Bios-3: Siberian Experiments in Bioregenerative Life Support

Attempts to purify air and grow food for space exploration in a sealed environment began in 1972

Frank B. Salisbury, Josef I. Gitelson, and Genry M. Lisovsky

When rocket science made it possible for humans to venture into space, it became apparent that human life support was the next pressing challenge. For the short term, this problem was solved by applying engineering approaches to provide a spacecraft atmosphere of suitable pressure and composition. Food and water were brought along, and wastes were stored or jettisoned. It soon became apparent, however, that long space voyages would benefit from waste recycling, possibly by using green plants (i.e., algae or higher plants) to remove carbon dioxide from the atmosphere, producing oxygen and even food, as on Earth. Transpired water vapor would be condensed and reused, and wastes from the crew would be at least partially recycled to the plants, the ecosystem's primary producers.

Ignoring the small amounts of matter that enter Earth's system as meteorites and possibly water ice (Frank and Huyghe 1990) and also the few hydrogen and, perhaps, other molecules (and, today, spacecraft) that may reach escape velocity and leave Earth forever, Earth is a system that is closed to matter, but open to energy. Vast quantities of radiant energy, mostly from the Sun, impinge on Earth and its many systems, driving dynamic processes in the earth's atmosphere, hydrosphere, biosphere (especially photosynthesis), and even lithosphere. Eventually, most of this energy degrades to heat, which is emitted back into space as thermal radiation. Some of the Sun's energy may be tied up for geologic intervals as chemical bond energy in such fossils as coal, oil, and natural gas.

The Bios-3 experiments demonstrated the feasibility of sustaining human life inside a small, essentially closed ecological system

Could a spacecraft or a colony on the Moon or Mars incorporate such a nearly closed (with respect to matter), bioregenerative life-support system, with plants using radiant energy to do much of the recycling? One way to find out is to attempt to design and construct such a system. In this article, we describe a relatively large-scale facility that was designed to include humans in a functioning, self-sustaining, closed ecosystem for continuous periods as long as six months.

This facility, which is called Bios-3, is located in the Siberian city of Krasnoyarsk (Figure 1). The ultimate reason for building this facility was to develop a bioregenerative life-support system for cosmonauts, possibly in space but more likely on the surfaces of the Moon or Mars. Learning to construct and to operate such a life-support system was a goal of the Soviet space program from its inception, and the space agencies of other countries share this goal. The US National Aeronautics and Space Administration (NASA) began to develop such a system in about 1960, when NASA was organized, but this program was dropped within a few years until about 1978, when NASA again began to fund a few projects relating to bioregenerative life support. The current program, which includes both biological and physicochemical approaches to life support, is called the Advanced Life Support Program. For several years, the program was called CELSS (for Controlled Ecological Life-Support System, Closed Ecological Life-Support System, or Controlled-Environment Life-Support System).

Some history of closed-ecosystem research

There have been many attempts to construct small, closed ecosystems. For example, Clare Folsome sealed small aquatic ecosystems consisting of algae, brine shrimp, and other organisms in glass flasks (Folsome and Hanson 1986). Although the flasks were prepared in the 1950s, some of them still retain fuctioning mini-communities (Nelson et al. 1993).[1] Furthermore, hobbyists have

Frank B. Salisbury is a professor emeritus in the Department of Plants, Soils, and Biometeorology in the College of Agriculture at Utah State University, Logan, UT 84322-4820. Josef I. Gitelson is director and Genry M. Lisovsky is chief scientist at the Institute of Biophysics, Academy of Sciences of Russia, Siberian Branch, Krasnoyarsk, Russia. © 1997 American Institute of Biological Sciences.

---

[1] M. Nelson, 1996, personal communication. Institute of Ecotechnics, Bonsall, CA.

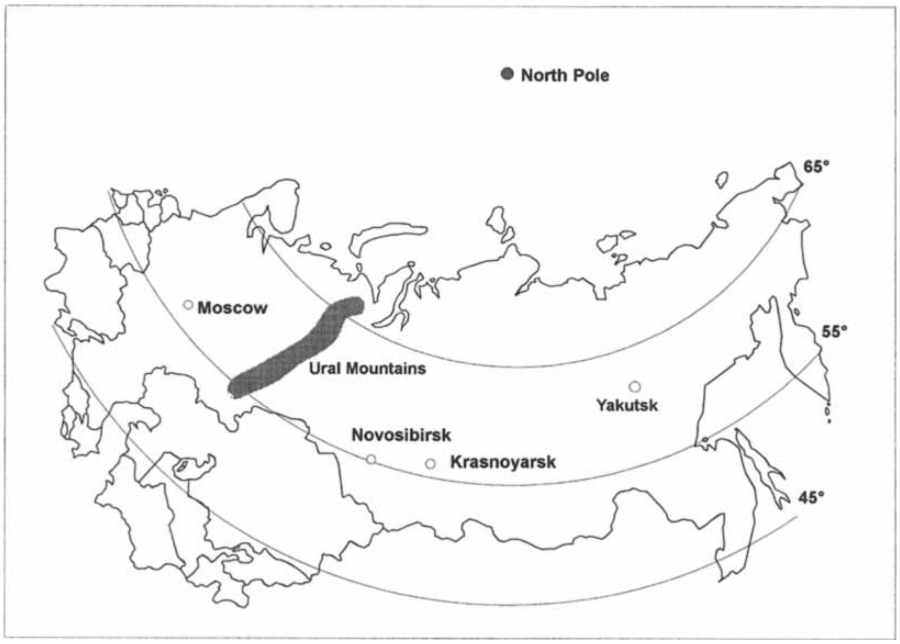

Figure 1. Map of the former Soviet Union, showing the location of Krasnoyarsk. Siberia, which is a geographical region within Russia, not a political entity, is generally considered to extend east from the Ural Mountains to and including Yakutsk. Russia beyond Yakutsk is referred to as the Far East.

built terraria sealed with soil, plants, microorganisms, and, no doubt, invertebrates, and these terraria have sometimes lasted for several years.

The $150-million Biosphere 2 facility, which covers 1.2 ha of desert in Oracle, Arizona, stands in stark contrast to these relatively simple systems (Nelson et al. 1993). Seven so-called biomes (ocean, freshwater and saltwater marshes, tropical rain forest, savanna, desert, intensive agriculture, and human habitat) attempt to mimic the biomes of Earth, or Biosphere 1. Approximately 3800 catalogued species of plants and animals live inside Biosphere 2, in which eight "biospherians" were sealed for two years (September 26, 1991–September 26, 1993). Although the project was plagued by publicity of both the gee-whiz and exposé types, many results were obtained that are of interest to scientists concerned with biospherics, a developing science that seeks to understand the ways in which a system that is closed with respect to matter can be stabilized and function indefinitely. Perhaps the most interesting observation (Nelson et al. 1993) was the unexpected decrease in oxygen concentration, much of which occurred as oxygen was used in respiration and in decay of organic matter sealed in the structure. This decay used much oxygen and produced much carbon dioxide. Some of the carbon dioxide combined with structural concrete inside the structure, and the result was a net loss of oxygen without an equivalent buildup of carbon dioxide. This phenomenon and others demonstrated that enclosing even a relatively large volume with thousands of species is not necessarily sufficient for spontaneous organization of balanced matter turnover.

It is ironic that publicity about the Biosphere 2 project emphasized its possible role in future space exploration. Such a relatively flimsy, pressurized structure could obviously not exist on the airless or nearly airless surfaces of the Moon or Mars, and it is unlikely that a stronger structure of such complexity could be built on the Moon or Mars even in the distant foreseeable future. The actual design of Biosphere 2 suggests that it was built to better understand the biomes of Earth. Bios-3, by contrast, was designed specifically as part of the Soviet space program. Although it was not initially concerned with understanding Earth's ecology, its operation has led to insights about the earth's biosphere.

Russian scientists credit Vladimir Ivanovich Vernadsky (1863–1945) with developing the concept of Earth's biosphere (e.g., Vernadsky 1989) and, hence, the foundation for bioregenerative life support. Konstatin Edwardovich Tsiolkovsky is also mentioned as the father of Russian space science based on his writings around the turn of the century. These writings, penned long before space travel was possible, included the concept of bioregenerative life support for long space voyages (Tsiolkovsky 1964).

More specific to the history of Bios-3 are the scientists who, for more than three decades, designed, built, and operated the structure. Sergey P. Korolyov sponsored the life-support studies in Krasnoyarsk, and Leonid V. Kirensky, Ivan A. Terskov, and one of us (Josef I. Gitelson) initiated the actual work, which began in 1961. The work was carried out in the Department of Biophysics in the Institute of Physics, part of Academic City in Krasnoyarsk. In 1981, this department became the Institute of Biophysics. Now a large organization, the Institute of Biophysics consists of a number of buildings and several laboratories, each headed by a specialist who supervises several technicians and graduate students.

In 1965, Bios-1 was constructed. This system regenerated the atmosphere for one human in a sealed 12 m^3 chamber connected through air ducts with an 18 L algal cultivator containing *Chlorella vulgaris*. Approximately 8 m^2 of the algal culture was irradiated with three 6 kW xenon lamps, which provided approximately 200–300 W/m^2 at the surface of the cultivator. The algal system, by removing carbon dioxide and producing oxygen, accounted for approximately 20% of the quantities (mass) of pure air, water, and food required by a single human; that is, the system achieved 20% closure. Food and water had to be taken into the system before a human could be sealed inside. In 1968, the Krasnoyarsk scientists achieved 80%–85% closure by recycling water. It became apparent, however, that to achieve a more complete regeneration, the team would have to replace *Chlorella* with something that was more edible. One of us (Genry M. Lisovsky) suggested that traditional

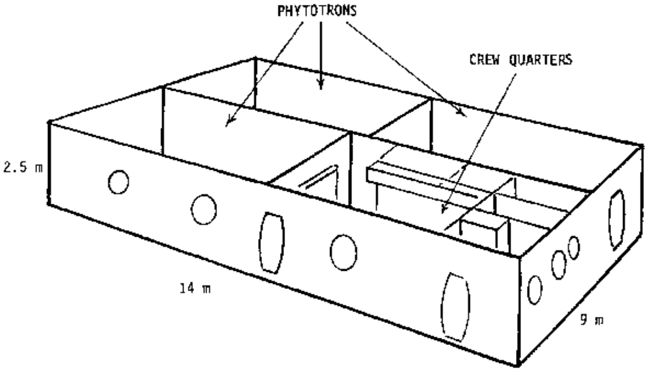

Figure 2. Drawing of Bios-3 showing the three phytotrons, the crew quarters, and some of the doors and windows.

food plants, such as vegetables and wheat, be introduced into the system. To this end, in 1968 the Bios-1 sealed chamber was attached to a 2.5 × 2.0 × 1.7 m chamber for higher plants and renamed Bios-2. (The builders called the chamber for higher plants a phytotron, a term that was coined in jest by James Bonner and Samuel Wildman in the 1940s to show that botanists could create something as imposing as the cyclotron that was then being contructed at the University of California–Berkeley.) A human could go through a sealable hatch from the sealed chamber into the phytotron to tend the plants and harvest the crops. In some experiments, the crop was wheat; in others, it was a set of vegetables (e.g., beetroots, carrots, cucumbers, and dill). Air purification was provided by both higher plants (approximately 25%) and algae (approximately 75%). This three-component system demonstrated the feasibility of direct gas exchange between humans and higher plants.

In 1972, Bios-3 (which will be described in detail in a subsequent section) was built by workers in the Department of Biophysics at a cost of approximately 1 million rubles (then, roughly equivalent to US$1 million), not counting the labor. All three Bios facilities were developed and operated by scientists with diverse backgrounds, including biology, engineering, chemistry, and agronomy. The chief designer of all three facilities was Boris G. Kovrov, a physicist who later became a biologist. The Bios-3 facility has been used almost continuously and in various ways since its construction, although only three full-scale experiments (i.e., with humans inside) have been carried out. The total time of closure—that is, the time that one or more crew members have been sealed in one of the three facilities—exceeds one year.

Actually, the first experiments to provide gas exchange for humans through photosynthesis of *Chlorella* were conducted in Moscow during 1960–1961 by Yevgeny Ya. Shepelev and Gana I. Meleshko at the Institute of Aerospace Medicine (Adamovich 1975, Gazenko 1967, Shepelev 1972). In a few studies, human volunteers were sealed in such systems for many months, and at least one study (in 1965) lasted a year. These experiments were intended to test physicochemical life-support systems, but a few plants were grown to provide some fresh food and vitamins and for their positive psychological effects.[2]

The Bios-3 facility

Bios-3 is completely underground and is reached by a passageway from the main building of the Institute of Biophysics. It is constructed of welded stainless steel plates to provide a hermetic seal. The structure (Figure 2), which is 14 × 9 × 2.5 m (with a volume of 315 m^3), is divided equally into four compartments (of nearly 7 × 4.5 × 2.5 m). Each compartment has three doors that are sealed tightly with rubber gaskets (which are the only rubber in the structure; cable insulation and other applications are silicon based). One door in each compartment leads to the outside, and occupants could escape within 20 seconds if necessary, but the need has never arisen. Each compartment can be sealed independently in combination with any other compartment. There are large, round windows in some doors and other large portholes in the living compartments (Figure 2).

The crew area, which occupies one compartment, is subdivided into three separate sleeping rooms, a kitchen, a lavatory, a control room, and a work area with equipment to process wheat and inedible biomass, make repairs and measurements, and purify water and air. During the early years of Bios-3, one compartment included algal cultivators, which provided enough air-revitalization capacity to support at least three crew members, although the remaining two compartments, which were used as phytotrons, did not provide enough space to grow the vegetables needed to sustain a crew of three. The algal cultivators were subsequently removed, and each of the three noncrew compartments was used as a phytotron to grow wheat, chufa (sedge nuts), and vegetable crops. The total growing area was 63 m^2, which provided ample air-regeneration capacity.

Each phytotron originally had 20 cylindrical, vertical 6 kW xenon lamps. The large total power requirement (approximately 400 kW) was met by a hydroelectric plant on the Yenisee River approximately 30 km away; the Yenisee also supplies water for removal of heat from lamps, compressors, and other equipment. Each lamp is surrounded by a vertical glass cylinder through which water circulates to cool the lamps. This "water jacket" is inserted through a hole that is cut in the ceiling, allowing the lamps to be changed from outside. Although these water jackets are tightly sealed, they could be a potential source of air leakage. By 1991, the number of lamps in one of the three phytotrons was doubled by inserting two lamps into each water jacket (Figure 3). With xenon lamps energized at 220 V, photosynthetic photon flux (*PPF*) at plant level varies from approximately 900 to 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ under single lamps and from approximately 1600 to 1850 $\mu mol \cdot m^{-2} \cdot s^{-1}$ under double lamps. Photon fluxes as high as 1300–1600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (single lamps) and 1600–2450 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (double lamps) can be achieved by adjusting the voltage. (Sunlight can reach approximately 2000 $\mu mol \cdot m^{-2} \cdot s^{-1}$.) High irradiances come at the price of air temperatures that are too high (approximately 27–30 °C) for the

---

[2] G. Meleshko, 1995, personal communication. Institute of Biomedical Problems, Moscow.

Figure 3. Inside one of the three phytotrons. In this room, two xenon lamps were installed in each water jacket. This photograph and that of Figure 4 were originally color slides. To make the double lamps barely visible in the photograph, the upper half of the picture was "burned in" (more than triple the exposure of the lower half) when the black-and-white print was made; that is, the lamps are much brighter relative to the plants than they appear in this print.

growth of many crops (including wheat). Consequently, the cooling system must be expanded if high light levels are to be used. In the experiments so far, the lamps were operated continuously, although some crops (e.g., tomatoes and potatoes) would have yielded much better with a daily dark period.

To maintain the pressure inside Bios-3 at close to atmospheric levels, which minimizes leaks, two air tanks are connected to the main structure. When pressure in the structure exceeds atmospheric pressure, air is automatically pumped into the tanks; conversely, air from the tanks is pumped into the structure when outside pressure is high. In the third full-scale experiment, the inside pressure was elevated slightly compared with the outside pressure to prevent contamination from outside pathogens. Before this experiment began, leak rates were estimated by measuring the amount of air that had to be pumped into the structure to maintain this slightly elevated pressure. At low outside barometric pressures, the air leak reached as high as 150–240 L/d, but the average leak rate was 60–80 L/d, or 0.020–0.026% by volume.

Air was circulated among the crew quarters and the phytotrons. It was partially purified by the plants, and a thermocatalytic filter (also called "catalytic converter") completed the purification by heating the air to 600–650 °C, which oxidized organic molecules to carbon dioxide and water. Transpired water was condensed and recirculated, mainly to nutrient solutions for the plants. Some of this water was boiled for washing and general cleaning, but water for drinking was further purified on ion-exchange filters. Small quantities of potassium iodide and fluorides were also added to this drinking water for health, and potassium chloride and some other salts were added to improve the taste.

The crew communicated with the outside world by phone or through the viewing ports. Samples of various kinds were passed outside through small airlocks for analysis. Electrical signals from sensors attached to the bodies of crew members to monitor various physiological parameters were transmitted to the outside through specially designed sockets. Crew members had privacy (they pulled the blinds) during their free time (e.g., to watch television) but were still monitored constantly for medical parameters. No health deterioration was evident after six months. Significant changes in the microflora of skin, mucous membranes, and intestines were observed, but these changes had no pathological consequences. No crew member developed allergies. Not only did the crew remain healthy, but the quality of air, water, and vegetables did not deteriorate during the period of closure.

All three closure experiments in Bios-3 were initiated during early winter to minimize pathogen invasion from the outside. The first experiment, which involved two men and one woman, lasted six months during the winter of 1972–1973. During the first two months of the experiment, the compartment filled with large algal tanks was sealed off, and the two phytotrons supplied oxygen and approximately one-fifth of the crew's calories. During the final four months, one phytotron was isolated, and the algal room was opened to the crew quarters to supply oxygen. At that time, an agronomist was replaced by an algal specialist, but the agronomist returned for the final two months, when the phytotron was filled with vegetable crops, and there were always three crew members in the facility (Gitelson et al. 1975, 1976). The second experiment, during the winter of 1976–1977, lasted four months. There were three male crew members, one of whom left during the experiment. The goal of the experiment was to test the ability of the enclosure to supply food (Lisovsky 1979). In the third experiment, two male crew members were sealed in the facility for five months, from November 1983 to April 1984. After that period, the facility continued to grow and observe plants for one month but was not closed (Gitelson et al. 1989, Kovrov et al. 1985).

Green algae or higher plants?

There was much discussion among both Soviet and US researchers about the advantages and disadvantages of cultivating algae or higher plants for use in a bioregenerative life-support system. Algae cultivation is relatively simple and highly reliable. If all but

Table 1. Bios-3 crops during the third experiment.

	Expected			Actual		
Crops	Diurnal needs of the crew (g)	Yield ($g \cdot m^{-2} \cdot d^{-1}$)	Area (m^2)	Area (m^2)	Harvest (g/d)	Harvest index (%[a])
Wheat (*Triticum aestivum*), grain (dry mass)	520	13	40.0	39.6	496	34.7
Chufa (*Cyperus esculentus*), tubers (dry mass)	234	26	9.0	8.6	120	48.1
Pea (*Pisum sativum*), grain	52	13	4.0	4.0	26	25.4
Carrot (*Daucus carota*), edible roots (fresh mass)	220	160	1.4	1.2	236	54.9
Radish (*Raphanus sativus*), edible roots (fresh mass)	110	125	0.9	0.9	266	59.8
Beets (*Beta vulgaris*), edible roots and leaves (fresh mass)	130	170	0.9	0.9	132	67.5
Kohlrabi (*Brassica oleracea gongylodes*), stems and leaves (fresh mass)	180	170	1.1	1.0	164	37.1
Onion (*Allium* sp.), leaves and bulbs (fresh mass)	120	170	0.7	0.6	110	90.1
Dill (*Anethum graveolens*), greens (fresh mass)	30	30	—[b]	—[b]	16	93.0
Tomatoes (*Lycopersicon esculentum*; fresh mass)	150	110	1.4	1.2	88	33.1
Cucumbers (*Cucumis sativus*; fresh mass)	100	250	0.4	0.4	276	54.6
Potatoes (*Solanum tuberosum*; fresh mass)	250	80	3.2	4.8	22	5.9

[a] Harvest index was calculated on a dry-mass basis.

[b] Area is not known because the crop was grown between other culture rows.

one cell of an algal culture should somehow be destroyed, that one cell could rapidly restore the whole culture. In one experiment, the initial growth rate was suppressed by 70% with ultraviolet radiation, but the culture recovered its growth rate in 24 hours (Gitelson and Rodicheva 1996). Moreover, *Chlorella* contains many food components necessary for humans, including all essential amino acids, sufficient lipids, and nearly all the essential vitamins. However, with these benefits come some disadvantages. Algae provide an unbalanced diet for humans because they contain virtually no carbohydrates. Furthermore, processing *Chlorella* or any other green alga to an edible form is difficult (Kamarei et al. 1986).[3] Use of large quantities of *Chlorella* in the diet of both test animals and humans has led to nutrient deficiencies and illness (Waslien 1975).

Higher plants, like green algae, remove carbon dioxide and add oxygen; they also transpire water, which can be condensed, simplifying water purification. Unlike algae, however, higher plant products are the basis of foods that people are accustomed to eating. In addition, higher plants may remove volatile and liquid contaminants such as benzene that form as a result of the presence of humans and machinery in the system, as in the so-called sick-building syndrome (Wolverton et al. 1984, 1989), and they provide an aesthetically pleasing environment for crew members. One problem with higher plants is that different crops may require different temperatures and, especially, different photoperiods. Moreover, it may be difficult to supply water and nutrients in microgravity (i.e., in an orbiting spacecraft); several groups are investigating possible solutions (e.g., Brown et al. 1992, Jones and Or 1996, Morrow et al. 1993, Salisbury et al. 1995, Yendler et al. 1996).

So far, no attempt to grow plants in space has been entirely successful. One of the authors (Frank B. Salisbury) has been principal investigator of a team that has twice attempted to grow Super-Dwarf wheat (a cultivar only 30 cm tall, ideal for small growth chambers) through a complete life cycle in the Russian Space Station Mir (Salisbury et al. 1995). The failure of four of six lamp sets in 1995 led to poor growth, but in 1996, with ample light (400 µmol · $m^{-2} \cdot s^{-1}$ *PPF*), plants grew vigorously and produced many heads. However, on return to Earth, it was discovered that all the heads were sterile.[4] The sterile heads and other symptoms (e.g., short stems, profuse tillering, and early leaf senescence) appear to have been responses to high levels of ethylene (1200 nmol/mol) in the cabin atmosphere. Results were encouraging, however, because of the vigorous growth. Furthermore, wheat grown in microgravity for ten days in the US Space Shuttle was comparable in virtually every way to controls grown in normal gravity (Lewis 1994). Hence, it appears likely that normal plants can be grown in space if environmental stresses (other than microgravity) are sufficiently reduced.

The role of higher plants in Bios-3

Table 1 lists the plants that were grown in the third experiment. Plants in Bios-3 were grown in artificial substrates with hydroponic solutions. For uniform oxygen emission and sustained oxygen production, each phytotron used a "conveyor" approach—that is, crops from three to

---

[3] See footnote 2.

[4] F. B. Salisbury, J. I. Gitelson, and G. M. Lisovsky, manuscript in preparation.

tato were low in Bios-3 (Table 1), probably because continuous light was present. These crops normally require a dark period to produce their fruits or tubers.

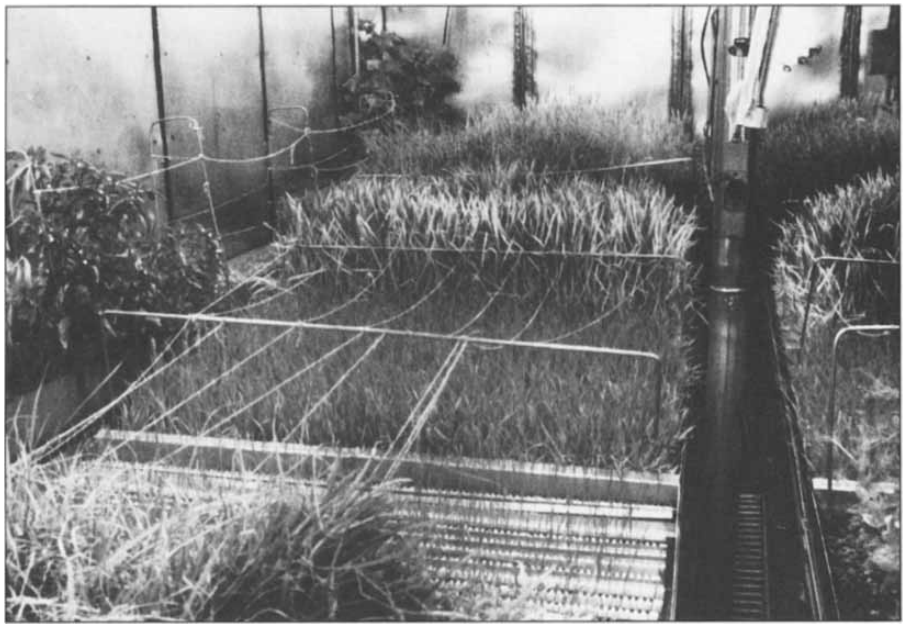

Figure 4. Wheat plants of different ages showing the "conveyor" approach that was used in the Bios experiments. Young wheat plants are in the foreground, with more mature plants toward the back. The aisle between benches is narrow (to leave as much space as possible for the crops). The post, with some environmental sensors attached, further obstructs the aisle. Crew members planted various herbs and other special plants in the corner and next to the wall on the left, space that would otherwise be wasted.

seven different ages were grown at once (Figure 4).

Chufa (*Cyperus esculentus*), sometimes called nut sedge, chufa flatsedge, or yellow nut sedge, was grown as a source of oil, which is present at high levels in its underground tubers. This sedge comes from Asia Minor, where it was used for millennia as a delicacy. Because its cultivation has not been mechanized, chufa is little known as a food today. Instead, it is considered to be a nasty introduced weed in most of the United States, especially in the Southeast. The closely related purple nut sedge, *Cyperus rotundus*, which has also been introduced to the United States, has been called the world's worst weed (Holm et al. 1977).

A variety of plants is needed. Starch-producing plants, such as wheat and potatoes, must be included to provide energy, and oil crops are necessary to provide the fats and oils required by humans. If these crops are properly chosen, there will automatically be the right amount of protein. Vitamins are supplied by grains, tubers, fruits, and salad crops, such as lettuce and cucumber. The harvest index or edibility coefficient (the percentage of total biomass that is edible) is lowest for grains and seeds, intermediate for tubers and roots, and highest for salad crops.

Special breeding programs were carried out in Krasnoyarsk to improve the harvest index of wheat in controlled environments from approximately 28–32% up to 38–42%. Values of 45% have been reached in similar studies at Utah State University (Bugbee and Salisbury 1988) and are commonly achieved in the field. Harvest indices of tomato and potato were low in Bios-3 (Table 1), probably because continuous light was present. These crops normally require a dark period to produce their fruits or tubers.

Table 2. Parameters of a life-support system with different degrees of closure.

Parameters	Degree of closure (%)		
	85	95	99
Regeneration of oxygen, water (%)	100	100	100
Regeneration of food (%)	35	80	100
Area occupied by plants per person (m^2)	13	30	56
Photosynthetically Active Radiation necessary for plants (kW/human)	2.0	4.6	8.5
Relative size of system	1.0	2.3	4.0

The role of humans in Bios-3

Humans occupy a multifunctional position in a bioregenerative life-support system. First, they are the object to be supplied with everything that they need. Second, they are metabolically intertwined with the system—that is, they need to qualitatively and quantitatively conform to the system's capabilities. And third, they control the system. Yu. N. Okladnikov, a physician, has been most responsible for the well-being of crew members in the Bios studies since the mid-1960s. He and his coworkers from the Institute and from a special laboratory from the Institute of Biomedical Problems in Moscow, which was established in Krasnoyarsk for medical support of experiments in Bios-3, were directly concerned with the health of the crew. Okladnikov and his coworkers also carefully considered the energy content of the crew's diet plus their energy expenditure, and they added thermal control because all of the energy inputs ultimately end up as heat.

The team calculated respiratory quotients (RQ, which equals the ratio of carbon dioxide exhaled to oxygen inhaled) of the crew members and assimilation quotients (AQ, which equals the ratio of oxygen given off to carbon dioxide taken up in photosynthesis) for the growing plants. When fat is metabolically oxidized, the RQ is 0.7, and the RQ of carbohydrate metabolism is 1.0. The average RQ for humans is approximately 0.89–0.90, depending on diet. The AQ for most crops is close to 1.0, but oil crops have a lower AQ. In the third Bios-3 experiment, the inclusion of the fat-producing chufa crop brought the crop AQ close to 0.95.

All of the Bios-3 crew members had four meals per day, with the menu repeated every five days. Animal products were lyophilized meats supplied through the airlock once each month and brought back to their natural condition with drinking water inside Bios-3; otherwise, the crew decided what they wanted to

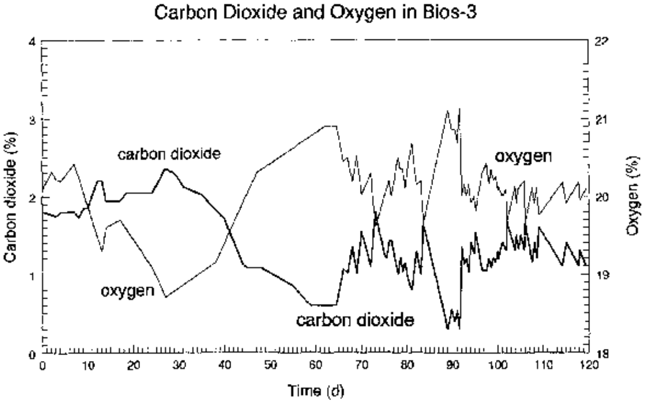

Figure 5. Dynamics of carbon dioxide and oxygen concentrations during the second experiment in Bios-3. Sharp changes during the second half of the run were caused by the burning of inedible plant wastes. Note the mirror-image responses of carbon dioxide and oxygen.

eat of the food that they were producing. The crew members found that they could not predict vegetable production accurately, but they were challenged by the experimenters to eat all that they produced. The program was so successful that the crew members in Bios-3 held their weights within ±830 g (in contrast to the biospherians of Biosphere 2, who initially lost on the order of 10 kg each; Nelson et al. 1993).

Animal products constituted approximately 25% of the mass of food consumed by the crew. There has been much talk about producing animals in a bioregenerative life-support system, but the efficiency of production can be as low as 10%, so the size of the system must increase considerably (Table 2). Thus, the Bios-3 scientists concluded that, because animal products store so well, it is best simply to supply them from outside. On the Moon or even Mars, it might be possible to include enough meat to last several years. An alternative that was virtually never considered in the Bios-3 experiments is for the crew members to be vegetarians—"Siberians must have their meat!"

Gas concentrations in Bios-3 remained relatively stable (Figure 5), suggesting a close metabolic balance between crew and crops. Carbon dioxide levels varied from approximately 0.5% (by volume), when crops were doing especially well, to a little over 2% shortly after the beginning of the experiment shown in Figure 5. Incineration of inedible biomass led to sharp increases in carbon dioxide, with a mirror-image drop in oxygen. Ideally, carbon dioxide should not exceed 1% of the air in a human habitat. Researchers at Utah State University found the optimum carbon dioxide concentration for wheat yields to be approximately 0.12% (Bugbee et al. 1994), and Lisovsky (1979) found that optimum growth in a dense canopy occurred when carbon dioxide exceeded 0.3%. Surprisingly, humans, which must expel carbon dioxide, easily tolerate levels that are well above those that are best for plants, which utilize carbon dioxide.

Crew members were selected based on four criteria: first, they had helped to build and work with the system and, therefore, had a meaningful interest in the experiment; second, they had desirable personal merits, including conscientiousness, efficiency, disposition, and the ability to prevent or avoid conflicts; third, they knew the experimental program well and had participated in decisions to modify a given program; and fourth, they had passed a board of special medical examiners. Before they were closed into the Bios-3 system, crew members underwent a detailed training session that included instructions about the system as a whole, the operation of individual components, safety features, cultivation techniques, treatment of biomass, cooking, and maintaining optimal everyday conditions.

The crew was monitored for one and a half to two months before an experiment with the same parameters that would be measured during the experiment, and thus individual baseline data for each crew member were obtained to use for comparison during the experiment. Crew members were also monitored for approximately a month after the experiment in case aftereffects should appear.

Human wastes were, for the most part, not recycled in the Bios-3 experiments. Feces were dried and stored, and the water re-entered the system as vapor. In the last experiment, urine was returned to the nutrient solutions for the wheat only, because the edible part of the wheat never came into contact with the solution, which would not be true for root and tuber crops. Addition of urine led to a buildup of sodium in the nutrient solutions and in the plants, but the sodium did not reach harmful levels.

Safety of the crew was of prime importance. There was around-the-clock medical supervision, special examinations, instructions to report any aches and pains, and five days of quarantine before closure. Some crew members took part in more than one experiment, one for a total of 11 months. All crew members remained perfectly healthy. Okladnikov summarized a discussion of crew health by saying that for 26 years his team had studied all the systems and organs of the crew and, in addition, a team of psychologists had studied the crew's mental health. In no respect did the crew members deviate from the norm.[5] Based on this experience, the team has tried to simplify procedures by decreasing the number of parameters to be monitored and establishing optimum times for examinations. The data need to be minimal, informative, and easily acquired.

Balance-sheet studies for Bios-3

Careful records were kept of most mass exchanges taking place inside Bios-3.[6] The Bios-3 scientists were aware of such apparently minor problems as the water introduced with canned meat (when it was used) and the water and various mineral elements removed with samples passed through the airlock for testing. Table 3 shows the requirements of the crew and of the ecosystem with the crew. In this case, the totals show that recycling reduced the crew requirements to only 4.6% of the requirements without recycling.

Mineral transport and balances of a number of elements were stud-

---

[5] Yu. N. Okladnikov, 1992, personal communication. Institute of Biomedical Problems, Moscow.

[6] Unpublished proceedings from a 1989 workshop held in Shushenskoye, Siberia.

Table 3. Daily requirements of one crew member without recycling and with recycling as in Bios-3.[a]

Substance	Crew requirements (g/d) (without recycling)	Ecosystem requirements[b] (g/d) (with recycling)
Food products (without water)	924	208
Oxygen	1283	—[c]
Chemical substances as components of the nutrient medium	—[d]	350
Potable water	5133	—[c]
Sorbents for water purification	—[d]	2.7
Sanitation water	5696	—[c]
Hygiene means	9.5	9.5
Common salt	28	28
Total	13,073.5	598.2

[a] Sampling of substances for analysis out of the system is not reflected in these values.

[b] When recycling is carried out, the requirements of the crew cannot be separated from the requirements of the ecosystem as a whole.

[c] Oxygen and water were recycled 100%; hence, they do not appear in this column.

[d] Not applicable because required by the ecosystem as a whole and not by individual crew members.

ied. These included both macroelements (N, S, K, Na, Ca, Mg, and P), and microelements (Fe, Mn, Cu, Zn, Sn, Pb, Al, Ti, B, Ni, Cr, V, and Co). All liquid and solid substances participating in internal and external exchange were analyzed once or twice monthly. Chemical methods were used for P and S, photometric methods for K and N, atomic absorption for Ca, Mg, Fe, Mn, Cu, and Zn, and flame-photometric methods for the other elements.

Nutrient elements for plants were introduced, of course, but some elements also entered the system as impurities in salts and with materials such as soap and toothpaste (i.e., Ni, Cr, Al, Pb, Sn, and Ti). Elements were lost from the system in dried feces, kitchen waste, inedible biomass (ash after incineration), and analysis samples. The closure of minerals in the third experiment was only approximately 20% on average (i.e., only 20% of minerals were retained in the system), although closure of nitrogen was approximately 40%. For some of the macroelements (i.e., K, Na, Ca, Mg, P, and S), however, input and output were nearly balanced, within the limits of error of analysis techniques (i.e., 10–15%). For the microelements, however, output exceeded input.

The biggest imbalances appeared during the second experiment. Some elements (Ni, Al, Cr, and Pb) were 10–20 times higher in the plants and nutrient solutions at the end of the experiment than at the beginning. Others (Sn, Ti, and Zn) were two to four times higher. These elements came mostly from construction materials. For example, solder produced Pb and Sn, especially the solder applied to the steel net used to collect the sewage water. New, untreated steel contributed Ni and Cr. A porous filter for water extraction contributed Al, Pb, Ni, Cr, Ti, Zn, Cu, and V. Catalysts in the thermocatalytic converter contributed Zn, Cr, V, Ti, Fe, Al, and probably other elements.

The major source of removal of minerals was the ash produced by burning inedible plant materials (mostly wheat straw). None of the mineral elements in the ash was returned to the nutrient solutions, although this recycling would probably have to be done in a functioning bioregenerative life-support system on the Moon or on Mars.

A number of the problems discovered in the second experiment were eliminated in the third experiment by using a modified catalytic converter as well as other metal nets and solders, and no new steel was introduced. By the end of that experiment, there was no accumulation of elements in the plants. Even in the second experiment, however, none of these elements reached harmful levels, nor did plant growth seem to be inhibited.

These results and others emphasize the important role of "deadlock substances": elements and molecules that may be unavoidably and irretrievably removed from the system. If such removal is inevitable, as it must be, then a bioregenerative life-support system can never achieve complete closure or total stability. Such a system on the Moon or Mars would always have to be replenished by materials sent from Earth or obtained from the immediate environment (e.g., carbon dioxide from the Martian atmosphere).

With advances in technology, many of the deadlock substances in the Bios-3 experiments could be reintroduced into the system and thus removed from deadlock status. The Krasnoyarsk researchers showed, for example, that many mineral elements required by plants could be extracted from inedible biomass simply by soaking the biomass in water. This result was confirmed by studies at the Kennedy Space Center (Garland 1992, Garland et al. 1993). Acid extraction could also be used to recover minerals from both ash and dry biomass, including that produced from human feces, but that procedure would require a source of acid. Thus, there will always be a price to pay for retrieving deadlock substances.

The role of microflora in Bios-3

At least half a dozen Bios-3 researchers have studied the microflora of nutrient solutions, plant root and shoot surfaces, solid media, and human skin and intestines (as feces samples; Gitelson et al. 1980, Somova 1996). They studied bacteria, fungi, actinomyces, and yeasts. A number of doctoral dissertations have been prepared as the result of these studies. Margarita Rerberg supervised the group of microbiologists, who placed a strong emphasis on microbial ecology. The researchers emphasized that, although stability was never achieved, populations of various microflora never exceeded the normal limits encountered outside of Bios-3. However, they found staphylococci on the skin, which indicates that human existence in the system could have been endangered.

In the first experiment, microbial communities varied according to the phases of the crops' life cycles and depended on cultivation conditions and environment. Weakened plants had 10–30 times more microorganisms than healthy ones. Some saprophytic organisms increased more than 1000-fold when plants suffered for one reason or another, but when the plants' condition improved, microbial populations decreased. The increases had the potential of stopping the system, so in the second experiment the researchers took measures to reduce these effects. In particular, the crew stopped washing linen (instead, they used only clean linen stored at the beginning of the experiment), and they added the thermocatalytic converter. Experimenters also treated the phytotrons and air with ultraviolet light before closing the system. Moreover, crew members wore gauze masks when they worked with the plants to avoid being exposed to potentially harmful organisms. In addition, as mentioned earlier, in the third experiment the atmosphere inside Bios-3 was kept under a slight positive pressure to keep air from entering through any leaks. As a result of these measures, no decline in crop production was observed compared with past experience, and the microbiological communities were somewhat more stable (although never completely stable). Most of the detailed microbiological work was done during the first two experiments: No new phenomena were observed in the third experiment, so the data obtained have not been prepared for publication.

Theory of closed systems

Many researchers at the Institute of Biophysics are physical scientists, and some have devoted considerable effort to understanding the theory of closed systems. Some such studies involve mathematical modeling of the Bios-3 results as well as of Earth's ecosystems. The following paragraphs summarize some of the topics that have been considered, with and without mathematical modeling.

One interesting relationship to come out of such theoretical studies is that between engineered and biological systems. Organisms are self-regulating. In an algal reactor, for example, there may be 10^{13} cells, any one of which could regenerate the system because the ability to do so is encoded in its genome. By contrast, engineered components have no such self-repair capability and hence are the weakest link in an artifically controlled environment. This point needs to be emphasized because most people assume that organisms are the weak link.

Balancing the requirements of humans with the food-production system provides interesting and critical challenges, as noted above. Could traditional food sources be replaced? At present, food choices are determined largely by tradition and available technology rather than by nutritional considerations (Salisbury and Clark 1996).

It is also important to consider trophic levels of the diet. Only 2.3–2.9% (lamb and beef) to 19% (turkey) of the energy contained in plant feed is converted to energy in meat (Ensminger et al. 1990), so the plant-growing area must be expanded if animal feed is included. Reducing the trophic levels is an obvious solution if resupply is difficult or impossible. This goal can be achieved by following a vegetarian diet or at least a more vegetable-based diet. One approach is to prepare vegetarian foods, if necessary those that mimic the biochemical composition and taste of meat. Alternatively, a few animals, such as fish or invertebrates, that can exist on plant biomass that is not suitable for humans might be included in a future bioregenerative life-support system without adding much to structure size or energy requirements.

A desirable goal would seem to be to reduce deadlock substances to the barest minimum. This reduction could be costly, however; it may be simpler and cheaper to resupply materials that are tied up in deadlock substances than to bring these materials back into the system with complex techniques. These studies lead to an appreciation of the balances that have existed for so long on Earth. The challenge in designing and building a functioning bioregenerative life-support system is to achieve these balances within a limited volume and with advanced technology to replace the large buffer sizes and often slow processes of Earth's ecosystems.

Achieving stability proved to be a serious problem in the Bios-3 experiments. The instabilities were mostly in microelements and microflora. Recognizing and evaluating these instabilities was a clear result of the Bios-3 experiments. Theoretical and experimental investigations are necessary to solve the stability problem in small, closed ecosystems. Microfloral instabilities pose a potential threat to such systems, and microbial communities may exhibit new processes not imagined in the system design. Viruses and plasmids were not studied in the Bios experiments, but they could also pose threats.

It is interesting that even with its size, complexity, and diversity of organisms, Biosphere 2 was unstable (Nelson et al. 1993), whereas Folsome's small, sealed flasks with their simple communities have continued to function for decades (Folsome and Hanson 1986). Intuition seems to tell us that complexity and diversity should lead to stability; in a diverse system, if one species dies out, another should be available to occupy its niche. But intuition could be wrong. Complexity and diversity increase the chances for some species to act in unforeseen ways, for example, by exhibiting positive feedback in their population growth. The role of diversity in ecosystem stability has often been discussed by ecologists (e.g., Barbour et al. 1987), who have concluded that this role depends on which definition of stability is being used and which ecosystem is being discussed.

Although a closed ecosystem does not need human participation, a functioning bioregenerative life-support system assumes a role for humans. Such a system is a biosphere that is under intelligent control. In a 1924 French monograph, Vernadsky called such a system a noosphere (Vernadsky 1989).

Where do we go from here?

All of the Bios experiments have demonstrated the feasibility of sustaining human life inside a small, essentially closed, ecological system. Events during the decade since the last manned experiment in Bios, including the Biosphere 2 experiment with its wide but controversial media coverage, have increased the interest in this field of experimental biology. The number of researchers working in this field has increased, perhaps enough to indicate a trend. Most international conferences on space problems held during recent years have sponsored several sessions devoted to such closed systems that have attracted large audiences (e.g., at the World Space Congress in Washington, DC, in 1992; at the International Astronomic Federation in Jerusalem in 1994; at the Committee on Space Research, or COSPAR, in Hamburg in 1994). A conference on closed ecosystems was held in Aomori, Japan, in 1992 in connection with opening of the Institute for Environmental Sciences, which will have a large experimental life-support facility similar to Bios-3 (Ashida and Nitta 1995, Tako et al. 1996). These developments show that the scientific community is increasingly convinced of the importance of developing artificial, closed ecosystems, not only for future life support in space, but primarily as tools to study the fundamental problems of biospherics—that is, to better understand the regularities of stable existence of Earth's biosphere.

What are the problems for the future? First, in our opinion, it is necessary to define the stability boundaries of a small, closed ecosystem like Bios-3. The goal of all experiments so far has been to maintain the ecosystem in a steady condition. To evaluate stability, the system should be perturbed from its steady state, which will allow transition–disturbance processes to be investigated. It should then be returned to its initial condition. Experiments of this type are required to develop a reliable control system for small, closed life-support ecosystems. Provided that the technology can be modernized, Bios-3 would be well suited for such experiments. Regretfully, the current economic situation in Russia makes this research impossible.

The next problem is to create a generation of new, experimental closed ecosystems to accelerate the gleaning of information. One next-generation system is being constructed at the Johnson Space Center in Houston, Texas: the Bioregenerative Planetary Life Support Systems Test Complex, now called BIO-Plex (Tri et al. 1996). Initially, the facility will consist of five cylindrical chambers, each 4.6 m in diameter and 11.3 m in length, joined by an interconnecting transfer tunnel and accessed through an airlock. Two more chambers can be added for future needs. Each chamber will have two decks and two hatches, one connecting with the tunnel and the other for emergency entry or egress. This structure will be NASA's state-of-the-art facility, with a series of tests planned to begin during this decade. Both physicochemical and bioregenerative life-support systems will be tested.

Finally, a third problem is to further enhance the degree of closure of experimental ecosystems—that is, to reduce the metabolic deadlocks. Inedible plant parts need to be transformed into edible materials in new ways, for instance, by biotechnological processing or genetic engineering, or by feeding the inedible parts to fishes or various invertebrates or using them to grow mushrooms. Efficient, inexpensive ways to recycle minerals from plant and human wastes back to the primary producers, the plants, also need to be developed. These processes and techniques are far from the maturity required to incorporate them into a closed life-support system; fortunately, such research is currently being sponsored by NASA and by the Japanese and European space agencies, although funding levels are minimal.

Based on the experience gained in the Bios studies, it is possible to make some suggestions about how a lunar base or other closed system should be constructed (Gitelson 1995). In an attempt to get the most use out of the Bios experience, an International Center for Closed Ecosystem Studies has been established at the Institute of Biophysics in Krasnoyarsk. The center's goal is to make the Bios experience accessible to the world scientific community and to facilitate the exchange of information among those scientists who are interested in the new science of biospherics. The benefits of such collaboration are self-evident, and we hope that our own joint work is a good case in point.

Acknowledgments

Preparation of this manuscript was supported by the Utah Agricultural Experiment Station (this is paper nr 4784) and by a grant to Frank B. Salisbury from NASA through Lockheed Engineering and Science at the Johnson Space Center (LESC-SOW-Y-5765). Work on this manuscript was performed while Josef I. Gitelson held an Associateship from the National Research Council at NASA Ames Research Center, Moffett Field, CA. We also acknowledge support from the Institute of Biophysics, Krasnoyarsk, Russia.

References cited

Adamovich BA. 1975. Non regenerative life-support systems for flights of short and moderate duration. Pages 227–246 in Calvin M, Gazenko O, eds. Foundations of space biology and medicine. Vol. 3. Moscow: Academy of Sciences USSR.

Ashida A, Nitta K. 1995. Construction of CEEF [Closed Ecology Experiment Facility] is just started. SAE Technical Paper Series nr 951584. 25th International Conference on Environmental Systems; 10–13 1995; San Diego, CA. Available from: SAE, 400 Commonwealth Dr., Warrendale, PA 14096-0001.

Barbour MG, Burk JH, Pitts WD. 1987. Terrestrial plant ecology. 2nd ed. Menlo Park (CA): Benjamin/Cummings Publishing Co.

Berner RA, Lasaga AC. 1989. Modeling the geochemical carbon cycle. Scientific American 260: 74–81.

Brown CS, Cox WM, Dreschel TW, Chetirkin PV. 1992. The vacuum-operated nutrient delivery system: hydroponics for microgravity. HortScience 27: 1183–1185.

Bugbee BG, Salisbury FB. 1988. Exploring the limits of crop productivity. I. Photosynthetic efficiency of wheat in high irradiance environments. Plant Physiology 88: 869–878.

Bugbee BG, Spanarkel B, Johnson S, Monje O, Koerner G. 1994. CO_2 crop growth enhancement and toxicity in wheat and rice. Advances in Space Research 11: 257–267.

Ensminger NE, Oldfield JE, Heinemann WW. 1990. Feeds and nutrition. 2nd ed. Clovis (CA): Ensminger Publishing Co.

Folsome CE, Hanson JE. 1986. The emergence of materially closed system ecology. Pages 269–288 in Polunin N, ed. Ecosystem theory and application. New York: John Wiley & Sons.

Frank LA, Huyghe P. 1990. The big splash. Secaucus (NJ): Carol Publishing Group.

Garland JL. 1992. Characterization of the water-soluble component of inedible residue from candidate CELSS crops. Washington (DC): NASA. Technical Memorandum nr 107557.

Garland JL, Mackowiak CL, Sager JC. 1993. Hydroponic crop production using recycled nutrients from inedible crop residues. SAE Technical Paper Series nr 932173. 23rd International Conference on Environmental Systems; 12–15 July 1993; Colorado Springs, CO. Available from: SAE, 400 Commonwealth Dr., Warrendale, PA 14096-0001.

Gazenko OG. 1967. Development of biology in the USSR. Pages 613–632 in Soviet science and technology for 50 years (in Russian). Moscow: Nauka Press.

Gitelson JI, Rodicheva EK. 1996. Self-restoration a specific feature of biological life support systems. SAE Technical Paper Series nr 961495. 26th International Conference on Environmental Systems; 8–11 1993; Monterey, CA. Available from: SAE, 400 Commonwealth Dr., Warrendale, PA 14096-0001.

Gitelson JI, Kovrov BG, Lisovsky GM, Okladenikov YuN, Rerberg MS, Sidko FYa, Terskov IA. 1975. Problems of space biology. Vol. 28. Experimental ecological systems including man (in Russian). Moscow: Nauka Press.

Gitelson JI, Terskov IA, Kovrov BG, Sidko FYa, Lisovsky GM, Okladnikov YuN, Belyanin VB, Trubachev IN. 1976. Life-support systems with autonomic control employing plant photosynthesis. Acta Astronautica 3: 9–10; 633–650.

Gitelson JI, Manukovsky NS, Pankova IM, Somova LA, Tirranen LS. 1980. Microbiological problems of closed ecological systems (in Russian). Novosibirsk, Russia: Nauka Press.

Gitelson JI, et al. 1989. Long-term experiments on man's stay in biological life-support system. Advances in Space Research 9: 65–71.

Gitelson JI, Blüm V, Grigoriev AI, Lisovsky AI, Manukovsky NS, Sinyak YuE, Ushakova SA. 1995. Biological-physical-chemical aspects of a human life-support system for a lunar base. Acta Astronautica 37: 385–394.

Holm LG, Plucknett DL, Pancho JV, Herberger JP. 1977. The world's worst weeds, distribution and biology. Honolulu: University Press of Hawaii.

Jones SB, Or D. 1996. Process-based selection of particulated growth media for optimal liquid and gaseous fluxes to plants roots in microgravity. 31st Scientific Assembly of the Committee on Space Research (COSPAR); 14–21 July 1996; The University of Birmingham, UK.

Kamarei AR, Nakhost Z, Karel M. 1986. Potential for utilization of algal biomass for components of the diet in CELSS. Pages 13–22 in MacElroy RD, Martello NV, Smernoff DT, eds. Controlled ecological life support systems: CELSS 85 Workshop. Available from: NASA Ames Research Center, Moffett Field, CA 94035. Technical Memorandum nr TM88215.

Kovrov BG, et al. 1985. Artificial closed ecosystems "man-plants" with a full regeneration of atmosphere, water, and ration vegetable. Proceedings of the XXXVI International Astronomy Congress; 7–12 Oct 1985; Stockholm, Sweden. New York: Pergamon Press.

Lewis NG. 1994. Plant metabolism and cell wall formation in space (microgravity) and on Earth. Pages 241–244 in NASA Technical Memorandum: 1992–1993 NASA space biology accomplishments. Washington (DC): NASA.

Lisovsky GM, ed. 1979. Closed system: man-higher plants (in Russian). Novosibirsk (Russia): Nauka Press. [Available in English as a NASA Technical Translation, 1981].

Morrow RC, Dinauer WR, Bula RJ, Tibbitts TW. 1993. The ASTROCULTURE™-1 flight experiment: pressure control of the WCSAR porous tube nutrient delivery system. SAE Technical Paper Series nr 932282. 23rd International Conference on Environmental Systems; 12–15 July 1993. Colorado Springs, CO. Available from: SAE, 400 Commonwealth Dr., Warrendale, PA 14096-0001.

Nelson M, Burgess TL, Alling A, Alvarez-Romo N, Dempster WF, Walford RL, Allen JP. 1993. Using a closed ecological system to study Earth's biosphere. BioScience 43: 225–236.

Salisbury FB, Clark MA. 1996. Choosing plants to be grown in a controlled environment life support system (CELSS) based upon attractive vegetarian diets. Life Support and Biosphere Science 2: 169–179.

Salisbury FB, Bingham GE, Campbell WF, Carman JG, Bubenheim DL, Yendler B, Jahns G. 1995. Growing super-dwarf wheat in Svet on Mir. Life Support & Biosphere Science 2: 31–39.

Shepelev YY. 1972. Biological life support systems. Pages 274–308 in Calvin M, Gazenko O, eds. Foundations of space biology and medicine. Vol. 3. Moscow: Academy of Sciences USSR.

Somova LA. 1996. Effect of closure on man-microbes interaction in different types of Earth-space missions. 31st Scientific Assembly of the Committee on Space Research (COSPAR); 14–21 July 1996; The University of Birmingham, UK.

Tako Y, Hosomi T, Nitta K. 1996. A mathematical model on physiological processes of candidate crops in CEEF. SAE Technical Paper Series nr 961499. 26th International Conference on Environmental Systems; 8–11 July 1996; Monterey, CA. Available from: SAE, 400 Commonwealth Dr., Warrendale, PA 14096-0001.

Tri TO, Edeen MA, Henninger DL. 1996. Advanced life support human-rated test facility: testbed development and testing to understand evolution to regenerative life support. SAE Technical Paper Series nr 961592. 26th International Conference on Environmental Systems; 8–11 July 1996; Monterey, CA. Available from: SAE, 400 Commonwealth Dr., Warrendale, PA 14096-0001.

Tsiolkovsky KE. 1964. Life in interstellar medium (in Russian). Moscow: Nauka Press.

Vernadsky VI. 1924. Biosphere and noosphere (in Russian). Moscow: Nauka Press.

Waslien CI. 1975. Unusual source of proteins for man. CRC Critical Reviews in Food Science and Nutrition 6: 77–151.

Wolverton BC, McDonald RC, Watkins EA Jr. 1984. Foliage plants for removing indoor air pollutants from energy efficient homes. Economic Botany 38: 224–229.

Wolverton BC, Johnson A, Bounds K. 1989. Interior landscape plants for indoor air pollution abatement. John C. Stennis Space Center (MS): NASA Office of Commercial Programs-Technology Utilization Division and the Associated Landscape Contractors of America. Report nr 22.

Yendler BS, Webbon B, Podolski I, Bula RJ. 1996. Capillary movement of liquid in granular beds in microgravity. Advances in Space Research 18 (4/5): 233–237.