MELISSA

TN 13

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PART I THE MELISSA LOOP CONCEPT

1. GENERAL REMARKS

The breadboarding phase of Melissa is expected to occur once the preliminary theoretical studies of its individual compartments have been carried out. The present phase of the study is performed in view of identifying the basic technical difficulties to be generated by the physical connection of the compartments and this technical note is due to provide a preliminary physical description of the loop.

The concept of the breadboard takes advantage of several characteristics of the involved biological actors :

* Their respective metabolisms are simple compared with those of superior plants. As a consequence, pertinent mathematical models can be built-up and simulations can be performed more easily, allowing fundamental characteristics of the subject to be investigated faster than through real experimentation.

* Their individual cultivation in aqueous medium can be conceived and realised as a quasi continuous process that can be managed automatically. For the same reason, the coupling of compartments to each other, in order to close (or nearly) a complete ecosystem loop, can be reasonably envisionned.

* The velocities of their reproduction cycles favourise somewhat fast experimental checks, likely to speed up significantly the preliminary phases of the feasibility studies. In addition it can shorten the studies of the long range effects of the exposure to space conditions (such as microgravity and cosmic radiations) upon the biological stability of the cultivations.

2. PRINCIPLES AND OPERATING CONDITIONS OF THE BREADBOARD

One of the aims of Melissa is the transformation of the wastes, CO2 and mineral salts, generated by a human-like consumer, into products consumable by the same consumer. Several compartments are necessary to close such a loop by ensuring anaerobe digestion, nitrification and oxygen production.

The compartments are physically installed in the same laboratory, they are connected to each other through automatic transfer mechanisms, controlled by a centralised system (1), which ensure the continuous operation of the loop.

Before the construction of the breadboard, the proper functionning of

the constituting compartments has been evaluated in normal laboratory conditions with the help of standard laboratory equipment and methods. All (or nearly) feasibility proofs have been experimentally provided, basic experimental procedures have been set up and measurements done concerning the conditions of cultivation of the species and their metabolic characteristics. Then comes the problem of coupling the compartments to each other.

The breadboard exercise is due to check the pertinence of the Melissa concept and the possibility of making it operating as a system. The present document aims to identify the technical problems created by the tentative realisation of the Melissa loop under normal terrestrial environment. This means in particular that the breadboard operation is conceived to be carried out under normal gravity (1g). Above all, the remark applies to the operations involving fluid management such as transfers of liquids, filtrations, separations, bubbling, stirring, etc..., which are very much simplified by this condition.

Other important restrictive conditions apply to the breadboard operation :

- The dietetic equilibrium of the consumers is partially reached by means of external food supply to the animals. In a first attempt it is not supposed to be 100 % ensured by the only resources of Melissa.

- In a similar manner, Melissa is not expected to provide drinkable water to the consumer at the very beginning. (Water management in a completely closed ecosystem is foreseen to be at least partially ensured by physico-chemical processes).

- Also to be considered as out of the scope of Melissa in it first phase is the atmosphere maintenance for the consumer.

- Conditions prevailing over the operation of the Melissa breadboard are "normal laboratory conditions". In particular Melissa will take benefit from the natural quasi regulation of the temperature around the conventional value of 22 C.

3. PHYSICAL DESCRIPTION

As already said, the Melissa breadboard loop is constituted by compartments physically installed in the same laboratory to be progressively connected together.

3.1. The consumer compartment.

For practical reasons, in order to limit the importance of extra investigations which could reveal not being strictly in line with the principal objectives of Melissa, it was established from the very beginning of the study that the "consumer compartment" would not exist completely as such. The dietetical needs of the simplest mammal are extremely sophisticated compared with the food production possibilities an "ecosystem" as much simplified as Melissa is. It was considered a sufficiently valuable compromise to utilize the animal-consumer principally as a "waste generator". As a consequence it will not be necessarily installed in the Melissa laboratory and its integration into the loop will be managed step by step.

Thus, due to the particular needs of the "consumer" which differ significantly from those of the other compartments, the Melissa loop cannot be initially closed. The possibility of a partial closure can

be considered however after verification is made that the animal can effectively be supplied by the oxygen, water and biomass elaborated by Melissa.

The animal chosen is the rat. The latter possesses many a quality in view of our study. It is robust and, as it has always been a conventional reliable laboratory cooperator, its physiology is well known. Moreover, its dietetic regime shares many common features with the human one.

At the moment there is no available information concerning the minimum size of a closed cage that would enable the animal to live safely for long periods, taking account of the room needed by the equipment for gas, liquid and solid management necessary to its life.

As far as we know, no tentative experiment was in fact performed to grow a rat under conditions compatible with Melissa, i.e. with 100% quantitative and qualitative physical and chemical control upon its consumption and its rejections. The indications given hereafter correspond to standard conditions adopted today in laboratories. In that respect, their accuracy could probably be improved after dedicated studies.

3.1.1 Growing conditions (2)

The growing conditions listed here below come from standard laboratory estimates. They must be considered as indications :

Dimensions of the cage : 50 cm x 50 cm x 50 cm

Artificial lighting : 12 h daylight/ day, preferably by fluorescent tubes.

Moisture : 50 TO 60 %

Temperature : 22 C +/- 1 C.

Ventilation : Air renewed about 10 times per hour (which is equivalent to 1250 litres/hour = 1/3 litre/s)

3.1.2. Transfer of wastes to the liquefaction compartment

As it was stated earlier the animals will be firstly used as a "waste generators" for testing the performance of the following compartment (liquefaction).
In order to ensure permanent, or quasi-permanent, feeding conditions of the liquefaction compartment, the process cannot be strictly continuous in quantity as the rat is not a "continuous animal" nor in composition because of the time variations of the animals metabolisms depending on a number of parameters. However, it will be rendered quasi continuous by preparing large quantities of rat wastes. This will average the properties of feces and urine produced over large periods of time (or large number of animals) and allow continuous operation during comparable periods.

In a first attempt the wastes will be prepared in a separate and isolated laboratory.

3.1.3. Equipment needed

The air conditionning will be performed by an air pulser and the

renewal ensured from open air.

Water supply is provided by gravity from an external reservoir. The consumption can be measured in volume via a direct reading on the reservoir.

Food is supplied manually to the rats in calibrated capsules or pellets. Measurement of quantities consumed is then possible.

Feces and urine collection : Collection is made by gravity and, if needed, separation between liquid and solid phases is obtained by filtration.

Concerning the growing of rats no specific device is required by Melissa as long as no study of the animal physiology is requested at the moment. It would go differently should the need of accurate knowledge on the subject be raised.

3.2. The photosynthesis compartment (IV)

It is constituted by a bioreactor in which is cultivated Spirulina under normal conditions (sunlight or equivalent) surrounded by the necessary equipment. As it was the first compartment of Melissa to be operated it is described hereafter in some detail and will serve as a model for the others, as far as their sizing is concerned.

3.2.1. Equipment needed

The values of masses and electrical consumptions given below and in the following paragraphs have not been measured. They are given as indications.

	Mass(kg)	Power(W)
Bioreactor 7 litres	16	
CO2 analyzer	20	200
Scrambling regulator	6	
Scrambling motor	1.6	200
Atmosphere conditioning	1.5	200
Anti-foam regulation	0.5	
pH control	0.2	
Temperature control	0.5	100
Flowmeter	0.2	
Electronics	10	200
Pump	1.3	33
Bioculture	5	
Light source	4	2000

Total	:	66.8	: 2933

3.3. The liquefaction compartment (I)

This compartment receives the waste generated by the consumer, essentially the biological polymers such as cellulose, proteins, polysaccharides, etc ...
These wastes include feces and urine and, in a further application, they could comprise some other products related to human activities.

It is constituted by a bioreactor in which Clostridium thermocellum and C. thermosaccharolyticum are cultivated. They produce fatty acids, ammonia, hydrogen and H2S.

The compartment must be operated at 60 C and thus it must be maintained at about 38 C above the laboratory ambiance by the supply of external heat.

3.3.1. Equipment needed

	Mass(kg)	Power(w)
Bioreactor 7 litres	: 16	:
Scrambling device	: 8	: 200
Atmosphere conditionning	: 1.5	: 200
Temperature control	: 4	: 1000
Electronics	: 10	: 100
Total	39.5	1500

3.4. The phototrophic compartment (II)

In this compartment are cultivated Rhodobacter capsulata and Rhodospirillum rubrum which transform fatty acids into biomass, which is a source of P.O.U. of high food value.

It needs light as a source of energy to ensure the photoheterotrophic growth of the cells.

A third species of bacterium, Thiocapsa roseopersicina, which treats H2S, is grown in the same bioreactor so that H2 and H2S are simultaneously suppressed and P.O.U., NH4, CO2 and mineral salts produced for use in the following compartments.

3.4.1. Equipment needed

	Mass(kg)	Power(w)
Bioreactor 7 litres	16	
Scrambling device	8	200
Atmosphere conditionning	1.5	200
Temperature control	0.5	100
Electronics	10	100
Light source	4	2000
Total	40	2600

3.5. The nitrification compartment (III)

The main function of this compartment is to recycle ammonia obtained from the wastes into nitrates the main N2 source for Spirulina.

The nitrification is realised by two different bacteria, Nitrosomonas and Nitrobacter. A third bacterium Thiobacillus will complete the sulphur cycle by oxydizing into sulphates the sulphides it uses to grow.

3.5.I. Equipment needed

	Mass(kg)	Power(w)
Bioreactor	16	
Scrambling device	8	200
Atmosphere conditionning	1.5	200
Temperature control	0.5	100
Electronics	10	100
Total	36	600

4 OTHER FUNCTIONS

Other pieces of hardware are needed corresponding to specific functions, should the realization of a more elaborated version of Melissa be undertaken :

4.1.The oxygen control loop :

As foreseen, the Melissa breadboard does not cope with the control of a complete oxygen loop. In particular it is neither in charge of maintaining the rat compartment atmosphere composition into its nominal range by trapping the CO2 produced by the animal breathing nor it ensures its reinjection into the cultivation media of species that would use it such as Spirulina.

A possibly simple means of coupling the rat breathing with an oxygen

control loop would consist of maintaining the animal in the same atmosphere as the photosynthetic bacteria taking care that the volume available should be such that the CO2 ratio is kept to safe values.

CO2 circulation would have to be forced by bubbling to accelerate its absorption in water. This circulation cannot be envisaged with CO2 alone but rather with the complete atmosphere. An extra pump would then be needed.

4.2.The water control loop :

Water management is treated in a similar manner as the air : the rat is supplied from its proper external drink source (fresh water) and, in a first step, the same policy as adopted for its food prevails.

However, the obtention of pure water from Melissa is normally possible without too many difficulties through physicochemical processes.

Extra hardware needed would then be : filters, pumps, purification unit (distillation) and control equipment.

5 TECHNICAL BREAKDOWN

As a preliminary estimate, all compartments have been tentatively quoted with equal sizes. This arbitrary choice is made provisionally until the reaction velocities of the individual cultivations are known with sufficient accuracy. Then the different bioreactors dimensions can be determined to ensure a coherent flow throughout the loop.

At first sight, the compartments are then namely constituted with standardized pieces of hardware. The technical breakdown stands as follows :

Compartment n	I	II	III	IV	Total mass (kg)	Total power (w)
Bioreactor	1	1	1	1	64	
Scrambling device	1	1	1	1	32	800
Illuminator		1		1	8	4000
Separation/Extraction	1	1	1	1		
Thermal regulation	1	1	1	1	5.5	1300
Electronics	1	1	1	1	50	500
Total..compartments.....					159.5	6600

Transfers and water loop :

I to II = A (volatile fatty acids)
I to IV = B (CO2)
II to III = C (NH4+)
II to IV = D (CO2)
II to V = E (biomass)

III to IV = F (NO3-)
IV to III = G (O2)
IV to V = H (O2)
IV to V = I (biomass)
V to IV = J (CO2)
V to I = K (waste)
water loop= L

	A	B	C	D	E	F	G	H	I	J	K	L	Total
Gas filter	x	x	x			x	x	x		x			7
Gas pump	x	x	x			x	x	x		x			7
Liquid filter			x						x				2
Liquid pump			x									x	2
Cooler					x								1
Dryer					x				x				2
Storage & packing	x	x			x				x		x	x	6
Fixation					x								1
Autoclave											x		1
Control	x	x	x	x	x	x	x	x	x	x	x	x	12

Physico-chemical conditions of operation (cultivating medium composition, temperature, pH) and all other parameters of the cultures such as growing rates are related in the previous issues of the present study.
The list of measurements performed and parameters controlled has been preliminarily settled and is regularly updated (4).

Measurements, treatments and transfers

The transfers of any of the substances (gas, liquid or solid) from one compartment to another were not considered parts of the individual compartments and the corresponding hardware elements were not quoted in the technical breakdown (masses and energy consumptions).

The purpose of the Melissa breadboard and the principles of its realisation have been described hereabove in Part I.
The present part of the note document underlines some of the main technical difficulties to be encountered during the construction of the loop.

1 THE REQUIREMENTS ONTO THE MELISSA BREADBOARD

Some of the requirements imposed on the characteristics of the Melissa breadboard are likely to generate direct important technical consequences. They are :

1.1 The constitution of a loop

Melissa is conceived as a system constituted by the assembly of a set of compartments initially tested individually. They must then be connected to each other so as to constitute a loop, even not completely closed, and this operation generates stringent matching conditions taking in consideration the differences of the reaction velocities and the appearance of new functions that were not necessary for individual cultivations.

1.2 The necessity of continuous operation

Melissa is due to prepare the study of the future regenerative life support systems such as those to be used for long human missions out of the Earth. For obvious safety reasons its compartments must be operating on a "non stop" regime and the conditions of continuous operation rank among the first to be mastered at the breadboard stage.

The ability of maintaining the parameters of a cultivation within definite ranges is thus a necessity which is rendered particularly difficult if some of the involved processes are not continuous in nature such as separations and transfers, for instance.

1.3 The automation

The continuous operation of the compartments requires that their processes are automatically driven. Considering the control of the entire Melissa system, such a great number of parameters must be monitored permanently for such long durations that it cannot be done but automatically.

1.4 The long duration of operation

The introduction of a recycling element in the life support system is justified by the time duration of the foreseen missions. A round-trip from the Earth to the planet Mars will take about 2 years for instance and this is typically the time basis after which Melissa should be designed.

This type of requirement for long lasting operations of the Melissa loop not only reinforces the need for its automatic control. It implies in addition that the cultivations must be protected against contaminations of any type.

The desired protection can be obtained via a systematic sterilisation of the cultivations media through physical means (autoclaving for instance), or chemical ones (via the regulation of the pH's).

1.5 The eventual applications in space

No spatial mission is scheduled for Melissa in the near future. However, it can be helpful that the technical difficulties anticipated to appear in the realisation of testing experimentations in space environment be simply identified and pointed out in advance.

Among them, microgravity with its particular effects on the behaviour of fluids and consequent difficulties of their separation, their filtration, their transportation and their thermal exchanges is one worrying concern.

The technical limitations of facilities offered on-board the currently available or foreseable spacecraft (in particular the limited amount of electrical power available in an orbiting laboratory and the thermal balance of the latter) must also be named here on the waiting list of problems to come.

2 TECHNICAL DIFFICULTIES

If left free under favourable conditions, a cultivation evolves towards a natural stop attained when any one of its parameters has reached a limit value. The role of the driving automatisms consists of preventing the occurence of these saturation phenomena. Technical problems are directly resulting from that necessity.

Concerning the cultivations proper, the controlled parameters are namely :

- The biochemical composition of the cultivations
- Their pH
- Their temperatures
- The growth rate of the microorganisms
- The characteristics of the light when used

They have been listed per compartment in (4). At first attempt, 16 parameters are to be controlled among 34 which must be measured. These numbers are to be reviewed when connections between compartments are considered and, later, when the realization of the loop has begun.

2.1 The maintenance of the cultivations and the continuity of operation

In order to regulate the composition of the cultivations, the products generated by the reactions must be extracted and removed regularly from the reactors. Inversely, the reactor must be resupplied with consumable products. These periodic operations must be performed automatically and, if possible, continuously. They can imply separation between any combination of gas, liquids, and solids.

2.1.1 The separation between solids and liquids

It will be obtained usually by filtration. The choice of the filter having been determined after appropriate studies, the necessity

remains, whence the filter is used continuously, to check permanently and automatically its efficiency.
The criteria of evaluation and the means of their measurement must be defined in that purpose, as well as the processes to recover the wanted efficiency, if necessary, by exchange of filter or cleaning.

2.1.2 The separation between fluids

It is a major difficulty if the mixture is nearly homogenous. Even after distinguishing between "separation of gases" on one hand and "separation of liquids" on the other, it is impossible to cover the subject in general terms.

The differences of the diffusion properties of gases and the differences of the densities of liquids have led to convenient and reliable techniques that could be applied via the use of selective membranes and centrifugation respectively.

The said techniques apply a-fortiori if separation between liquid and gas has is envisaged.

2.3 The transfers

After one of the said separations has been performed it is then necessary to transfer the separated product to the place of it utilisation, normally another compartment.

2.3.1 ansfer of fluids

It can well be ensured by pumps and would take benefit from the use of ballast reservoirs, precisely if the extracted fluid has to be reinjected into another compartment.

2.3.2 Transfer of solids

There is no real problem if the product must (or can) be transferred as wet. Such a case would be equivalent to the transfer of a fluid as long as the degree of dilution of the solid particles into the bearing liquid allows it.
It goes differently if the solid has to be dried. Then a drying step must be foreseen and processes for transfer be set up, constituting potentially a particular unit.

2.3.3 Measurements related to separation and transfer operations

The weighing of the quantities transfered is hardly done continuously unless it can be replaced by the measurement of a volume in the case of fluids.
To be performed continuously, chemical analyses of chemical controls must be done on fluid phases.

2.4 The light

Some of the incoming photons are not used in the photosynthetic processes and most of them simply warm the cultures up.
The lighting is then in tight relation with the thermal control of the cultures and, more generally, with the thermal control of the laboratory itself, especially if the latter is tentatively conceived in view of its utilisation in space ambiance.

2.4.1 Light production

Independently from the normal conditions of lighting of the laboratory, some of the microorganisms of Melissa require specific lighting to grow normally : Spirulina and Rhodobacter.

The light sources differ from each other by their photonic yield which can be quoted by the number of interesting photons obtained per unit of energy taken from the electrical power source. All photons not involved in the photosynthesis effect will inevitably be transformed into heat, and as a consequence the photonic yield must be optimized. It should at least be measured.

Several physical effects are possible candidates for producing light : Joule effect in conventional electric lamps, electrical discharge in gas tubes, solid state diodes, etc...

Their respective merits must be compared concerning many of their characteristics in both domains of their biological use (spectral adaptation) and their technical properties (Weight, size, electrical efficiency, reliability, operating temperature, life time and maintenance easiness).

2.4.2 Light transmission

Driving the photons to the needed organisms, i.e. into the medium with which they interact can be ensured by several conventional devices like mirrors or optical fibers.
Optical devices able to optimize the interaction between photons and aqueous microorganisms are still to be imagined. The long term drawback of

2.5 Thermal regulation

Melissa is far from being an isothermic system. The compartment of Clostridia must be maintained at 60 C, for instance, although the temperatures of other compartments should remain at 22 C.

The thermal control of the system needs these differences between compartments temperatures be maintained while the products themselves are expected to be exchanged continuously from one compartment to another, creating thus systematic thermal flows. The thermal consequences of these exchanges must obviously be taken in account when setting up the system steering laws and proper technical means be envisioned to control them.

First, the consequences of temperature variations on the growth of all concerned microorganisms must be known, then, the thermal conditions of the exchanges must be mastered. The ranges of admitted temperature variations depend upon the growth velocities in the concerned compartments, and they can be in such discrepancy that the use of thermal adapters could reveal necessary to ensure that exchanges between compartments are quasi-isothermic.

In all cases the thermal breakdown of the system might be assessed in view of preparing the eventual use in space.

2.6 pH regulation

The regulation of the pH value is performed as for individual cultivations, it must not be considered as a particular problem.

2.7 Sterilisation and axeny maintenance

As stated in the case of the rat feces (3) autoclaving is a convenient way of sterilizing a product in a homogenous manner . This implies a procedure "in batch" as no simple way exists of autoclaving on a continuous basis. The processes are not different from individual cultivations but then the energy, including its thermal consequences, and the time necessary for the complete operation of sterilization must be counted.

Axeny is quite different a problem as there is no easy way to measure the axeny of a cultivation and to maintain it actively against a contamination of unknown origin without sterilising the cultivation itself.
The protection of the cultivation against contaminants by complete isolation of the compartments presents in all cases the major drawback that no warning signal can exist before a contaminating agent is already acting there. One must then accept the risk as not 100% avoidable and rather rely on measures to correct the eventual consequences. Redundancy can be helpful which consists of resterilizing the contaminated half of a doubled reactor if one wishes really a complete continuity of operation.

PART III CONDITIONING OF THE RAT FECES

1 The role of the consumer compartment during the breadboarding phase of Melissa

The first experimentations to be settled with the Melissa breadboard are due to demonstrate the possibility of coupling its compartments to one another, once they have been tested individually, without disturbing their behaviour, while maintaining them in operation for long periods (months).

The initial duty of the consumer compartment consists then of providing Clostridia with rat feces in a controlled manner (Quantities and chemical composition of the feces being measured).

In a further step, when its initial purpose has been reached, the capacity of the Melissa system to treat cellulosic wastes will be checked.

Later-on the possibiity of the system to treat the CO2 produced by rats respiration can also be investigated.

However, for practical reasons, it is not intended immediately to try growing rats in a completely closed compartment, controlled automatically, as it is the case for the microorganisms, the other acting members of Melissa. As far as we know this type of experimentation has not yet been reported or even been tentatively performed and such a venture can be considered premature for the benefit of the Melissa study at the moment.

2 Growing conditions of the rats.(*)

A certain number of conditions apply to the production of rat wastes and to their supply to the Clostridia compartment. The following assumptions are made in consequence, concerning the rules of discipline to be observed concerning the rats growing along the breadboard operation :

The rat wastes will not be more or less simulated and generated through any artificial fabrication process. So they will not take benefit from the regularity of their chemical composition that an artificial/industrial production would easily provide. They will be natural feces generated by living rats and thus their chemical composition will normally depend upon the physiological condition of the animals, likely to vary from day to day.
It is desirable that these eventual variations be kept to a minimum and, when not completely inevitable, be identified and recorded.

For this reason, the rats of the Melissa breadboard will be "dedicated" ones, and normally will not be changed during the duration of the experimentation.

The animals will be grown under constant conventional laboratory conditions :

- the ambiant temperature of the cages will be regulated at 22 +/- 2 C

- they will receive constant standardized food and water from sources external to Melissa in measured and recorded quantities.

- they will breathe ambiant laboratory air renewed by external atmospheric supply and the gasses rejected by the effect of their respiration will be left for normal dilution in the atmosphere and not be collected.

- They will not normally be fed with the Melissa-produced food (Spirulina Rhodobacter etc...). However, the non toxicity of Spirulina and Rhodobacter/Rhodospirillum produced by Melissa as biomass will be checked experimentally as well as their tolerance and their free acceptance by the animals. The latter check could be done "off-line", possibly on different individuals if judged more convenient. The capacity of Melissa as a food producer constitutes itself the subject of a separate study.

The subject is rather complex and includes, beyond the check of non-toxicity, of obvious concern, the study of the long range effects of Spirulina/Rhodospirillum ingestion upon the animal metabolism. The corresponding experimentation must not necessarily be carried out in line with the present one (production of wastes). In particular it might be envisaged that the study of the rat as a consumer is performed with different individuals.

The capacity of Melissa to treat other wastes than rats feces can be wished. Then it might appear necessary that other products be added to the rat wastes (cellulose for instance) in addition to the practically inevitable parts of unconsumed food.

(*) These conditions are staying well within the possibilities of the CEN/Mol animalry.

3 Conditionning of the feces.

Several techniques can be envisionned for providing the feces to Clostridia in a controlled manner. Their respective merits concern :

- the regularity of the process so that the flow rate of waste delivery to the Clostridia compartment can be adjusted, maintained and measured.

- the handling easiness and safety

- the obtention and the maintenance of sterilisation

- the easiness of the chemical analyses

3.1 Pulverisation

Feces can be reduced into powder by grinding, either or not after complete drying, and homogenization can be reached by means of mechanical mixing. This operation can probably be rendered more efficient if the operation of mixing were performed in liquid phase as the chemical analysis, necessary for checking the homogenity, implies rehumidification.

Should the intention be raised of mixing feces with cellulosic additives then the pulverisation process would still apply, (use of chopping grinder recommended to treat paper or straw, if necessary after experimental test).

Calibrated distribution of pulverized feces to the Clostridia compartment seems to present difficulties. If one wishes to control the flow with accuracy, it is quite mandatory that the density of the product is either maintained constant or permanently measured. This is difficult if the feces are maintained as a powder.
Due to the adherence of the powder onto the tubing walls it seems also difficult to maintain a really continuous flow of waste to the Clostridia reactor.

3.2 Fluidization

Once they have been collected and possibly separated from urine, the feces can be fluidized i.e. mixed with such quantity of water that the mixture behaves as a liquid. Agitation ensures homogenity of the mixture and must be maintained permanently until the preparation is delivered to the Clostridia culture.

The process is classical and needs no particular description. One can remark that it would well fit with a procedure "in batch". The need for homogenization necessitates that the volume of the preparation exceeds a certain minimum value and the interest of maintaining constant the composition of the product leads to prepare in advance the total quantity of wastes necessary for a complete experimental sequence.

The distribution to the Clostridia compartment can be rendered automatic through the simple effect of gravity, possibly regulated by means of a peristaltic pump.

Fluidization facilitates sterilization by U.V. and flow control. The only potential drawback of the process is the addition of water.

3.3 Encapsulation

In spatial applications, which need often a 100 % degree of automaticity or nearly it has been a current practice, for supplying regularly some compartments , to use calibrated capsules or pellets.

This has been the solution retained by CNES to feed the two monkeys of the "Rhesus" experiment and also by NASA for some experiments involving animals.

The pellets correspond to a calibrated quantity of matter. They can be isolated from external influence by a protective individual envelope soluble in water. This type of conditionning calls for techniques currently in use in pharmaceutical industry. The pellets are easy to handle manually or automatically. This technology, if applied to the rat feces, would probably fit most of the technical requirements of Melissa.

Some drawbacks appear to be underlined, however :

Before starting manufacturing the pellets many technical difficulties must have been resolved. A mandatory step is the quantization of identical weight-calibrated elements, and it seems that a significant financial investment should be made for covering the cost of developing and testing a dedicated apparatus. The corresponding expenditures look excessive when compared with the possibilities of its use.

The time duration to perform this side activity is also to be considered. In addition the necessity of evaluating the possible chemical effects that the constituents of the encapsulating membrane could generate upon the action of the following compartment would also be time and money consuming. It makes finally the "pellet" solution rather complicated and possibly expensive.

4 Comments

A mammal life support system is far from being as easily manageable by automatisms as can be microorganisms. The introduction of the rat into Melissa brings in several sources of difficulty.

The chemical composition of the wastes, which should ideally be kept constant through the duration of the experiment, is likely to depend upon environmental circumstances and to vary from one individual to another.

Although in nominal operation Melissa should be able to cope with normally expectable variations of the composition of the wastes generated by the consumer , it is desirable, for the benefit of the study, that the basic experimentations be carried out with minimum disturbance. In that respect the preparation of batches of wastes, of averaged chemical composition, even slightly different from one another, is the recommended procedure. It is the condition of accurate measurements.

For practical reasons, it is recommended that the total time duration does not last too long so that not too many intermediate operations of sterilisation of the feces are needed during the batch constitution.

In that purpose, fluidization appears to be the most practical technique from the viewpoint of the preparation of the wastes, their chemical analysis, their homogenization, their sterilization, and their regular distribution to the next compartment (Clostridia).

The requirement for including a drying and pulverisation step in the sequence of operations of waste preparation seems in no case mandatory. Feces can be ground directly even as slightly wet matter and the drying phase appears unnecessary. However a re-watering operation has to follow, when fluidisation is needed for transportation and distribution.

The addition of other wastes, such as paper or cellulosic products, will imply the use of a knife equipped grinding machine currently commercially available.

5 Particular technical problems

5.1 Continuous supply

Any waste production process (technical or physiological) is generally not continuous at all. The preparation of wastes, to be processed in a continuously operating system like Melissa, must comprise a step of storage either to take account of the conditions generated by an "in batch" regime or coming from any needed "off line" treatment, such as milling, in the present case.

The transition between continuous and non-continuous regimes is a source of specific difficulties. Loading/unloading activities are likely to generate mechanical problems the automatic control of which can be complicated.

5.2 Cleaning

Although the requirement can probably not be mandatory at the very beginning of Melissa history, hardware to be utilised during the study of ecological systems, as well "on-line" as "off-line", must

leave the possibility of being cleaned. Cleaning can well be performed as non-continuous operation, piece by piece however the system should preferably be continuously cleanable as a whole, in view of future utilisation in space.

An on-line-continuously-operating equipment can be cleaned by water. The only potential drawback seems to be the change of the dilutions of the cultivations generated by this addition of water.
This type of operation could be usefully combined with the eventual necessity of adjusting the chemical composition of the next cultivation medium.

5.3 Sterilisation

To be properly identified and mastered the bioreaction of Clostridia requests that the other bacteria are destroyed before the medium is seeded with the chosen strains.

Several methods can be thought of for sterilizing : ultra-violet and gamma rays can be used with a continuous flow, many chemical products also are commonly employed, as well as autoclaving.

In the present case the sterilisation process must be such that its efficiency is absolute and complete as long as Clostridia have not been introduced in the reactor and its action must stop as soon as they have been introduced. The latter requirement eliminates the chemical solution. The necessity of stopping the sterilisation would lead to operating difficulties comparable with the sterilisation itself, it would imply difficult measurements of chemical concentrations and of total quantities and generate risks of long terms effects and of consequences on the following compartments.

Autoclaving and irradiation by U.V. or gamma rays do not suffer from these drawbacks, no long lasting effect is to be feared after use. They constitute valuable candidate means for sterilisation of the Melissa breadboard. Among them, autoclaving seems the most efficient with no risk of shadowing effect as can present the radiations.

6 Flow chart of solid wastes preparation

Many of the operations listed hereafter have not yet been calibrated in terms of their respective durations and even performances. The successful sequencing of the said operations supposes that each of them has been validated by previous proper trials.

6.1 Collection of rat feces

Although it should ideally be conceived as a continuous, or nearly continuous, process, which would facilitate keeping the cultivation of Clostridia free of oxygen, in reality the solid waste preparation will follow an "in-batch" sequence.
For the present study the rats are grown conventionally, in standard cages. Automatisation of the physical processes would imply at least that specific hardware is developed on purpose. Such an option would be time and money consuming and moreover not evidently in line with the study objectives.

The preparation of solid wastes, including the collection of rat feces and the introduction of external wastes is then to be performed partly manually.

6.2 Transfer into grinder

This is as well an "in-batch" operation. Its periodicity which is determined by the capacity of the grinder. Normal ordinary use of existing machines implies manual loading and unloading.
Like feces collection, loading the grinder cannot easily be automatised. Automatisation would lead to study and develope special hardware and would be also a time consuming activity.

6.3 Grinding of wastes

It is naturally performed automatically, possibly in a grinder equipped with marbles, and it can be done either before or after the sterilisation.

6.4 Sterilisation

This constitutes also an off-line sequence of activities, essentially not continuous, with a time constant dictated by the temperature cycling itself.
It is wished that the sterilisation process and the grinding are carried-out in the same container so that the number of transfers is minimized and so the risks of exposure to open air.

6.5 Filling of the Clostridia compartment feeder

Manual or partly manual. The following operation, which consist of regularly feeding the Clostridia culture, is continuous, but the present one is not. Unless a dedicated apparatus is conceived and built, allowing grinding, autoclaving and regular distribution of ground feces to be ensured by the same equipment unit.

6.6 Storage

It is naturally a completely passive phase. The precaution to be imperatively taken concerns the maintenance of anoxy.

7 Remarks

As long as some basic data like the reaction velocity of Clostridia are not known, is not possible to make proper estimates of some important physical parameters of the feces conditionning such as the duration of the sequences of preparation, the degree of grinding, and the size of the batch in particular.

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