Pergamon

Adv. Space Res. Vol. 18, No. 11, pp. (11)111–(11)117, 1996

0273–1177/96 $15.00 + 0.00

PII: S0273–1177(96)00097–X

MELISSA: A POTENTIAL EXPERIMENT FOR A PRECURSOR MISSION TO THE MOON

Ch. Lasseur,* W. Verstraete,** J. B. Gros,*** G. Dubertret† and F. Rogalla‡

* *Thermal Control and Life Support Division, ESA/ESTEC, 2200 AG Noordwijk, The Netherlands*
** *Laboratory of Microbial Ecology, Coupure Links, 9000 Gand, Belgium*
*** *LGCB, Université Blaise Pascal, Av des Landais, 63177 Aubière, France*
† *LBFMV, CNRS, Av de la Terrasse, 92198 Gif sur Yvette, France*
‡ *Anjou Recherche, Chemin de la digue, 78600 Maisons Laffitte, France*

ABSTRACT

MELISSA (Micro-Ecological Life Support System Alternative) has been conceived as a micro-organism based ecosystem intended as a tool for developing the technology for a future artificial ecosystem for long term space missions, as for example a lunar base. The driving element of MELISSA is the recovering of edible biomass from waste, CO2, and minerals with the use of sun light as energy source. In this publication, we focus our attention on the potential applications of MELISSA for a precursor mission to the Moon. We begin by a short review of the requirements for bioregenerative Life Support. We recall the concept of MELISSA and the theoretical and technical approaches of the study. We present the main results obtained since the beginning of this activity and taking into account the requirements of a mission to the Moon we propose a preliminary experiment based on the C cycle of the MELISSA loop.

INTRODUCTION

Establishment of a long term manned base on the Moon implies the development of a reliable life support system including food supply and waste management. Due to the mission duration, supplying all food, oxygen and water from the Earth will result in a tremendous cost, therefore the life support system has to become in large part regenerative.

Presently, physical /chemical processes are available to regenerate air and water by appropriate treatments. The air loop can be closed by regenerating oxygen from carbon dioxide via the use of molecular sieve, carbon-dioxide reduction by Bosch reactor, and water electrolysis. The water loop can be partially closed by employing evaporation systems or membrane filtration techniques. However, the physical/chemical techniques consume a lot of energy and cannot produce food, which must still be resupplied from Earth. Food production can be only achieved by biological means, and the introduction of biological techniques opens a new area of solutions for other life support requirements, such as atmosphere, water and waste managements. In fact, nearly complete loop closure can be foreseen in a closed ecological system, such as MELISSA.

Due to the fact that artificial ecosystems are related to various disciplines (radiation biology, genetic, plant physiology, control theory, biotechnology,...) they can be considered as one of the best tool for the scientific utilisation of the Moon base. Moreover, the establishment of an artificial ecosystem during a precursor mission to the Moon is justified by the necessity to evaluate the adaptive strategies of organisms to the lunar environment via multigeneration experiments.

PRELIMINARY REQUIREMENTS FOR A BIOREGENERATIVE LIFE SUPPORT SYSTEM

Artificial ecosystems have been studied by man for several decades now /1/, in an attempt to increase understanding of both the evolution of, and the behaviour of, natural ecosystems; and in a more engineering approach to design a stable ecosystem ensuring human living conditions, in the frame of an example based on long-term manned space missions.

Preliminary requirements for the etablishment of an artificial ecosystem on the Moon have been defined earlier by ESA /2/ and NASA /3/. Regarding its main functions (recycling of the waste from the crew, and production of food and respiratory gases for the crew), several recommendations have been already made. They concern :

- the components of the ecosystem (microalgal and/or higher plant for the food and oxygen production ; microbial cultures including photoheterotrophic compartment for the waste recycling);
- the scientific objectives (mathematical model, system theory, dynamics and control);
- technical points (implementation of a complex monitoring and data acquisitions system and computer simulation).

When considering the design of artificial ecosystems, two fundamentally different approaches are often put forward. From the one side, the holistic approach proposes to take advantage of the self-regulatory characteristics of a natural/complex ecosystem by enclosing groups of species known to be associated in

natural ecosystems and allowing them to reorganise in a new, self-maintaining ecosystem. From the opposite, purely engineering side, the strictly reductionist approach is based on the separation of each elementary component of the ecosystem, development of separate control systems for each, and the use of the individually controlled elements to construct an entire system.
The choice of either an holistic or a reductionist approach is of great importance for the design of manned, closed ecological systems capable of sustaining human life. Indeed there is doubt, arising from ecological considerations, that the holistic approach is in fact the simplest and most efficient way of realising a artificial ecosystems sustaining humans. Indeed, if the ecosystem is simply allowed to evolve without any control, it will evolve following its intrinsic laws; given the generally limited understanding of its evolution patterns, which are governed by non-equilibrium dynamics and non-linear reactions to disturbance, this evolution will be extremely difficult to predict.
The two concepts (holistic and deterministic) can be compared on essentially two features: efficiency and stability : efficiency is generally linked to the yield calculated on two or three parameters, stability being in the frame of life support, a major criterion of safety.

In the field of ecosystem studies, the optimization of the process can be performed with several goals: to sustain human dietary needs to reduce the consumed energy; to increase the efficiency of the loop (output/input); and to increase both efficiency and safety. This optimization implies the mastery of the process behaviour to maintain the "ideal" external conditions.
Because of its interest for the understanding of the interrelations between the various components of an ecosystem (characterisation of the interfaces) and the deterministic control that it makes possible, the compartmentalized approach has been selected for the preliminary developments of artificial ecosystems by ESA.

MELISSA : AN EXAMPLE OF ARTIFICIAL ECOSYSTEM

The MELISSA (Microbial Ecological LIfe Support System Alternative) project has been set up to be a model for the studies on ecological life support systems for long term space missions /4/. The compartmentalisation of the loop, the choice of the micro-organisms and the axenic conditions have been selected in order to simplify the behaviour of this artificial ecosystem and allow a deterministic engineering approach. In this framework the MELISSA project has now been running since the beginning of 1989.

MELISSA is a joint venture involving five independent organisations (SCK/CEN MOL, University of Ghent, CNRS at Gif sur Yvette, University of Clermont Ferrand, Matra Espace) four associated members (CNES, ADERSA, TNO Leiden, Generale des Eaux) and ESA. The driving element of MELISSA is the recovery of edible biomass from waste, carbon dioxide and minerals, with the use of light as a source of energy for biological photosynthesis. Light-dependence is minimised by the incorporation of anaerobic steps in the waste recycling loop, allowing the usual carbon-oxidation reduction loop to be partially short-circuited.
MELISSA has four successive micro-biological compartments (fig 1) colonised respectively by

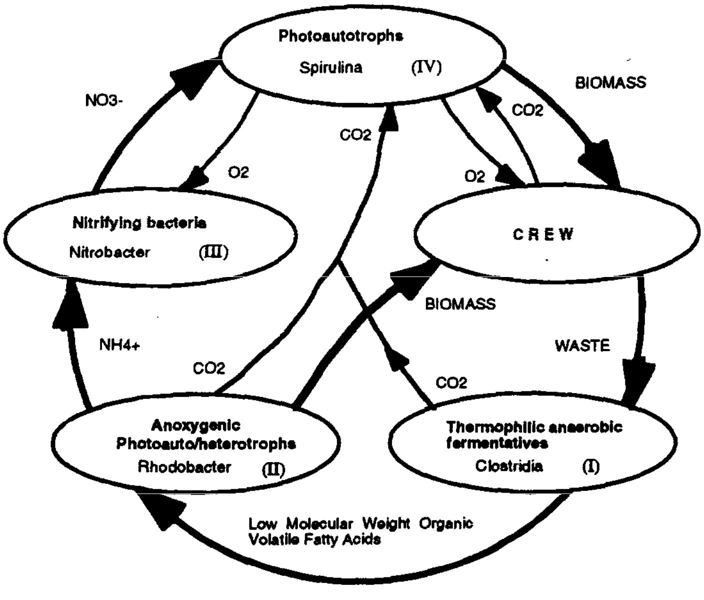

Figure 1 : Concept of MELISSA

Table 1 : Activities of the 3 strains selected for the liquefying compartment.

	Clostridium Thermocellum	Clostridium thermosaccharolyticum	Coprothermobacter proteolyticus
Cellulolysis	Y	N	N
Proteolysis	N	N	Y
Saccharolysis	Y	Y	Y
CO2 production	Y	Y	ND
H2 production	Y	Y	Y
VFA production	Y	Y	Y
Ethanol production	Y	Y	Y
NH4+ production	Y	Y	Y

thermophilic Clostridia for waste liquefaction, anaerobic Photo-rhodochromogens for the removal of soluble organics, nitro-bacteria for the nitrification of ammonium ions and the cyanobacteria Spirulina for food production and carbon-dioxide recycling.

In the first phase of the MELISSA contract, an important bibliographic review was carried out to check the validity of the concept : theoretical efficiency, safety, biomass quality and interconnected compartments toxicity have been studied. Pure cultures of each micro-organism were prepared to study their metabolism and prepare the modeling. Tests of toxicity were realised, as well as the preparation of the instrumentation and control hardware for the physical closure of the loop.

- Liquefying compartment : In the MELISSA loop, wastes (faeces, paper, kitchen wastes,...) would be first liquefied by thermophilic Clostridia. Tests have been undertaken to study the growth of collection cultures Cl. thermosaccharolyticum (LMG 2811) and Cl. thermocellum (ATCC 27405). The formation of volatile fatty acids, ammonia, CO_2 and H_2 have been measured, on different carbon and nitrogen sources. The Clostridium strains are growing well and different substrates have been fermented (glucose, starch, cellobiose, tryptone, yeast extract, urea, NH_4Cl) with production of CO_2, H_2, ethanol, acetic, lactic and butyric acid (Table 1). At the same time, isolation and characterisation of a third strain " Thermobacteroides proteolyticus" has demonstrated proteolytic activities. Tests are now running to identify the best co-cultures from these 3 strains, in terms of efficiency.

- Phototrophic compartment : This compartment is colonised by non-sulphur purple bacteria such as Rhodobacter capsulatus and Rhodospirillium rubrum, which have the ability to grow either in photoautotrophic or photoheterotrophic conditions. The energy source of these photobacteria is light. The growth performances on different C and N sources are presented in Table 2 which shows that both strains are able to grow in heterotrophic conditions. However, due to its ability to metabolise a larger number of C and N sources, the Rsp. rubrum has been selected for the heterotrophic compartment.

- Nitrifying Compartment : The main function of compartment III is to recycle ammonia into nitrate. First tests on non-fixed cultures have clearly pointed out the very slow growth rate and the risk of inhibition. For this reason, a specific study has been dedicated to identifying a reliable and efficient support. The following requirements have been highlighted : very long life duration, small retention time, roughness of the surface, weight, chemical stability. Four different supports have been compared in real conditions. The support "Biostyr" has been clearly identified as the best. At the same time, a comparison of two bioreactor designs, fixed-bed and fluidized bed, has been realised during four months of continuous culture using active charcoal as support. Two remarks can be made : - the fixed-bed reactor presents the best yield in terms of NH_4^+/NO_3^- ratio, and at the same time the highest NH_4^+ eliminated load.

Table 2 : Comparison of the C and N sources assimilation for R.rubrum and R.capsulata. /12/.

Nitrogen Sources	Carbon Sources	R. capsulatus	R.rubrum
NH4+	Acetic	+	+
	Lactic	+	+
	Isobutyric	+	+
	Propionic	+	+
	Butyric	+	+
	Valeric	+	+
	Isovaleric	+	+
	Caproic	+	+
	Isocaproic	+	+
	Ethanol	-	+
Urea	Lactic	+	+
	Acetic	-	+
	Ethanol	-	+
	Lactic + Ethanol	+	+
	Acetic + Ethanol	-	+

Table 3 : Comparison Model experiment for the biomass compounds of the photosynthetic compartment for different incident flux.

Mean radiant incident flux (W/m2)	% of EPS in total Biomass	Experimental global formulae of Spirulina platensis	Spirulina Global formuale given by the model	Standard Deviation
10	10	C=1 H1.579 O0.435 N0.162 S0.0064 P0.0057	C=1 H1.574 O=0.459 N=0.173 S=0.0063 P=0.0057	C/H=0.3 C/O=5 C/N=6 C/S=1.5 C/P=0
100	20	C=1 H=1.590 O=0.493 N=0.140 S=0.0073 P=0.0043	C=1 H=1.583 O=0.514 N=0.154 S=0.0072 P=.0050	C/H=0.4 C/O=4 C/N=9 C/S=1.4 C/P=6
160	27	C=1 H=1.6000 O=0.541 N=0.120 S=0.0078 P=0.0043	C=1 H=1.589 O=0.552 N=0.140 S=0.0078 P=0.0046	C/H=0.7 C/O=2 C/N=14 C/S=0 C/P=6.5
230	34	C=1 H=1.607 O=0.580 N=0.105 S=0.0084 P=0.0039	C=1 H=1.594 O=0.590 N=0.126 S=0.0086 P=0.0042	C/H=0.8 C/O=1.7 C/N=16.7 C/S=2.3 C/P=7.1

- Photosynthetic Compartment : Following the extensive study realised to establish the stoichiometric equations in optimum conditions and under N, S, P limitation /5/, attention has been paid to study of the light interaction in a photo-bioreactor. Study of the interactions between physical limitations with light in a photo-bioreactor leads to very complex equations. A simple model based on the one-dimensional equation of Schuster for radiation transfer has been used for a flat surface reactor /6/, and has been adapted to a cylindrical bioreactor to be consistent with the ESA hardware. Table 3 presents a preliminary comparison of the biomass composition in the reactor with the values calculated by the model for different light intensities.

- Crew Compartment : In the MELISSA model, the rat was chosen as a simple and well-known representative of the consumers and as a producer of excreta. These rat excreta are used as fermentation substrates. Studies have been performed to investigate the food acceptability of Spirulina and its suitability as a component of the consumer diet in the MELISSA ecosystem. Results obtained during a sixteen week period have confirmed that Spirulina may be used up to 40% as a component of the rat diet /7/.

- Modelling : There is only a small number of artificial ecosystems projects dealing with the important task of modelling (mass balance, dynamics,...). The mathematical modeling of the MELISSA loop is performed following a modular approach. This means that each compartment is first considered apart with its own inputs and outputs. In a second step, the different compartments are connected in order to check the mass balances and to define additional inputs to, and outputs from, the system. For MELISSA the technique for modeling consists of separate stoichiometry and kinetics, as is usually done for chemical reactor design. An extensive description of the MELISSA modelling approach has been already presented in /8/, and first results of the percentage of closure of the loop are presented in figure 2.

- Instrumentation and Control : With the goal of progressive physical realisation of the MELISSA loop, several problems of hardware have been identified and studied : axenic and continuous cultures, on-line analysis, architecture of control, reliability. However, the main part of this study has been focused on understanding the dynamics and control policies. Indeed the mathematical model, previously developed has been reappraised to reflect specific hardware characteristics. Figure 3 presents our actual level of accuracy for a predictive control of the Spirulina growth rate.

EXPERIMENT PROPOSAL

Regarding the well known space requirements (energy, weight, volume,..) and in order to improve our knowledge about artificial ecosystems, a simplified loop of MELISSA is proposed as an experiment for a precursor mission to the Moon. This experiment, presented in figure 4, consists of the photosynthetic (spirulina) and the consumer compartments.

Indeed, we propose to limit this study to the C cycle. The CO_2 produced by the crew compartment is recycled by photosynthesis into O_2. At the same time, we propose to use the resulting biomass to provide the main

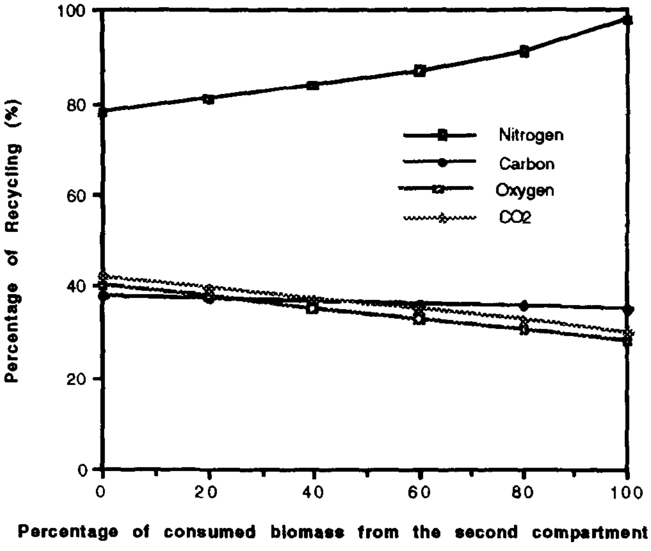

Figure 2 : Percentage of recycling of the complete MELISSA loop.

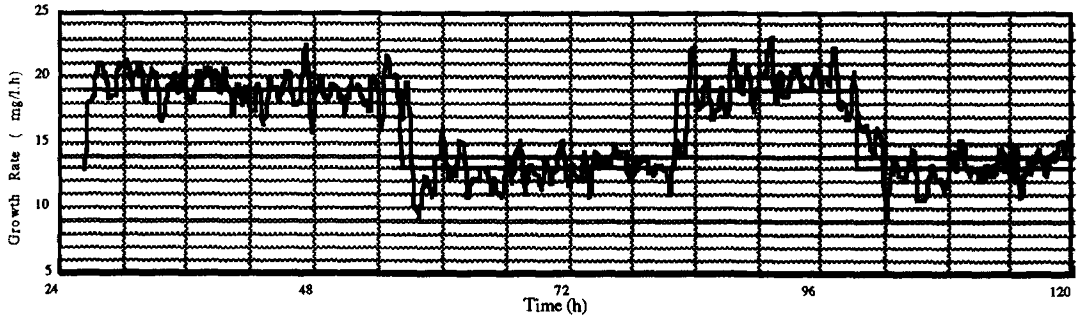

Figure 3 : Evolution of the growth rate for two set points 13 and 19 mg/l.h. (Standard deviation 1.1)

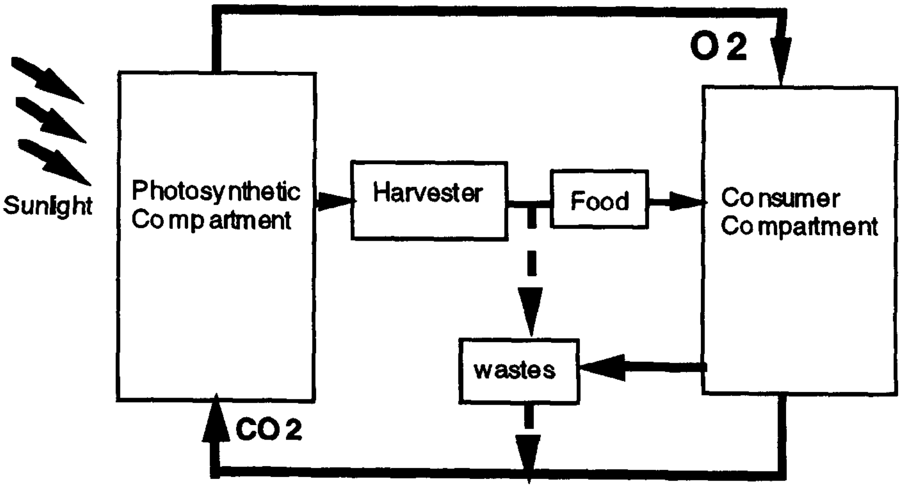

Figure 4 : proposal of experiment based on the C cycle of the MELISSA loop

part of the consumer diet. A value of 40 % based on previous experiments /7/ has been selected. The energy will be supplied by sunlight.

The goal of this experiment is to evaluate the influence of the lunar environment (radiation, reduced gravity, temperature cycle, duration of the photo-period,...) on the micro-organisms during a multigeneration experiment. This experiment could also be used to validate solutions to specific technical problems on the Moon such as radiation shielding, temperature control, energy supply during the night,...

On the basis of our current knowledge, some technical data can be already evaluated :

- crew compartment : the choice of the consumer will determine the size of the experiment. Taking into account the constraints of this mission, and the necessity to obtain reliable data, we propose a consumer compartment of 3 mice. Several publications /9,10/ allow us to evaluate the size and the weight of this compartment, at around 15 kg.
- photosynthetic compartment : in order to close the C cycle, the size of this compartment is related to the O_2 consumption of the animal. Assuming an average of weight of 10 g, a VO_2 of 0.065 ml/g and illumination of 200 W/m^2 we arrive at a production of spirulina of 50 mg/l.h. A volume of 20 liters will therefore be required. It must be clear that this volume will be considerably reduced if we assume a higher available illumination power. For example a total light energy of 300 W/m^2 allows to decrease the volume from 20 to 18 liters.
- temperature and Sun light availability : it is clear that the amplitude of the temperature cycle (+170 to -110 dgC), and the duration of the night (14 days) introduce important technical difficulties : first maintaining the experiment in the range 10 to 40 dgC; second, providing the minimum energy necessary during the night period. For example, 50 W during 15 days requires more than 100 kg of conventional batteries. For these reasons, we believe that the equator is may be not the most suitable site for a life sciences experiment. A polar site should reduce the temperature cycle and a minimum of energy will be probably available. On the other hand, it is apparently rather difficult to obtain accurate data about the environmental conditions at the lunar poles.
- radiation : this remains an important problem for life sciences experiments. Bacteria, for example, must receive a dose lower than 1 Krad /11/. During an experiment of 90 days, respecting this value should not cause an important shielding mass, but a compromise will have to be found between the utilisation of the direct sun light, the mass of the radiation shielding, and the mass of the hardware for the energy supply and the temperature control.

CONCLUSION

The MELISSA loop was conceived 5 years ago to be a model of artificial ecosystems for long term space missions. It has already provided the following major results :

- demonstration of the validity of the concept. All the functions expected (proteolysis, cellulolysis, saccarolysis, heterotrophic activities, nitrification, photosynthesis) have been demonstrated during real tests.
- the biomass production (Spirulina) has been validated as an important part of the consumer diet during a period of 16 weeks.
- no toxicity has been identified between two succesive compartment.
- high theoretical percentage of closure of nitrogen (99.5 % of the total production is reconsumed).
- modelling of the complete MELISSA loop in terms of mass balances allowing to calculate the % of recycling for each chemical compounds (C,H,N,O,S,P).
- demonstration of the robustness of the predictive control approach during tests of several months.
- creation of a pilot plant in ESA where the experiments can be tested and evaluated. Three compartments of the MELISSA loop are currently running in this Lab.

Thus, the achievements made so far form an excellent base for a precursor scientific mission. Regarding the requirements of such a mission on the Moon it is proposed to limit this experiment to the the photosynthetic and consumer compartment of the MELISSA loop.

ACKNOWLEDGEMENTS

I would like to thank B.Aalders, M.Davidson, L. Deparolis, H.Evans, P.Poinas and all the MELISSA partners for their technical advices.

REFERENCES

-1- TAUB F."Closed ecological systems" Anal. Rev. Ecol. and Systematics, 4, 1974.

-2- BONNET R. "Mission to the Moon, Europe's priorities for the scientific exploration and utilisation of the Moon" ESA SP 1150, June 1992, 190p.

-3- LOCKHEED "Lunar base controlled ecological life support system" final report, NASA CR-188479, 1991, 207 p.

-4- MERGEAY M., VERSTRAETE W., DUBERTRET G., LEFORT TRAN M., CHIPAUX C. , BINOT R.A. "MELISSA - A micro-organisms based model for CELSS development" Proceedings of the 3rd European Symposium on Space Thermal Control & Life Support System, Noordwijk, the Netherlands, 3-6 Oct 1988.
-5- CORNET J.F., DUBERTRET G. "The Cyanobacterium Spirulina in the photosynthetic compartment of the MELISSA artificial ecosystem" Workshop on artificial ecological systems, CNES/DARA, Marseille, France, 24-26 Oct 1990, p91-97.
-6- CORNET J.F., DUSSAP C.G., DUBERTRET G. "A structured model for simulation of cultures of the cyanobacterium Spirulina Platensis : I coupling between light transfer and growth kinetics" Biotechnology & Bioengineering, Vol 40, p 817-825, 1992.
-7- TRANQUILLE N., EMEIS J. "Food acceptability studies for MELISSA" Final report, projet ESA, 113088, 1991, Noordwijk, The Netherlands.
-8- DUSSAP C.G., CORNET J.F., GROS J.B. "Simulation of mass fluxes in the micro-organisms based ecosystem MELISSA" 23rd International Conference on Environmental Systems, July 12-15, 1993, Colorado Springs USA.
-9- BONTING S.L., KISHIYAMA J.S., ARNO R."Facilities for animal research in Space with special references to space station freedom. ICES conference 1990,
-10- DALTON B.P., JAHNS G.C., HINES M.L. " Rodent habitat concepts towards the future" ICES conference 1993, Colorado Springs, USA.
-11- GRIGORIEV Y.G., RYZHOV N.I., KRASAVIN E.A., VOROZHTOSOVA S.V., KPSHEEVA L.A., SAVCHENKO N. " The radiobiological effects on heavy ions om mammalian cells and bacteria" Life Sciences and space research XI, proceedings of the COSPAR, Madrid May 1972, p 247-259.
-12- ALBIOL J., GICQUEAU B., LASSEUR C."Preliminary studies on the performance and behaviour of the MELISSA photoheterotrophic compartment" 24 th ICES, Friedrichhafen, Germany, june 1994.