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1 INTRODUCTION

The advance of the MELISSA project as a tool for the study and development of Advanced Life Support Systems (ALSs), requires to set up an integration loop, in a dedicated facility, such as the MELISSA Pilot Plant. During previous phases, the MELISSA pilot plant work has been devoted to the development of the individual compartments of the loop. To this purpose a systematic approach has been followed in a wide range of tasks. Those include the selection of strains, study of their growth kinetics at different conditions, design and set-up of the corresponding bioreactors and associated instrumentation, development of the corresponding mathematical models, characterisation of the continuous operation of the compartments and test of control laws. The research done, has been mainly focused on compartments II, III and IVa. As a main result, the interconnected operation of these compartments at two different sizes, bench scale and pilot scale, has been achieved for long periods of time ranging from weeks to months.

In order to complete the objectives envisaged since the elaboration of the project concept, and in order to demonstrate the validity of MELISSA as a model system for biological ALSs, the closure of the loop of compartments in the Pilot Plant has to be completed. This goal will be reached in a gradual way following a step by step approach during the following years.

Attainment of this goal is a very complex task that requires 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 has previously been done as a starting point (Godia *et al* 2001) and its description is going to be refined in this and future technical notes.

This technical note is devoted to the description of the preliminary engineering design of the liquid loop to be installed in the MELISSA Pilot Plant laboratory and is complementary to technical notes 62.4 and 62.5 describing correspondingly the design of the gas loop and the solid loop components of the MELISSA complete loop. Its purpose is to describe in more detail the different elements, of key relevance in the

treatment of the liquid phase components of the MELISSA loop concept as well as the necessary elements for its proper interconnection. This description will be the base line for its physical implementation in the upgraded MELISSA laboratory to be built in the next 18 months, with the main goal of the final demonstration of the complete MELISSA loop.

2 COMPARTMENT SET UP DESCRIPTION

2.1 General overview

The liquid loop comprises all the interconnections of the MELISSA loop were a liquid fluid is treated or interchanged. It comprises either liquid nutrient solutions, purified recovered water or any liquid fluid with suspended biomass. Although of closed loop nature, it can be considered that its initial step is located in compartment I were all the organic wastes produced by the crew and all the non consumed biomass is introduced. Treatment of solid wastes is described in TN 62.5. The crew produced urine is an important source of nitrogen and minerals that must be considered as well. At this point urine is foreseen to be treated either chemically or enzimatically to obtain an

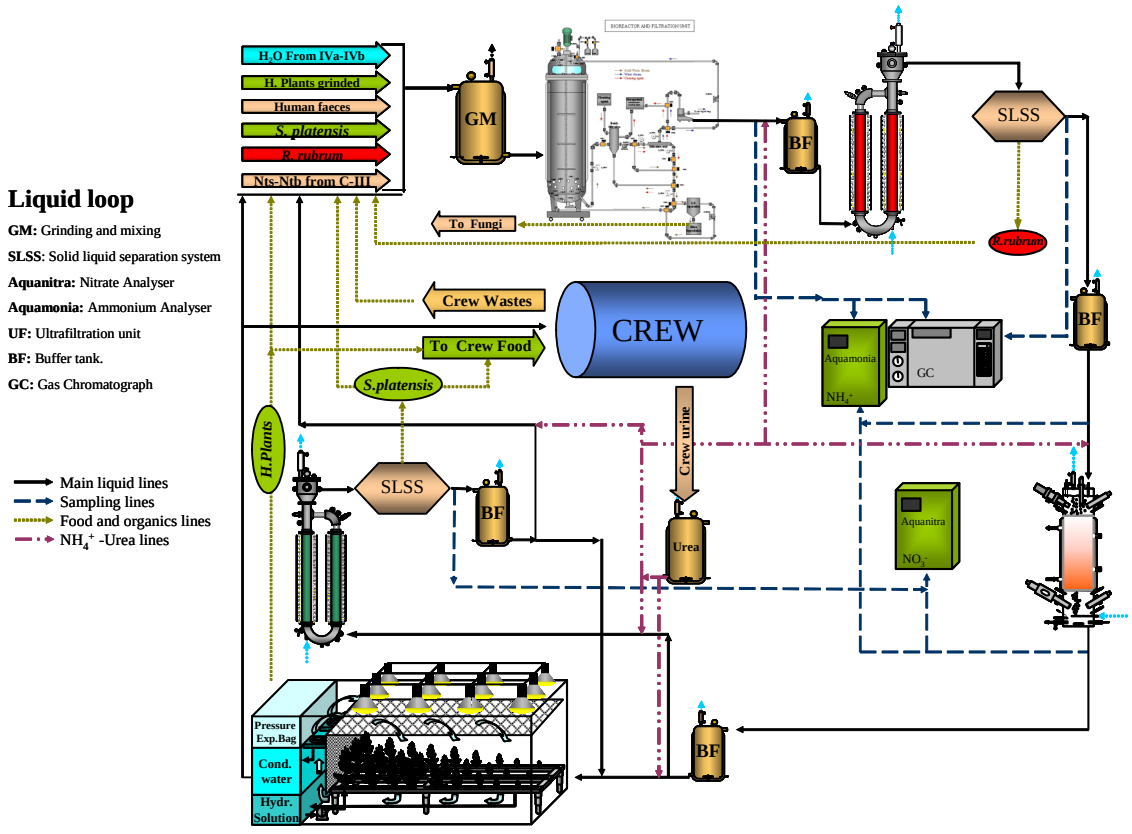

Figure 1: General overview of the MELISSA liquid loop

ammonia rich effluent to be used as nitrogen source in any compartment as required because ammonia can be consumed either in compartment I, II, III , IVa or IVb at different levels.

A general overview of the liquid loop interconnections is depicted in figure 1.

In the following paragraphs a description of the different components and interconnections of the liquid phase of the loop will be done.

2.2 Compartment I

This compartment has as main task to decompose or liquefy the biomass components and macromolecules generated in the other compartments. Its final design and operational conditions will be defined, following the degradation studies and final design performed at Univ. of Gent. However as a main operational constraints it can be considered that the operation of the compartment will be performed in anaerobic conditions and temperatures around 55ºC.

Preparation of the input to the compartment will require as a first step, to collect the biomass from different sources and if necessary its proper storage to avoid

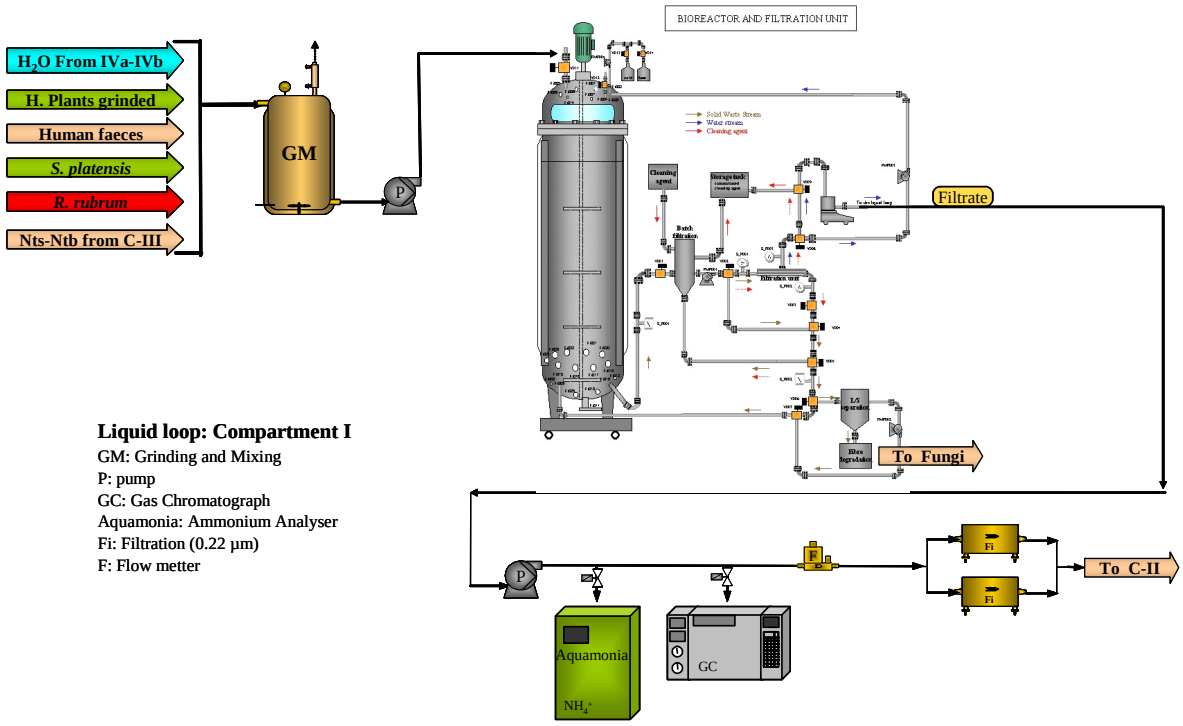

Figure 2: Compartment I liquid phase interconnections.

degradation.

Microbial biomass, mainly *Spirulina platensis* and *Rhodobacteria*, can be stored either freezed, freeze dried or as a liquid at low temperature in high biomass concentration, but still containing a significant amount of water. If storage is to be done for extended periods of time, drying or freeze drying could be the preferred choice. Freezing might be a good compromise between storage requirements and energy expenditures. However, if the biomass storage periods are not expected to be long, storage at low temperature is a possible alternative. Higher plant biomass will have to be grinded and human faeces homogenized. In case of using dried or freeze-dried biomass it can be stored in plastic containers under vacuum. A more detailed description of the procedure for biomass treatment before its use in this compartment can be found in TN 63.5.

Depending on the biomass origin or storage strategy decided, the appropriate dilution of the biomass will be performed previous to its use. As a reference, and according to the previous studies preformed in Gent, it can be considered that the mixture of human faeces, plant biomass and microbial biomass will be diluted about 10 times its wet weight.

Preparation of the mixture is proposed to be done batch wise and feed to the bioreactor of compartment I in a semicontinuous mode. The organic matter mixture can be prepared and feed periodically. As an example a feeding period of 3 days can be considered. Each of those days, the liquor contained in the bioreactor can be recirculated through the ultrafiltration unit until a volume equal to the feed one is filtered and removed from the bioreactor. Then the recently prepared mixture can be introduced into compartment I. Currently, and according to the agreements taken in Paris 01, sterilization of the introduced organic matter mixture is not considered necessary.

In order to facilitate its preparation, it is proposed to set up one stainless steel tank with grinding, mixing and weighing capabilities. In this way the preparation procedure will consist in the addition of the biomass to the tank until the desired weight is reached and the further addition of water to complete the desired final weight and therefore the proper dilution. Once completed, the tank should be closed and anaerobic

conditions implemented. After this step, the tank liquor will be used to feed compartment 1.

The reference operational conditions of the main bioreactor of this compartment can be found in table 1, as described in technical note TN 56.3.

Parameter	Value
Temperature (ºC)	55
Hydraulic retention time (days)	23.5
Sludge age (days)	30
Load (kgCOD/(m^3·d)	1.2
Dry weight in feed (gDW/L)	17
Daily load (gDW/Day)	238
Volatile Fatty Acids (g/D)	59

Table 1: Summary of feeding characteristics according to TN 56.3

Therefore the bioreactor will be operated at an hydraulic retention time of 23.5 days (dilution rate: 0.0425Day^{-1}) and about 238 gDW/Day of biomass dry weight will be supplied in the input medium. The total bioreactor volume will depend on the amount of urine recycled through the first compartment and at this moment is still not completely decided. However the volume is expected to range between 25 and 330 litres. As an example, assuming all the nitrogen required is obtained by degradation of the wastes, using a 330 litres tank would require a 14 litres/day feeding. If a 3 days semicontinuous scheme is used, a liquor of 42 litres containing 716 g of dried biomass should be prepared and fed every 3 days.

Measure	Type	Required for
Temperature	on-line	Local temperature control
PH	on-line	pH control
Pressure	on-line	Pressure safety valve
Gas Flow	on-line	Monitoring and control of performance
CH_4	on-line	Monitoring and control of performance
CO_2	on-line	Monitoring and control of performance
Redox	On-line	N_2-gas addition
HCO_3^-	titration	
Solids	On-line	Monitoring and control of performance
Liquid Level	On-line	Local flow control

Table 2: list of measurements to be performed in compartment 1.

As described above the bioreactor liquid content will be recycled through a microfiltration unit with a tubular polyvinylidene fluoride membrane of 30 nm pore size (WWF 4385) according to TN 56.3. A cleaning loop, allowing to bypass the bioreactor during cleaning procedures, will be also set-up. Due to accumulation of slowly biodegraded compounds, the bioreactor will be periodically purged to remove the dry weight excess. The liquid effluent from the membrane used will be sampled for the analysis of NH_4^+ and volatile fatty acids.

The microbial contamination tests performed previously indicate that after the microfiltration unit, no bacterial contamination will be present. However a second safety filtration step, to assure that microbial contamination will not affect the subsequent compartment, is proposed. After it, the effluent will be introduced in the second compartment. A pictorial description of the interconnections of this compartment can be found in figure 2. In table 2 a list of the expected measurements to be performed in the bioreactor of this compartment is shown. A more detailed description of their characteristics can be found in TN 56.3.

In order to properly operate the microfiltration unit and the bioreactor, several measurements will be required. The list of sensors to install at the output line of the filtration unit is described in table 3, according to TN 56.3 were a more detailed description of their characteristics can be found.

Sensors	**Type**	**Required for**
Pressure	on-line	Local control
Level	on-line	Local control
Flow	on-line	Local control
VFA	on-line	Monitoring and control of performance
NH_4-N	on-line	Monitoring and control of performance

Table 3 List of sensors to install at the output line of the filtration unit, according to TN 56.3 .

As already mentioned after the microfiltration unit a measurement should be done for the concentration of ammonium and volatile fatty acids. If the range of analysis allows it, the same analysers could also measure these variables at the output of the second compartment.

2.3 Compartment II

The main role of compartment II is to consume the volatile fatty acids (VFAs) generated in compartment I. The accomplishment of this task is attained by the use of photoauto/heterotrophic bacteria, such as *Rhodospirillum rubrum* or *Rhodobacter capsulata*. Those organisms use the light energy to convert the VFAs into edible biomass.

The output flow of compartment I can be regulated by acting on the performance of the microfiltration unit. However, in case that compartment I is operated semicontinuously or if flow into compartment II has to be decreased for any other reason, a buffer tank will be required. It is therefore proposed to install an in-situ sterilizable buffer tank that can be used to temporarily store the liquid effluent of compartment I. The liquid input to this tank should be filter sterilized to avoid microbial contamination. From this tank a pump will draw its contents at the required flow to attain the desired dilution rate for compartment II. At this time it can be expected that this dilution rate will range between 0.01-0.12 h^{-1}, which for a reactor liquid volume of 50L would be between 0.5 and 6 litres/h. The flow rate will be measured and filter sterilized as a security measure to assure the axenity of the bioreactor. The final volume of the bioreactor is not fixed yet since it is currently being designed. The preliminary design of this bioreactor, taking into account the available kinetic data and the expected

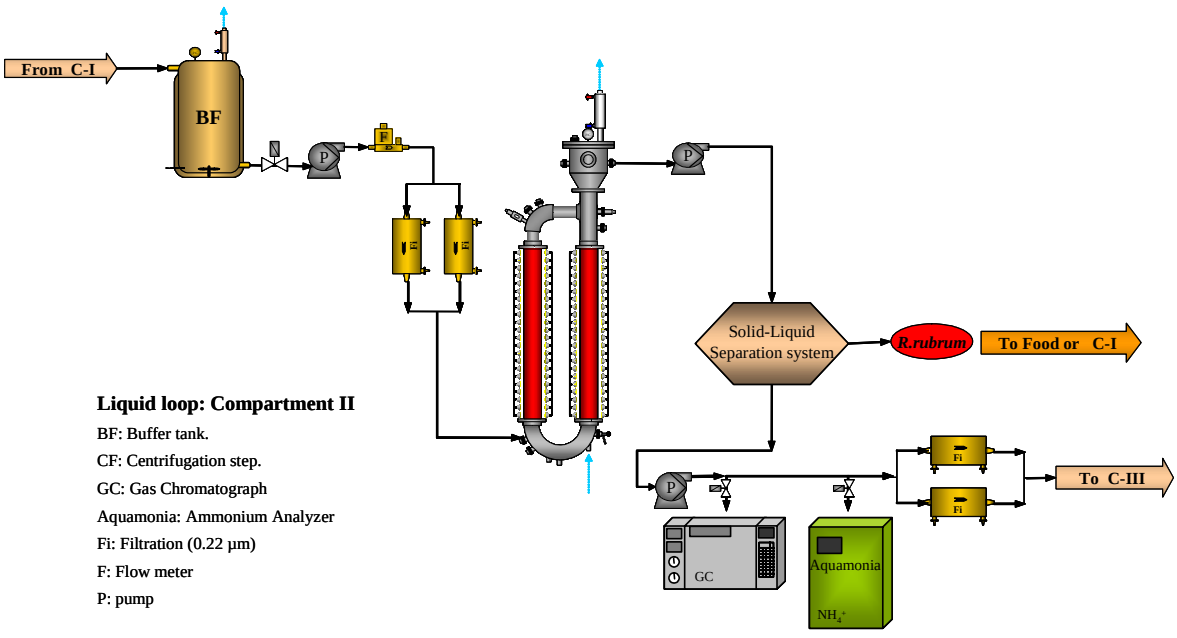

Figure 3: Compartment II liquid phase interconnections

composition of the feed, indicates a volume of around 50 litres. Final volume will depend on the final design constraints and its effects on the ratio between illuminated volume and total volume. A general view of interconnections of compartment II can be seen in figure 3.

The output flow of this compartment will contain a significant amount of biomass that has to be removed. At this point the specific system configuration, to separate the biomass from the liquid effluent, is still under study and therefore not defined. Depending on the separation system finally decided an *in situ* sterilizable buffer tank to temporarily store the compartment II effluent might be necessary to attenuate any flow rate variations.

For the proper operation of this compartment, several variables are foreseen to be measured as described in table 4.

Variable	Measured	Required for
Temperature	on-line	Local temperature control
pH	on-line	Local pH control
Pressure	on-line	Pressure safety valve
Gas Flow	on-line	Monitoring and control of performance
Input Liquid Flow	on-line	Monitoring and control of performance
Input/output CO_2	on-line	Monitoring and control of performance
Input/output VFA (gas)	on-line	Monitoring and control of performance
Input/output VFA (liq.)	on-line	Monitoring and control of performance
Input/output NH_4^+	on-line	Monitoring and control of performance
Agitation (rpm)	on-line	Local agitation control
Biomass concentration	on-line	Monitoring and control of performance
Liquid Level	on-line	Local flow control

Table 4: List of variables required in compartment 1I.

In case that an *in situ* sterilizable buffer tank is installed at the output of compartment II, it will also require several sensors for key variables, either during its operation to verify proper agitation, temperature and liquid level, as well as for sterilization were temperature and pressure measurements are also required

As the objective of this compartment is to completely consume the VFAs, it is convenient to measure the output level of those compounds. This will allow to detect any unexpected increase in their level and take appropriate actions. Either at the output

of this compartment or before the input to compartment III, the levels of NH_4^+ will also have to be measured as it is the key nitrogen source for the other compartments.

2.4 Compartment III

This compartment has to convert the ammonium, produced in the first compartment and not consumed in the second one or obtained from urine, into the nitrate required for compartments IVa and IVb. The bioreactor used will be the one already existing in the Pilot Plant. A general view of interconnections of compartment III can be seen in figure 4.

Variable	Measured	Required for
Temperature (jacket)	on-line	Local temperature control
pH- (top and bottom)	on-line	Local pH control
Pressure (top)	on-line	Pressure safety valve
Input Gas Flow	on-line	Gas flow control
Input Liquid Flow	on-line	Liquid flow control
Dissolved O_2 (top and bottom)	on-line	Gas flow control
Input/output CO_2	on-line	Monitoring and control of performance
Input/output NH_4^+	on-line	Monitoring and control of performance
Output NO_3^-	on-line	Monitoring and control of performance
Input/output VFA (liq.)	on-line	Monitoring and control of performance
Agitation (rpm)	no	Local agitation control
Liquid Level	on-line	Liquid flow control

Table 5: List of variables required in compartment III.

After the previous steps to eliminate biomass from the effluent of compartment II, the biomass free liquid can be stored, in a buffer tank. This will allow to supply a stable and regulated flow into compartment III. This fluid will have to be introduced into compartment III in a stable and controlled flow of known composition with respect to its key variables. This means at least to know the NH_4^+ supplied and the possible input of other organic carbon sources such as VFA's. Those measures are coincident with some of the ones necessary for compartment II and therefore measuring them only at one point is recommended. To consume the NH_4^+ it is necessary to provide a sufficient amount of oxygen to the compartment. This is attained via the gas phase supply which is described in TN 62.4. At the output of this compartment determination of the levels of NH_4^+ , NO_2^- and NO_3^- is necessary in order to evaluate the operation of

the reactor. It is also foreseen the use of a nitrite estimator software to predict and be able to correct the generation of unwanted levels of NO_2^- . This software will require an on-line measurement of the amount of the different nitrogen sources taking part in the process. At this moment a measurement system for NH_4^+ and NO_3^- is foreseen. A summary of the variables to measure for this compartment can be found in table 5.

As biomass is immobilized in the reactor it is not foreseen obtain a significant amount of it in the output effluent. However, as biomass increases in the fixed bed, its accumulation in the bioreactor will impair fluid circulation and as a consequence its performance. To extend the operational time of the bioreactor, a periodic cleaning procedure was proposed. In this one biomass is released with a reverse flow and conveyed to the output of the bioreactor. It is therefore necessary to separate this biomass from the liquid flow and collect it for its recycling in the first compartment. The definitive biomass separation procedure is still not defined.

The biomass free liquid effluent of this compartment can be used either in compartment VIa or in compartment IVb. The exact distribution for which, will be done depending on the exact necessities of each compartment at a given moment.

Either in the input or in the output effluents of this compartment a combination

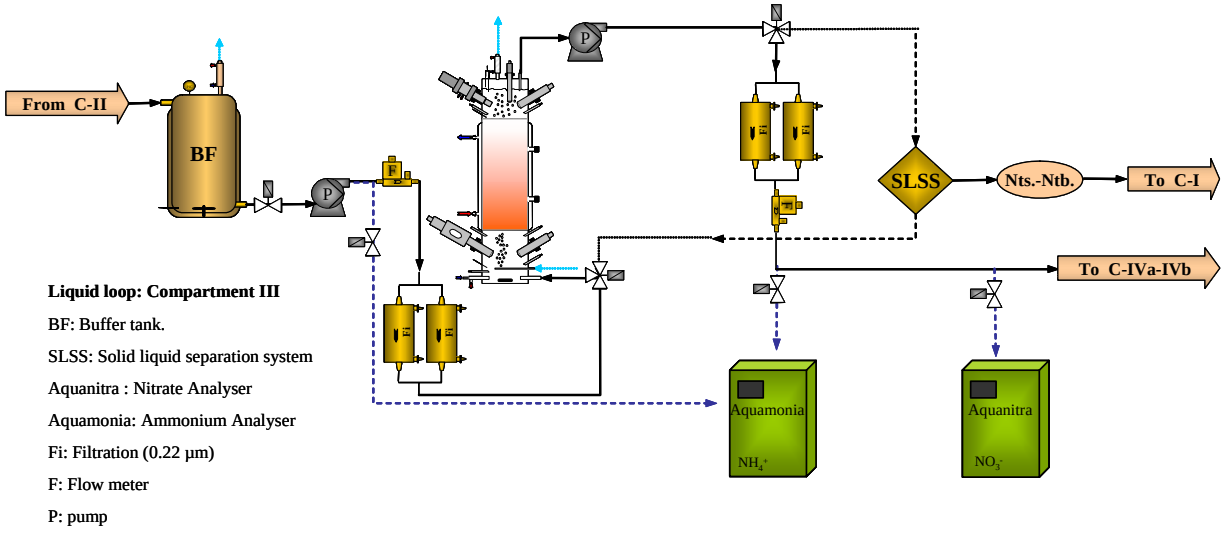

Figure 4: Compartment III liquid phase interconnections

is possible with the NH_3^+ rich liquid effluent resulting from the urine degradation in order to adjust the nitrogen source necessities of this compartment or the following ones.

2.5 Compartment IVa and IVb

The following compartments are of key importance for the process due to its role in edible biomass generation and oxygen production. The combined operation of both compartments will allow the survival of the crew . For its operation it is necessary to supply a liquid flow containing all the mineral nutrients and nitrogen source together with a gas flow with the carbon dioxide that will be converted into biomass.

2.5.1 Compartment IVa

The liquid flow rich in NO_3^- from compartment III will provide the minerals and nitrogen source to this compartment. If necessary it can be combined with the ammonium rich liquid flow from urine degradation. To improve stability of flow control and allow dampening of any possible flow oscillations, together with improving the mixing with the ammonium rich flow, it is proposed to set up a buffer tank at the input of this compartment. It will be *in-situ* sterilizable and have, as the other storage tanks, agitation, temperature, pressure and level measurement capabilities. The output of this tank can be used either in compartment IVa as well as directed to compartment IVb. The liquid effluent flow for compartment IVa will be measured and filter sterilized to

Variable	Measured	Required for
Temperature	on-line	Local temperature control
pH	on-line	Local pH control
Pressure (top)	on-line	Pressure safety valve
Input Gas Flow	on-line	Gas flow control
Recirculation Gas Flow	on-line	Loop circulation
Output Gas Flow	on-line	Pressure control
Input Liquid Flow	on-line	Liquid flow control
Dissolved O_2	on-line	Monitoring and control of performance
Input/output CO_2	on-line	Monitoring and control of performance
Output NO_3^-	on-line	Monitoring and control of performance
Biomass concentration	on-line	Biomass productivity/optim
Liquid Level	on-line	Liquid flow control

Table 6: List of variables required in compartment 1Va.

avoid any microbial contamination.

The compartment IVa container is an illuminated airlift loop bioreactor. The key measurements for this compartment are summarized in table 6, and besides the most common ones such as temperature and pH the control of gas flows and compositions with respect to O_2 and CO_2 together with the liquid flows and biomass concentration, are of key importance.

The biomass growth and productivity will be regulated by means of the light intensity. This value is not measured currently because regulation relies on the value of the voltage supply to the lamps and previous calibration measurements.

A general view of interconnections of compartment IVa can be seen in figure 5.

2.5.2 Compartment IVb

This compartment is devoted to the higher plant cultivation for crew consumption and oxygen generation. For its cultivation special higher plant chambers are being designed. Therefore at this moment only an estimation of its main characteristics can be provided.

Variable	Measured	Required for
Temperature	on-line	Local temperature control
pH of nutrient solution	on-line	Local pH control
Pressure	on-line	Pressure safety valve
Input/output Gas Flow	on-line	Gas flow control
Input/chamber CO_2 concentration	on-line	Monitoring and control of performance
Output Gas Flow	on-line	Pressure control
Input Liquid Flow	on-line	Liquid flow control
NO_3^- in nutrient solution	on-line	Monitoring and control of performance
NH_4^+ in nutrient solution	on-line	Monitoring and control of performance
Nutrient Solution Level	on-line	Liquid flow control
Air humidity	on-line	Local air control
Light intensity	on-line	Monitoring and control of performance
Air Flow	on-line	Local air circulation control
Nutrient Solution Conductivity	on-line	Local air circulation control

Table 7: List of variables required in compartment 1Vb.

Higher plants will be grown in higher plant chambers in hydroponic solutions. the biomass free liquid effluent from compartment III mixed with the resulting components of the degradation of the crew urine will be used as minerals and nitrogen sources. This liquid influent will be introduced in the hydroponic solution were the nutrient content and pH will be monitored. The number and type of nutrients monitored is at this time undefined but it can be expected that ammonia and nitrate will be monitored together with other elements. Conductivity measurements can be used as an indication of the global mineral content. Alternatively it is possible to envisage the use of more specific ion detectors in the liquid feed of the higher plants. This fact depends on the availability of the specific technology and the final identification of the key ions to follow. In the same way as is done in compartment IVa the ammonium rich effluent obtained from the urine treatment tank can be used in this compartment as a nitrogen source.

This hydroponic solution will be circulated trough the different plant containing trays allowing them to uptake the mineral nutrients. The carbon source will be supplied trough the gas lines as carbon dioxide. The oxygen produced will also be removed via

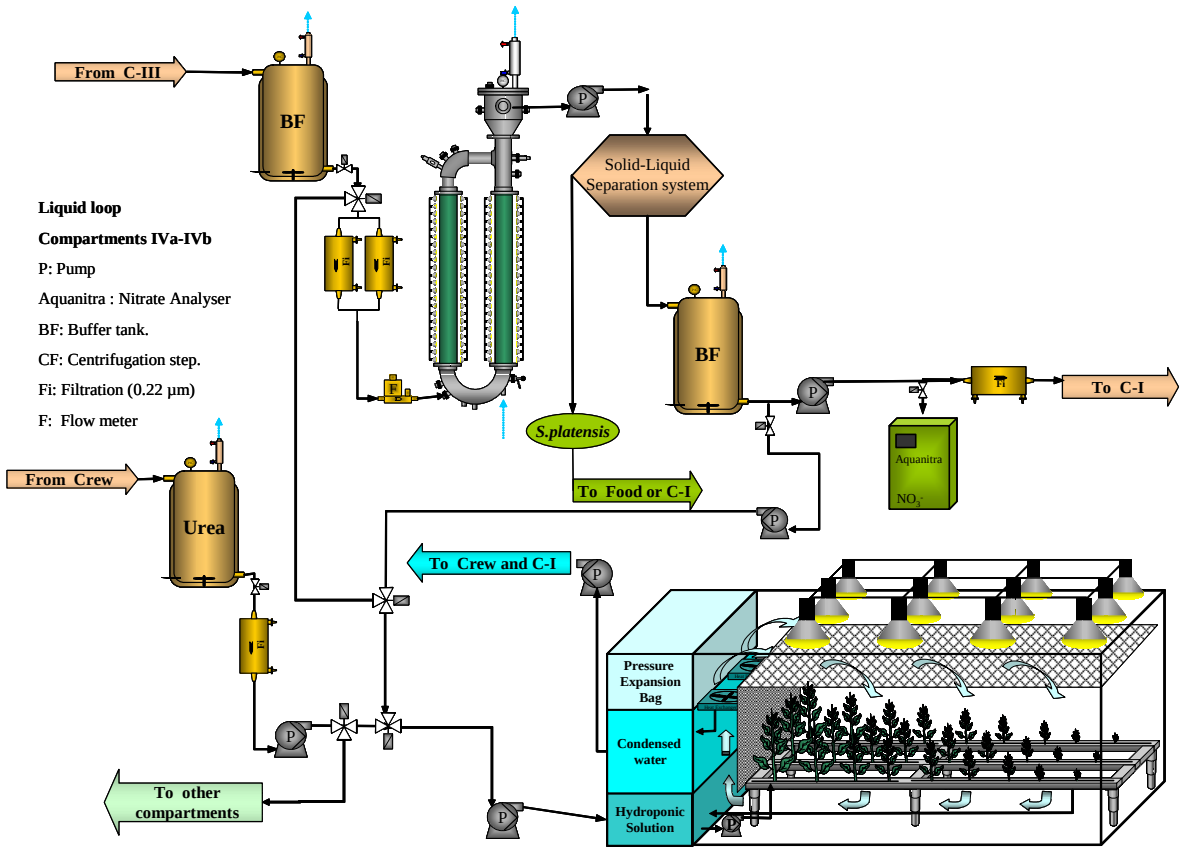

Figure 5: Compartment IVa and IVb liquid phase interconnections

the gas lines.

As a result of the higher plant activity air humidity increases and several heat exchangers, for water condensation and temperature maintenance are necessary. The condensed water will be stored in clean water tanks and used preferentially as drinking water, although other uses might also be foreseen.

The plant chambers will be illuminated, preferentially by external lamp banks and light intensity at the plants level will be monitored and recorded. In this case, light quality and photoperiod will be as important as light intensity. Light quality will be defined at the time of purchasing the lamps, but photoperiod will require an automatic system to periodically establish light and dark periods.

Prior to its final design several variables are foreseen as being of interest and are listed in table 7 .

A general view of interconnections of compartment IVb can be seen in figure 5.

The higher plants produced will either be consumed by the crew or grinded and introduced in the first compartment as described in TN62.5.

3 REFERENCES

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