Monday, June 9

Session MP2
Room 2
2:30 - 5:30 p.m.

Biological Life Support Systems

CREW REGENERATIVE LIFE SUPPORT IN LONG-DURATION SPACE MISSIONS

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The paper deals with the status and prospects of spacecraft and base crew life support. A key problem governing human stay and activities in long-duration space missions and planet exploration is the development of regenerative life support systems (LSS). The use of systems for water recovery and air revitalization and in prospective food from the end products of life as well as an integrated bioengineering system enables the crew to be provided with water, oxygen, and food, thereby creating a habitat environment on spacecraft or base. In Russia (former USSR) extensive research has been done to prove the feasibility of integrated long-life regenerative chemical/physical and biological systems. The first chemical/physical systems were installed on Salut orbital space stations to recover potable from humidity condensate. The Russian Mir space station incorporates systems for water recovery from humidity condensate, from urine reclamation and hygiene waste water processing, a system for oxygen generation by electrolysis, a system for the removal of CO_2 and other trace contaminants. The systems allow a considerable reduction in specific mass water and oxygen supplied from the Earth. A modular construction of the regenerative systems provides for their updates. The Mir updated systems complemented with a system for CO_2 collection and concentration and a Sabatier CO_2 system followed by a vitamin greenhouse are planned to be installed on the Russian segment of the International Space Station (ISS). The ISS LSS will be a baseline of new regeneration spacecraft and planetary base LSS. Advanced LSS will be based on the water recover efficiency, low energy and mass demand, LSS reliability enhancement with a gradual transition from physical/chemical to integrated physico-chemical/biological systems.

For successful space exploration and missions to the Moon and Mars a R&D program for building new generation LSS should be developed. Experience gained on development of ISS shows that the most effective way to accomplish this is international cooperation and partnership.

BIOCONVERSION SYSTEMS FOR FOOD AND WATER ON LONG TERM SPACE MISSIONS.

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INTRODUCTION

Regenerative biosystems are logistical and economic requirements for long duration space missions on which expendables are often expensive and resupply is not tenable. Therefore wastewater recycling and crop plant generated waste biomass conversion to food would prove beneficial. We fabricated and laboratory tested both a biological wastewater reclamation system (BWWR) and a waste cellulose to edible mushroom conversion system (CMCS) with simulated waste products. The BWWR is designed to remove bacteria, microalgae and other microbiota from water without the use of ionizing radiation, disposable filters, intense heat or toxic chemicals and convert them to a harmless cellulosic product. The CMCS converts the waste cellulose anticipated from the BWWR and plant crop waste cellulosic biomass, such as the ligno-cellulose stalks and other non-food plant parts from controlled ecological life support systems (CELSS), into edible mushrooms. The CMCS test substrate was hay treated with a variety of mulching techniques and inoculated with straw mushroom spawn.

METHODS

The pilot scale BWWR consists of two modules which are designed to process the contaminated water sequentially. The first consists of two connected 19-L plastic tanks one of which serves as a holding tank and the other as a reactor vessel. The reaction chamber contains a mixing paddle composed of four vertical panels. Sampling ports are located at four different levels. The biologically active components of the first module are the non-pathogenic Dictyostelium amoebae which prey on other microbiota such as bacteria. These are added to microbially contaminated water in the holding tank. This water is then transferred to a mixing chamber where the relative numbers of amoebae and contaminating microbiota are monitored. Predation is allowed to continue until a marked reduction in microbial contaminants is detected in the mix. Bacterial numbers are determined by standard plating techniques on 1% lactose-peptone agar (LPA) and recorded as colony forming units (CFU). The liquid is pumped from the mixing chamber and fed into the second module, an environmentally controlled "dry" reaction chamber. In this chamber, the liquid is spread onto perforated stainless steel surfaces. Here, the amoebae (having converted engulfed microbiota into Dictyostelium cell substance) respond to their genetic programming for life on a solid substrate, in the presence of light, and differentiate into mature cellulosic stalks which can be harvested and added to the feed stock for the CMCS.

Parametric bench-top experiments studied the dynamics of stirred vs. static binary cultures of E. coli and D. dictyostelium in cell substance) respond to their genetic programming for life on a solid substrate, in the presence of light, and differentiate into mature cellulosic stalks which can be harvested and added to the feed stock for the CMCS.

Parametric bench-top experiments studied the dynamics of stirred vs. static binary cultures of E. coli and D. discoideum in nutrient poor vs. enriched media. The data, in terms of reduction of bacterial numbers over time were applied to BWWR liquid reactor experimental design. The superiority of perforated stainless steel over porus plastic test surfaces was also determined in bench-top studies carried out by inoculating candidate surface materials with liquid reactor effluents in water agar petri plates.

The CMCS consists of a chamber with programmable controlled temperature, relative humidity, air exchange, simulated sunlight lux levels and substrate moisture. The substrate and mushroom spawn are housed in a perforated rotation cylinder divided longitudinally into four compartments to enable comparative studies and to provide for even exposure to the chamber environment. When mushrooms appear they can be harvested. The design of the experiments which were carried out in the CMCS was based on a series of trials of various spawning media and substrate preparation/mulching techniques.

RESULTS

BWWR: As expected, bacteria continued to exist in water with extremely low levels of nutrients for protracted periods of time (in excess of 17 days). In the liquid reactor, contrary to the usual logarithmic growth curve anticipated in a closed system, the counts of CFU from samples in the mixing tank described a saw-toothed course, the graph of CFU vs. time looking much like a fever chart. The number of CFU plunged from a high of over 400 colonies down to 2 CFU in 3 days. It rose again to the same level in 5 days and then plunged down to 7 CFU at 6 days. It peaked again at 6 days, dropped down to 350 CFU at 7 days and rose again to over 400 CFU at 8 days when the experiment was terminated. In the holding tank,

starting from a low of 7 CFU at 1 day, the numbers rose to 10 CFU at day 3 and dropped to 1 CFU at day 4. They then rose precipitously to over 400 CFU on day 5 and were down to 2 CFU by day 7. The number of CFU fluctuated between 4 and 2 until day 11 when they rose to 400 CFU, dropping to 1 CFU on day 14. On day 16, a dose of over 1000 Dictyostelium amoebae were added to the holding tank. On day 17 the experiment was terminated and the count was 1 CFU.

When liquid reactor effluent was inoculated onto the surface of perforated stainless steel inserts, in the "dry" reactor, growth was not detected by visual observation until day 19. At that time, mature cellulose stalks and intermediate Dictyostelium stages were detected on the stainless steel surfaces.

CMCS: Examination of the four compartments of the rotating cylinder showed that, in order for mushroom primordia to appear, special care must be take to provide adequate moisture to the substrate. This was dramatically demonstrated by the lack of growth in the cylinder chambers where substrate moisture was allowed to dissipate during primordium formation. Primordia appeared only in the chamber where substrate moisture had been maintained by plastic covering and frequent misting.

CONCLUSION

With proper manipulation and augmentation, the BWWR appears to provide a potential for the safe biological removal of microbes from waste water. Similarly, the CMCS has demonstrated a possible means for effectively converting biomass to food. Both deserve further exploration.

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NOVEL LABORATORY APPROACHES TO MULTI-PURPOSE AQUATIC BIOREGENERATIVE CLOSED-LOOP FOOD PRODUCTION SYSTEMS

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INTRODUCTION

The Closed Equilibrated Biological Aquatic System (C.E.B.A.S.) is an artificial (man-made) aquatic ecosystem which was primarily developed to study the long-term influence of space conditions on several subsequent generations of aquatic animals and plants the „evolution" of which was consequently reported on all IAF-congresses and IAA Man in Space Symposia since 1989. Its development was directed by an international scientific program in which 5 German and 3 U. S. American universities, the Institute of Biophysics of the Russian Academy of Sciences in Krasnoyarsk and the Institute for Medical-Biological Problems in Moscow are involved. C.E.B.A.S. is operative in 2 different versions: the „Original C.E.B.A.S." with a volume of more than 150 liters and the „C.E.B.A.S. MINI MODULE" with about 9 liters volume. Based on the latter a spaceflight version fitting into a spaceshuttle middeck locker is currently under construction and ground test which is dedicated to two different spaceshuttle missions in late 1997 and early 1998.

CONSTRUCTION PRINCIPLE AND RESULTS

Based on the construction principle of the Closed Equilibrated Biological Aquatic System (C.E.B.A.S.) two novel combined animal-plant production systems were developed in laboratory scale the first of which is dedicated to mid-term operation in closed state up to two years. In principle both consist of the „classic" C.E.B.A.S. subcomponents: animal tank (Zoological Component), plant cultivators (Botanical Component), ammonia converting bacteria filter (Microbial Component) and data acquisition/control unit (Electronical Component). The innovative approach in the first system is the utilization of minimally three aquatic plant cultivators for different species. In this one the animal tank has a volume of about 160 liters and is constructed as an „endless-way system" surronding a central unit containing the heat exchanger and the bacteria filter with volumes of about 1.5 liters each. A suspension plant cultivator (1 liter) for the edible duckweed *Wolffia arrhiza* is externally connected. The second plant cultivator is a meandric microalgal bioreactor for filamentous green algae. It consists of 3 x 2 subunits and may be as well exposed directly to sunlight with an automated oxygen level-dependent shading as illuminated with fluorescent lamps. The third plant growth facilitiy is a chamber with about 2.5 liters volume for cultivation of the „traditional" C.E.B.A.S. plant species, the rootless buoyant *Ceratophyllum demersum*. Both latter units are illuminated with 9 W fluorescent lamps. In the current experiment the animal tank contains the live-bearing teleost fish *Xiphophorus helleri* and the small pulmonate water snail *Biomphalaria glabrata* because their physiological adaptation to the closed system conditions is well known from many previous C.E.B.A.S. experiments. A part of the animals derives from a 13 month test of the C.E.B.A.S. prototype #3. The water temperature is maintained at 25°C and the oxygen level is regulated between 5 and 8 mg/l by switching on and off the plant cultivator illuminations according to a suitable pattern thus utilizing solely the oxygen produced by photosynthesis. The animals and the micoorganisms of filter and biofilm provide the plants with a sufficient amount of carbon dioxide. Oxygen concentration, pH value, temperature and redox potential are on-line recorded. Ion concentrations and numbers of living germs in the system water are determined twice monthly in the laboratory from samples taken from a special „sample removal module"; the sample volume is automatically replaced from an reservoir container. A rotatory pump produces a water flow of about 38 l/min. System malfunctions are transmitted by an alert device to the person in duty who is able to control the system status and to perform certain settings *via* a modem. Figure 1 shows the construction scheme of this system. For a similar smaller test system with approx. 10 l volume developed from the C.E.B.A.S.-MINI-MODULE a novel indirect solar energy supply is tested which has a buffer capacity to maintain the system for 7 days in darkness under central European climate conditions also in winter. This time span may be increased by the implementation of additional batteries to simulate, e. g. a lunar night. I contains only a single plant cultivator which is operated with *Wollfia arrhiza*. This lemnacean plant is able to produce large amounts of plant biomass in a short time by vegetative reproduction *via* daughter fronds. This easy-to-handle apparartus is dedicated to be operative more than 4 month. The experimental animals and microorganisms are the same as in the large system. The lecture pesented here provides detailed information on the system construction principles and the biological, physical and chemical data of the first 7 month of the test runs of both systems.

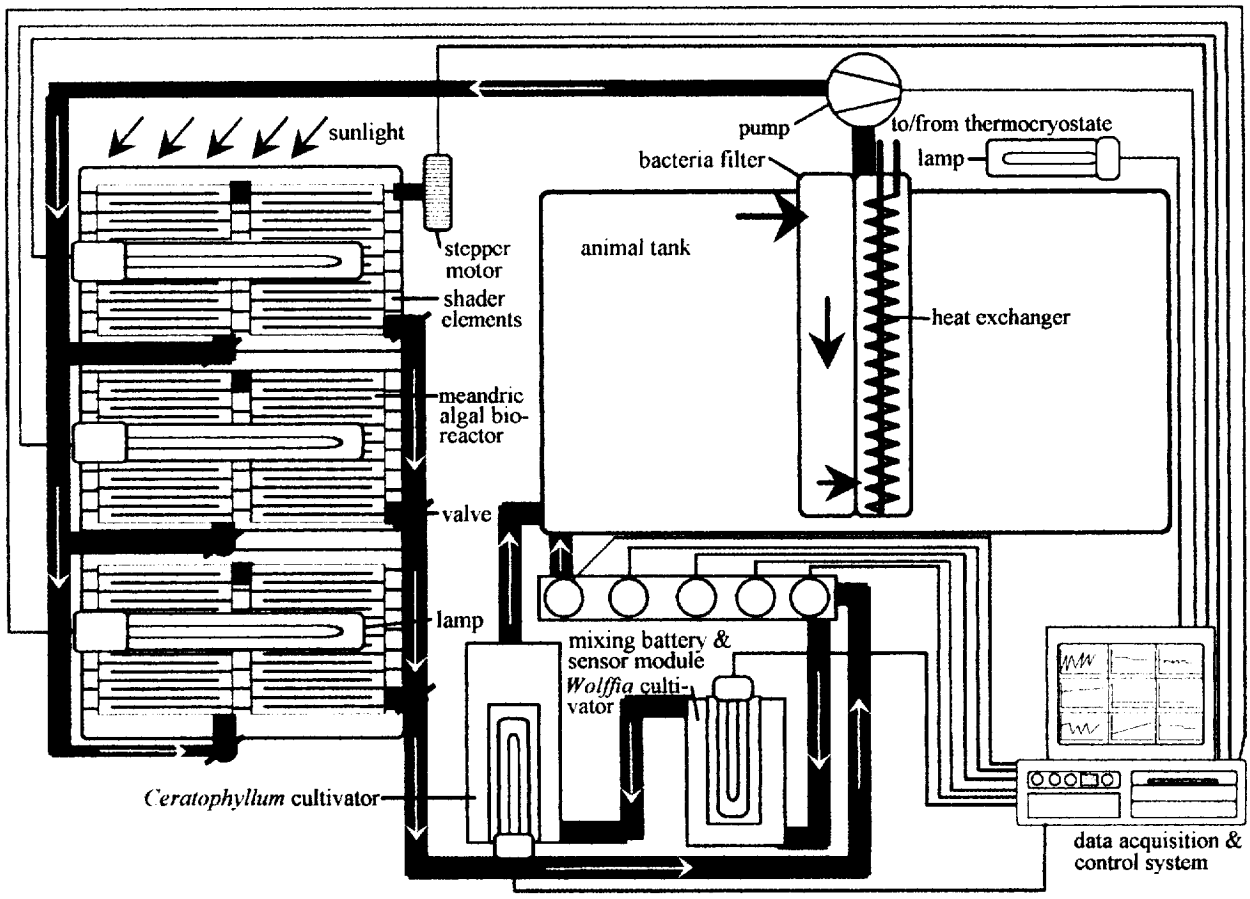

Figure 1: Construction scheme of the C.E.B.A.S.-based animal-plant production system

CONCLUSIONS

The test results from both systems will provide valuable information about first attempts to convert the laboratory devices into closed-loop production sites with herbivorous fishes which are fed with plants inedible for humans, mainly the *C. demersum*. Furthermore, the utilization of *Wolffia arrhiza* for human nutrition can be evaluated more precisely. Models for the combination of intensive aquaculture systems with higher plant hydroponics can be developed for terrestial tests and actual biomass production. The data collected with the solar energy supply system allow serious calculations for the construction of those in larger scale for real production sites. Finally initial careful attempts can be made to develop dispositions for the implementation of aquatic food production modules into bioregenerative life support systems of a higher degree of complexity for a lunar or planetary base.

ACKNOWLEDGEMENTS

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ARTIFICIAL NEURAL NETWORK DERIVED PLANT GROWTH MODELS *

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The goal of the Advanced Life Support Systems (ALSS) is to provide self-sufficiency in life support for productive research and exploration in space, for benefits on Earth and to provide a basis for planetary explorations. Part of this objective is to be able to grow crop plants in one or more controlled environments for the purpose of providing life essentials to a human crew, such as oxygen, potable water, and food. To do this reliably and efficiently, it is necessary to achieve control of the rates of various plant physiology processes. including: net exchange of exhaled carbon dioxide for oxygen (net photosynthesis), purification of water (transpiration), and food production (biomass production rate and harvest index).

To develop an efficient control system that will be able to manage, control, and optimize plant-based life support functions, system identification and modeling of plant growth behavior must first be done. We have developed a plant growth (physiology) model using artificial neural networks. Neural networks are very suitable for both steady-state and dynamic modeling and identification tasks, since they can be trained to approximate arbitrary nonlinear input-output mappings from a collection of input and output examples. In addition, they can be expanded to incorporate a large number of inputs and outputs as required, which makes it simple to model multivariable systems. Thus, unknown nonlinear functions in dynamical models and controllers can easily be parameterized by means of multilayer neural network architectures.

Artificial neural networks are composed of simple albeit numerous non-linear processing elements (modeled after biological neurons) interconnected through a complex network of variable strength connections (modeled after biological synapses). The topology of interconnections and the synaptic strengths essentially dictate the functionality of a given network. A typical network is capable of receiving a large number of analog/digital inputs (e.g., sensor signals) in parallel, and after a complex nonlinear transformation operation, provides the outputs (e.g., predicted growth, biomass). The unique strength of such neural network architectures emerges from their ability to build up their own rules through learning from examples the underlying input/output transformations in ill-defined problems.

In this paper, we will describe our approach to developing these models, the neural network architecture, and the results. With the use of neural networks, these complex, nonlinear, dynamic, multimodal, multivariable plant growth models will be able to better interpolate between all the various environmental conditions and parameters and be able to simulate both short-term (day-to-day) and long-term (plant life cycle) growth of various plants.

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SIX-MONTH SPACE GREENHOUSE EXPERIMENTS - A TEP TO CREATION OF FUTURE BIOLOGICAL LIFE SUPPORT SYSTEMS

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INTRODUCTION

SVET Space Greenhouse (SG) - the first automated facility for growing of higher plants in microgravity conditions was designed in the eighty years under the joint Bulgarian-Russian project "Study of the ways and means for use of higher plants in Biological Life Support Systems" for future long term manned missions in Space. The first successful 54-days experiment with vegetable plants was carried out on the MIR Orbital Complex (OC) in 1990.

The experiments in SVET SG were resumed in 1995. An American Gas Exchange Measurement System (GEMS) was added to the existing Bulgarian plant life support system. A three-month wheat plant experiment was carried out as part of MIR-NASA-3 fundamental biological program.

A set of SVET-2 SG equipment (a greenhouse of new generation) was developed by Bulgarian scientists and launched on board the MIR OC and successful six-month experiments for growing up of two crops of wheat were conducted in 1996-97 as part of MIR-NASA-5 program.

METHODS

Some optimizations in the SVET-2 SG hardware have been made to improve the environmental conditions in the 1996-97 experiments. A new, optimized Light Unit with considerably improved technical and biotechnical characteristics and a new Secondary Pump Power Supply have been designed. Software improvements in the Control Unit made the substrate moisture measurement more precise and provided a possibility for individual, consecutive and independent measurement of each sensor. Another software improvements enable the LP parameter (duration of the lighting period) to be changed.

The American GEMS system has the additional capability to measure a wide range of environmental parameters, except the gas exchange measurements that give a possibility to calculate photosynthesis, respiration and transpiration.

The upgraded basic plant life support system SVET-2 SG as well as the new GEMS system that increased the information possibilities of the equipment were an important precondition for achievement of the experiments goals to grow wheat through a complete life cycle, to document the environmental parameters that might impact plant growth (in addition to microgravity); to collect samples for analysis on the ground; to improve conditions for plant growth as much as possible.

RESULTS

The Space Greenhouse Complex was used to grow a fully developed wheat crop for 4 months during 1996. In the space experiment duration of the full cycle of ontogenesis for the "Super-Dwarf" wheat plants as well as their specific stages was similar to that in ground controls. Nearly 300 heads were developed but no seeds were produced. After the harvest of the first planting, a second crop of wheat was planted in the SVET-GEMS system (with CO_2 measurements in the plant leaf area). The result was again a vigorously developing canopy. The plants were harvested after 42 days, frozen in liquid nitrogen for biochemical investigations after landing of the Shuttle STS-81 in the early 1997.

CONCLUSION

The results of these six-month experiments proved that normal technical and technological conditions for plant growth in microgravity had been provided. Only now the reasons for the lack of seeds will be considered. One of the hypothetical causes is the presence of harmful ingredients in the air - for example the gas, ethylene, probably produced by fungus growing in MIR on the walls. And maybe the microgravity is the principle factor that hinder the seed formation - we will find out about it through long investigations in future space and earth experiments.