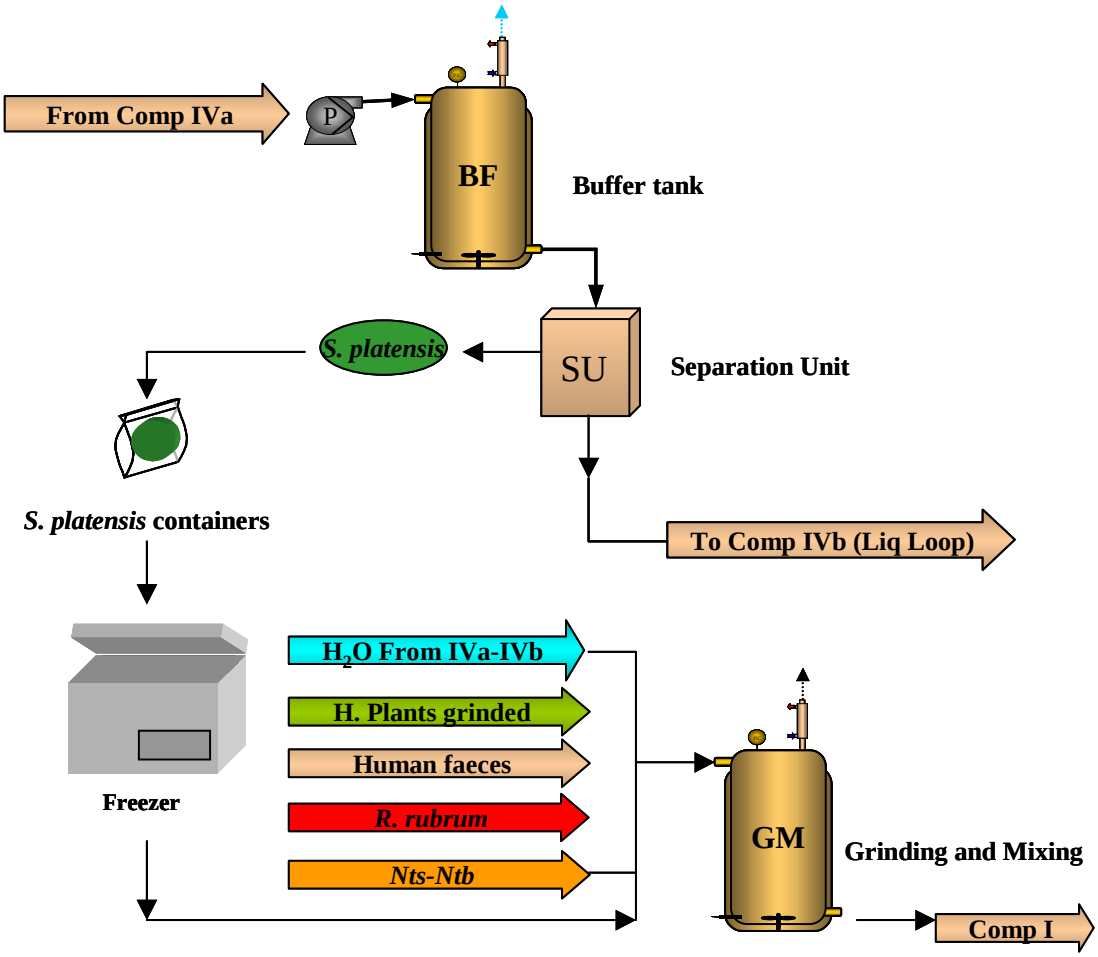


**Figure 4.-** Treatment of the cells coming from compartment IVa

## 2.7.- Compartment Ivb

The higher plant compartment, or compartment IVb, is the basic food supplier for the crew.

As plants will grow hidroponically, the basic solids related to this compartment are the seeds and the edible and non edible part of the plants.

Seeds will be vacuum-stored.

The plant collection as well as the separation of the edible and non edible parts of the plant will be done manually.

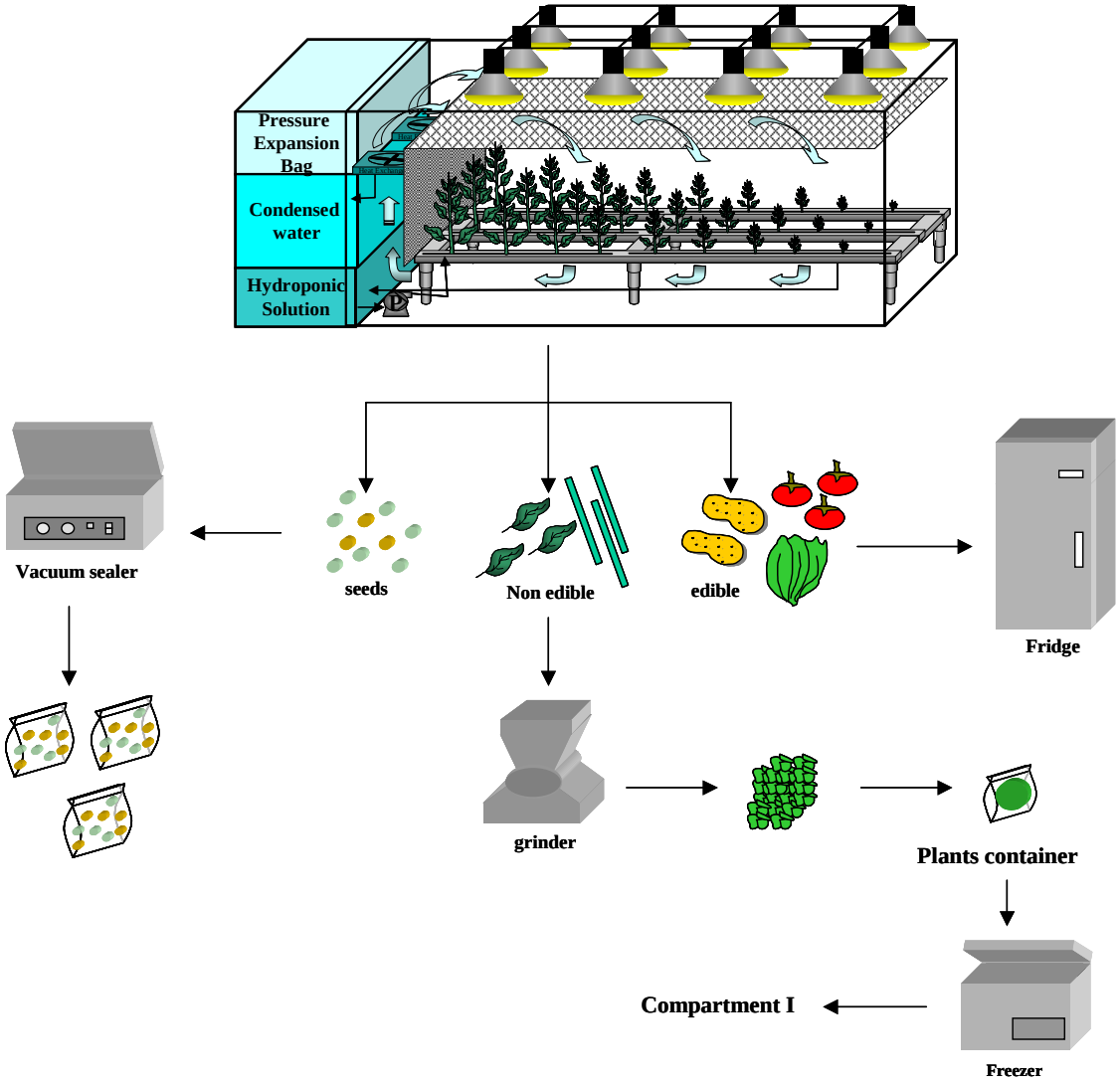


**Figure 5.-** Scheme of the higher plant treatment procedure

The edible parts of the plants will be stored under low temperatures and they will be, in a future, used either in the volunteers diet or the rats diet.

The non edible part of the plants will be first grinded and then frozen and stored in containers in order to avoid degradation. A sample will be previously taken in order to determine its water content.

In figure 5 a scheme of the plant collection, separation and storage is presented.

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