

# Past, Present, and Future of Closed Human Life Support Ecosystems - A Review

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During the development of human space exploration, the idea of simulating the Earth's biosphere to provide human life support led to the convergence of space biology and the field of ecology to develop closed manmade ecological systems. A space habitat can be thought of as an ecological system of human beings exchanging energy and material within a spacecraft. In order to understand and control material and energy exchange processes, one must combine basic ecological principles with the knowledge gained through Bioregenerative Life Support Systems (BLSS) research to date. Experimentation in closed manmade ecosystems for spacecraft life support began in the 1960's and over the years has shown biological life support systems to be realizable. By building on lessons learned, we can identify and prioritize future research objectives. Future development should focus on improved reliability of mechanical components, autonomous ecosystem control, closure of the carbon cycle (food generation and waste recycling), and the maintenance of long-term stability and robustness. A common need identified throughout all closed ecological life support systems (CELSS) research is mass and energy exchange models that enable intelligent autonomous control and design optimization. To validate mass balance models, integrated system level experiments are needed, but full-scale tests are time consuming and expensive. Small closed experimental ecosystems (microcosms) could allow observation of stability limits and response to perturbation with repeated short duration experiments. However, they must have proper scaling to represent larger system dynamics. Thus, the definition of non-dimensional ecological parameters (or *invariant* system descriptions) that define similarity between biological life support systems of different temporal and spatial scales is a potentially critical yet little studied research area that will enable prediction of mass and energy exchange for various system configurations and operating scenarios.

## Nomenclature

<i>AE</i>	=	Artificial Ecosystem
<i>ALSSIT</i>	=	Advanced Life Support Systems Integration Test Bed
<i>BIO-Plex</i>	=	Advanced Life Support System Test Bed
<i>BLSS</i>	=	Bioregenerative Life Support Systems
<i>CAB</i>	=	Controllo Ambientale Biorigenerativo
<i>CEBAS</i>	=	Closed Equilibrated Biological Aquatic System
<i>CEEF</i>	=	Closed Ecological Experimental Facility
<i>CES</i>	=	Closed Ecological System
<i>CELSS</i>	=	Controlled Ecological Life Support System
<i>DDT&amp;E</i>	=	Design, Development, Test, and Evaluation
<i>ECLSS</i>	=	Environmental Control and Life Support System
<i>ELT</i>	=	Ecological Law of Thermodynamics
<i>ESA</i>	=	European Space Agency
<i>ESM</i>	=	Equivalent System Mass
<i>IBMP</i>	=	Institute of Biomedical Problems, Moscow
<i>LCC</i>	=	Life Cycle Cost
<i>LMLSTP</i>	=	Lunar-Mars Life Support Test Project

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*MEERC* = Multiscale Experimental Ecosystem Research Center  
*MELiSSA* = Micro-Ecological Life Support System Alternative  
*MES* = Experimental Mini-ecosystems  
*MPP* = MELiSSA Pilot Plant  
*NASA* = National Aeronautical and Space Administration  
*P<sub>GMS</sub>* = probability of supporting equipment failure  
*P<sub>LSS</sub>* = probability of catastrophic ECLSS failure  
*P<sub>QL</sub>* = probability of human fatal mistake due to life support quality  
*P/R* = photosynthesis to respiration ratio  
*PALACE* = Permanent Astrobase Life-support Artificial Closed Ecosystem  
*R* = index of system closure  
*RQ* = Respiratory Quotient  
 THESEUS = Towards Human Exploration of Space: a European Strategy

## I. Introduction

**D**URING the development of human space exploration in the 20<sup>th</sup> century, the idea of simulating the Earth's biosphere to provide human life support led to the convergence of space biology and the field of ecology to develop closed manmade ecological systems.<sup>1</sup> The purpose of this effort was to model and understand Earth's sustaining life support mechanisms in order to simulate them for sustaining human life in space. An Environmental Control and Life Support System (ECLSS) for spacecraft maintains an environment "suitable for the well-being of men and systems during the mission" in an isolated volume.<sup>2</sup> The ECLSS must satisfy the physiological needs of the crew by revitalizing the atmosphere (removing CO<sub>2</sub> and providing O<sub>2</sub>), maintaining temperature, regulating humidity, providing potable water and food, and removing hazards posed by wastes.

As early as the end of the 19<sup>th</sup> century, K.E. Tsiolkvsky was considering the possibility of biological life support systems for people in space, but experimentation did not begin until the 1950's.<sup>1</sup> Space life support development in the 1960's spurred research in closed manmade ecosystems in the US, Japan, Germany, England, France, and the Soviet Union.<sup>1</sup> Humans were included in the first prototypes, showing biological life support systems to be realizable. However, to this day researchers still have opposing opinions about the feasibility of creating stable closed biological systems for space life support. Most agree that simulating the Earth's biosphere as a whole is not valid to solve the problem. Due to limitations of size, weight, and energy, using the biosphere as the model for simulating sustainable mass and energy exchange becomes inefficient in an artificial closed system. There have also been significant advances in physicochemical methods to provide life support. However, these systems are not without their difficulties, such as reduced reliability over time. The benefit of biological systems is that they can serve multiple functions (processing human waste while recycling it to generate oxygen, water, and food). They can operate at normal cabin pressures and temperatures. Moreover, living systems can reproduce themselves, allowing continuous functioning and self-repair. Estimates of the true advantages and disadvantages of physicochemical and biological systems require comprehensive theoretical and experimental investigations, which will likely result in combined systems. The creation of closed human ecosystems will allow humans to exist in space with only the input of energy and little to no release of material to the surroundings. Such technology can also improve sustainable closed loop living in extreme environments on Earth.

## II. Basic Principles: Ecology and Closed Ecosystems

### A. Principles of Ecology & Ecosystem Theory

The principles of ecology and ecosystem theory are invaluable for studying the material and energy exchange processes between biological entities and non-biological components of a spacecraft life support system. Merriam Webster dictionary defines *ecology* as a "science that deals with the relationships between groups of living things and their environments," and an *ecosystem* as the "complex of a community of organisms and its environment functioning as an ecological unit." An ecological system is the "structural and functional whole including organisms and their habitats, in which processes of biological material and energy exchange go on, to support sustainable functioning of the self-contained system."<sup>2</sup>

Biological systems for space life support are by definition ecological systems, or ecosystems. In fact, one could consider a spacecraft habitat as an ecological system of human beings exchanging energy and material within the spacecraft environment. Ecosystems have natural or manmade boundaries, which are open to constant fluxes of energy and mass (water, air, etc.) Living components are *autotrophic* (producers) or *heterotrophic* (consumers or

decomposers). A *habitat* is the species' address, while a *niche* is a species' job, or 'function'. If multiple species fulfill the same niche, there is functional redundancy. *Diversity* is the variety of species present. Many ecologists theorize, with much debate, that species diversity in an ecosystem enhances stability. If this theory is true, then diversity is an important design factor for bio-regenerative life support systems for space. Abiotic processes that make an ecosystem operational are energy flow and material cycling.<sup>2</sup> Materials (such as carbon, nitrogen, and phosphorous) recycle back and forth between abiotic and biotic components, through *biogeochemical cycles*. *Liebig's law of the minimum* says that the nutrient that is least available with respect to need limits growth, also known as a *limiting factor*. This results in dynamic equilibrium conditions where inflows balance outflows over the long run, with tolerance to perturbation in environmental conditions.

*Ecosystem Theory*.<sup>3</sup> *Systems ecology* uses a systems approach to explain the characteristic processes and reactions of ecosystems, similar to how physics explains physical phenomena.<sup>3</sup> Ecosystem theory attempts to predict reactions to well-defined changes in forcing functions, by understanding emergent ecosystem properties and underlying processes. Ecosystems, like all other systems, must follow the first, second, and third laws of thermodynamics and are constrained by biochemical reaction rates. An interesting theory about ecosystem sustainability is that processes, reactions, or exchanges between components are self-regulated to move the system as far as possible away from thermodynamic equilibrium, or to maintain the highest capacity for work or power, also known as exergy.<sup>3</sup> To do this, ecosystems can grow: in biomass, in structural complexity, and in genetic information. This idea, known as the *ecological law of thermodynamics* (ELT), is a translation of Darwin's theory into thermodynamic terms.<sup>3</sup> Ecological thermodynamics is "the study of energetic efficiencies of ecosystems and of their energetic pathways."<sup>4</sup> Ecosystem theory ascribes ecosystems with seven characteristic properties: open systems far from thermodynamic equilibrium, hierarchical organization, high diversity, high buffer capacity, networked organization, high information content in organism genomes, and high levels of organization resulting in emergent properties.<sup>3</sup>

## B. Properties of Closed Ecological Systems (CES)<sup>1</sup>

Ref. 1 describes a life support system as an artificial ecosystem (AE) designed to support a specific species: human beings. An AE is subdivided into its components or *links*, which are species, or groups of species, that fulfill a specific function of energy and material exchange. Artificial ecosystems exchange energy with the outside, and can be either open or closed to matter exchange, by storing needed substances (open) or regenerating them (closed).<sup>1</sup> If material cycling is well coordinated, it can continue indefinitely.<sup>1</sup> In order to evaluate various designs for life support systems (biological or physicochemical) there are three important characteristics to consider. The first is performance or efficiency in terms of system requirements (how much food is produced, what gas exchange rates can be achieved, etc.) The second is the cost of the system, usually approximated by the system mass, which is proportional to launch cost. Other costs might include crew time required for operation and maintenance, or total life cycle costs. The third characteristic is robustness, or the ability to maintain habitable conditions for crew survival and productivity over the mission lifetime.<sup>5</sup> The *Advanced Life Support System Evaluator* (ALiSSE) metric,<sup>6</sup> developed by ESA simultaneously considers many of these criteria, including power, energy, efficiency, reliability, and/or risk to humans, and crew time.

### 1. System Mass

Equivalent System Mass (ESM) is NASA's standard measure for evaluating ECLSS system cost, and it is by far the mostly widely used metric for ECLSS trade studies in technical literature. Originally developed by Ref. 7, ESM includes the mass of a system and associated infrastructure given a specific mission location, duration, and crew size.<sup>8</sup> ESM approximates system launch cost as a sum of actual mass (initial infrastructure plus consumables) and equivalent masses of volume, power, cooling, and crew time. Ref.'s 7-9 define the metric in detail and provide guidance for implementation. If the technologies under comparison have the same function at the same quality, reliability, and safety, the best option becomes that with the lowest ESM.<sup>7</sup>

A major driver of system mass for long duration exploration missions is the level of closure (how much material is recycled versus stored). The *Index of System Closure* ( $R$ ) characterizes the portion of expended substance that is recycled (not stored) per unit time:  $R = 1 - m/M$ , where  $m$  is the mass of stores consumed, and  $M$  is the total mass consumed.<sup>1</sup> Biological or physical components can achieve material closure. Ideally closure is full (100% of needed resources are cycled), but can be partial, where some stored substances are needed.<sup>1</sup>

### 2. Life Cycle Cost (LCC)

Because costs for development, launch, and operations vary depending on the mission destination and duration, LCC is a more inclusive and accurate cost estimate than equivalent mass.<sup>10</sup> According to the Systems Engineering Body of Knowledge (SEBoK), life cycle cost is "the total cost of implementation and ownership of a system over its useful life. It includes the cost of development, acquisition, operation, maintenance, support, and, where applicable,

disposal.”<sup>11</sup> LCC is a standard systems analysis metric used by NASA in mission design and throughout the aerospace industry.<sup>10</sup> For space exploration missions, LCC will include design, development, test, and evaluation (DDT&E); launch and emplacement; and operations. Ideally, system disposal or retirement should also be included in LCC. Ref. 10 and Ref. 12 provide an overview of methods for estimating the different components of LCC.

### 3. Robustness

Definitions of robustness vary widely across science and engineering disciplines. However, a common thread is the persistence or consistency of an outcome given uncertain, changing conditions. Probably the most well-known application of robust design is in industrial engineering, where robustness typically refers to the invariance of a manufactured product output to variance in inputs or environmental conditions. In a more general engineering context, Merriam Webster defines robustness as “capable of performing without failure under a wide range of conditions.” For this reason, Ref. 5 defines ECLSS robustness as the *ability to maintain habitable conditions for crew survival and productivity over the mission lifetime under a wide range of conditions*. This wide range of conditions under which an ECLSS must provide consistent habitability includes ordinary usage; finite occasional disturbances, perturbations, or disruptions; and longer term, sustained changes in the system or mission context.<sup>5</sup> Given the increased uncertainty and sources of variability inherent in space exploration missions, Ref. 5 proposes that spacecraft life support robustness should expand upon the traditional notion of reliability to include invariance to environmental perturbation (a.k.a. resistance or stability), resilience to disturbances or adverse events, and even survivability during catastrophic failures. As mission distance from Earth increases, robustness may become a more important design criterion than mass efficiency.

*Reliability:* The Systems Engineering Body of Knowledge defines reliability as “the probability of a system or system element performing its intended function under stated conditions without failure for a given period of time.”<sup>11</sup> A *failure* is the inability to perform as intended. Ref. 13 defines *integral reliability* as the summed probability of catastrophic ECLSS failure ( $P_{LSS}$ ), human fatal mistake due to life support quality ( $P_{QL}$ ), and failure of supporting equipment such as power ( $P_{GMS}$ ).

*Stability:* Ecologists sometimes describe stability as synonymous with robustness. Stability is the ability to return to an equilibrium state after a temporary disturbance.<sup>14</sup> Stability requires a steady state mass balance for each element in the system. Non-zero mass into the ECLSS must equal mass out or else deadlocks will result, leading to system failure. Ref. 15 cautions that the index of closure can only be meaningful if there exists a settled stationary state and the total mass of the system is constant. Systems of differential equations describing mass flow dynamics between system compartments allow stability analysis. There are generally two schools of thought on the characteristics that contribute to artificial ecosystem stability. The first (an engineering perspective) is that stability depends upon the accurate and prompt control of processes maintaining material cycles and the prohibition of evolutionary transformation.<sup>1</sup> This is because an artificial closed system with small dimensions has reduced buffer capacity with which to maintain stability and must be genetically stable for reliable mass and energy exchange rates.<sup>1</sup> The second school of thought is that of systems ecologists. According to Ref. 16, the most promising life support system for space is an ecosystem that has self-organized to include humans and a minimum of technology. There has been great disagreement about the *carrying capacity* for a human, i.e. the minimal area, energy, and materials required per person for a closed ecological system. What level of diversity and/or control brings stability?

Both camps (proponents of technological versus ecological complexity) agree that the method to answer these questions lies in experimentation with closed artificial ecosystems containing all of the required links or functions, to inform model development. Often this data is time consuming and expensive to collect with full-scale integrated facilities. It would take a very long time to test all variations of environmental conditions that could occur over the lifetime of the system.

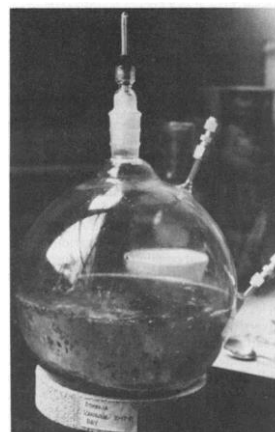
### C. Experimental Ecosystems for Biospheric Research

Analysts use mathematical models to predict the performance of a specific AE design. However, ecosystem models are not yet able to describe mechanistic processes for material and energy exchange accurately, due to system complexity.<sup>1</sup> Hence, model development and validation require experiments with real, physical, closed ecosystems. Microcosms are small, controllable, replicable, low cost experimental ecosystems. They are typically small containers of different sizes, shapes, and compositions. Microcosms are widely used in modern ecology because of their controllability, replicability, and low cost.<sup>16</sup> They can be open or closed to material exchange and usually contain many species. Metabolic measurements of CO<sub>2</sub> and O<sub>2</sub> flux or micro calorimeters allow inference or direct measurement of metabolic rates and thermodynamic energy exchange.<sup>16</sup> Microcosms are scalable in order to make inferences about larger similar systems. Their use dates back to 1851, and some sealed microcosms from the 1970’s are still alive today. Knowledge gained from microcosm experiments contributed to some of the first space

life support system models.<sup>17</sup> In 1986, Ref. 4 pioneered the study of materially closed ecosystems showing that closed systems 100 to 5000 mL in volume can persist for decades and perhaps indefinitely.

Figure 1 shows a picture of one of Folsome's microcosms. Initial data suggested that these sealed microcosms were truly self-sustaining and metabolically stable.<sup>4</sup> Closed ecosystems possess no external material reservoir and hence must achieve a dynamic mass balance in order to persist. A microcosm is persistent if balanced redox activity sustains regardless of species survival.<sup>4</sup> Ref. 4 found that biologic assemblages of even modest diversity continued redox activity indefinitely after material closure, adjusting their own metabolic pathways to maximize material cycling and tending towards maximum entropy production.<sup>4</sup> Redox balance might therefore be an index of ecosystem stability.<sup>4</sup>

According to the Oxford dictionary, a mesocosm is "an enclosed and essentially self-sufficient (but not necessarily isolated) experimental environment or ecosystem that is on a larger scale than a laboratory microcosm." Three decades of controversy remain unresolved about how much technology, biodiversity, hierarchy, and energy can sustain a human containing mesocosm.<sup>16</sup>



**Figure 1. An Algal-microbial Materially Closed Ecosystem<sup>4</sup>**

#### **D. Designing a Closed Ecological Life Support System**

The one common objective of any environmental control and life support system for human spaceflight is to maintain an environment "suitable for the well-being of men and systems during the mission" in an isolated volume.<sup>2</sup> The ECLSS purpose is to maintain the habitability of the spacecraft. It must provide for the crew's needs, in order to keep them alive (at a minimum) and preferably healthy, happy, and productive (to achieve mission objectives).<sup>18</sup> Beyond this commonality however, there is no one-size fits all ECLSS for human space exploration. The primary drivers of ECLSS design are the crew's metabolic inputs and outputs, environmental conditions (largely determined by mission destination), and mission objectives (dictating the duration, return time to Earth, ability to resupply, and crew activities). The crew consumes resources (food, oxygen, and water) and produces waste (CO<sub>2</sub>, urine, feces, trace contaminants, trash, etc.) In addition to metabolic needs, the crew requires protection from the hazardous space environment characterized by extreme temperatures, high vacuum, micrometeoroids, reduced gravity, and high levels of cosmic and solar radiation. The purpose of a closed ecosystem for human life support is to maintain an optimal ecological habitat for humans through material exchange, compensating for disturbances created by human metabolic activity.<sup>1</sup> The system is a metabolic counterbalance or reciprocal to the humans living in it, consuming human excreta, and restoring what humans consume.<sup>1</sup> Several NASA references document detailed human requirements for food, water, and energy; and expected metabolic exchange rates.<sup>19,20,21</sup>

Currently the only means of achieving 100% material closure is with some combination of people, animals, plants, and microorganisms with mechanical and physicochemical support hardware.<sup>1</sup> Physicochemical technologies can close the water and oxygen loops of a habitat, but not the carbon loop (i.e. food production). Ideally, the heterotrophic link decomposes all compounds synthesized by the autotrophic link. Humans are the main heterotrophic agents, oxidizing organic substances, while phototrophs primarily synthesize organic substances from inorganic material. Participation of other heterotrophs is less necessary, but they can perform other functions, like decomposition and mineralization of by-products that humans or phototrophs cannot utilize. If food will be synthesized it must in turn be decomposed. However, no single autotroph exists that can use all human wastes and produce biomass for food. Thus, a multi species system of both phototrophs and heterotrophs is necessary to support human material exchange requirements.<sup>1</sup> Diverse combinations of microorganisms, plants, and animals can be proposed. Ref. 1 details the advantages and disadvantages of photosynthetic algae, higher water plants, higher land plants, chemosynthetic microorganisms, animals, fish, fungi, and other heterotrophic microorganisms for use in closed artificial ecosystems. Component selection should take into consideration human dietary requirements first, as well as compatibility with humans (i.e. non-toxic), their controllability, energy efficiency, and other mission specific constraints or design drivers.<sup>1</sup> Ref. 1 suggests that other design objectives might include safety, reliability, comfort, manufacturability, and minimal consumption. Besides biological components, or links, AE design must also consider mechanical and electrical support systems and autonomous control for environmental regulation; buffer sizing to store gases and allow changes in process rates; redundant modularity to increase reliability; and architectural flexibility to accommodate changes in mission characteristics.<sup>1</sup>

### III. Biological Life Support System History and Development

The observations made and data collected over decades of integrated AE experiments and research have proven the feasibility of reliable and safe biological human life support systems. Vernadsky and K.E. Tsiolkovsky were conceptual predecessors for experimental work in closed ecological systems in Russia. In 1926, Vernadsky devised the concept of the biosphere as an essentially closed material cycle on a planetary scale, with the conclusion that it is feasible to sustain life indefinitely in a system with a closed material cycle.<sup>22</sup> Tsiolkovsky published books in 1895 and 1926 in which he foresaw the use of plants to regenerate the human environment and produce food for humans on board a spacecraft.<sup>1</sup> Interest in using biological systems for life support began in the 1950's. Research explored the use of green algae in closed systems to produce oxygen for airplane pilots and closed system experiments used small test animals to observe gas balance.<sup>1</sup> Experimental work on Bioregenerative Life Support Systems (BLSS) with closed material cycles began in the 1960s.<sup>1</sup>

#### A. 1960's

##### 1. *The Department of Biophysics at the Institute of Physics in Krasnoyarsk (1961-1965)*<sup>1</sup>

A research team formed to design manmade ecosystems for a prototype biological life support system. This team developed technology for continuous micro algal culture.

##### 2. *BIOS-1 at the Institute of Biomedical Problems (IBMP) in Moscow (1964-1966)*<sup>1</sup>

The BIOS facility in Moscow conducted the first experiments with micro-algae coupled to human beings in 1964. BIOS-1 was a 2-link system of human-microalgae with closed gas and water exchange. The facility contained two main compartments: an algal cultivator and the human cabin. Human food was provided externally, and solid human waste was removed from the system. Algal nutrient medium was also supplied externally and in turn, dried algal biomass was removed. Recycled urine (using ion exchange resins, permeable membranes, vacuum distillation and sublimation, air-drying, evaporation, and biological purification) and other condensate supplied drinking water. Operators recycled process water for algal cultivation after removing micro algal suspension. Additional algal nutrients came from urine and vacuum distillate from human hygiene water. The algal cultivator purified the air (including other pollutants like CO). In 1964, 12-hour and then 24-hour experiments with one human test subject tested direct gas exchange. In 1965-1966, experiment durations increased to 14, 30, 45, and 90 days for direct gas exchange. Gas closure reached 90%, with continuous algal cultivation but CO<sub>2</sub> imbalance occurred because of respiratory quotient (RQ) differences between microalgae and humans. Investigators discovered that human dietary adjustment (ratio of lipids to carbohydrates to proteins) could correct the RQ discrepancy. Photosynthetic rates in the cultivator remained continuous, regulated by human CO<sub>2</sub> production.

##### 3. *BIOS-2 (3-link) at the Institute of Biomedical Problems (IBMP) in Moscow (1969)*<sup>1</sup>

In the BIOS 2 facility, investigators added a greenhouse (called a phytotron), to form a 3-link system of humans, micro-algae, and higher plants. Human food was >50% phytotron produce and <50% imported food. Solid excrement left the system untreated. The algal and human link had closed water exchange. The algal cultivator processed human liquid waste and its condensate provided drinking water. The higher plant link condensate provided hygiene water to the crew, who replaced the plant nutrient medium weekly. Autotrophs provided 100% oxygen and consumed all human CO<sub>2</sub>. Ninety-day experiments showed the feasibility of entirely balanced gas-water exchange and steady state regeneration of vegetable food for humans. Agreement between plant assimilatory quotient and human respiratory quotient was attainable by selecting appropriate crop species. Chlorella and higher plants provided 26% of the human diet. It was determined that 2.5 m<sup>2</sup> growing area should support human requirements for fresh vegetables.

##### 4. *BIOS-2 (4-link) at the Institute of Biomedical Problems (IBMP) in Moscow (1969)*<sup>1</sup>

To increase food chain closure, IBMP added other crops (like wheat) and a microbial cultivator to oxidize human solid waste. The BIOS-2 facility was now a 4-link system with humans, microalgae, higher plants, and a microbial cultivator. The phytotron grew two wheat cultivars with conveyer cultivation. Human food was part phytotron grain and part imported. The microbial cultivator oxidized solid excrement. The algal cultivator processed liquid waste. Algal cultivator condensate provided drinking water, while the higher plant condensate provided hygiene water. Water closure was complete with the exception of samples removed for analysis and replaced with distilled water. Gas exchange was 100% closed. In a 73-day experiment, the need for mineralization of inedible plant biomass became apparent. If vegetable food production were to increase, the inedible plant biomass would result in excess O<sub>2</sub> production and CO<sub>2</sub> deficit, unless mineralized.

##### 5. *NASA Meetings and Microbial Studies, US*<sup>1</sup>

NASA held meetings to discuss closed ecosystems studies and published the proceedings. Consensus was growing that physicochemical methods were most reliable and understood, but participants acknowledged the

promise of investigating bio-regenerative means. Two approaches considered were algae photosynthesis or chemosynthesis by hydrogen bacteria. From 1954-1968, studies of closed systems with algal cultures of *Chlorella* coupled to mice for gas exchange showed that 2.3kg of *Chlorella* were needed per person to supply oxygen and consume CO<sub>2</sub>. These early experiments revealed that the ratios of carbon dioxide produced versus oxygen used are not the same between the algae and the mice. In 1964, Ref. 23 conducted a mice/algae system called microterrella, which used algae to generate oxygen, microbes to decompose mammal wastes and provide plant nutrients, condensed water vapor for drinking water, and dried algae for mice food. Nutritional food supplements were necessary. The most surprising find was the self-regulating properties of the system. It remained in balance despite population changes and was able to buffer sudden environmental changes. In 1962, investigators conducted similar experiments with duckweed, though they found it to be unsuitable for human consumption due to high amounts of oxalic acid. Studies of hydrogen bacteria and algae explored the human food value of microorganisms. Green algae caused digestive problems even at small fractions of the diet, while cyanobacteria were digestible. The difference was in the presence of a cell wall for algae. Investigators discounted hydrogen bacteria as a life support food source, due to digestive disturbances.

## **B. 1970's**

### *1. Two Month Experiments in BIOS-3 at the Institute of Biophysics in Krasnoyarsk, Siberia (1972)<sup>1</sup>*

In parallel with BIOS-1 and BIOS-2, The Biophysics Division of the Institute of Physics in Siberia was assembling another facility called BIOS-3. Crew controlled BIOS-3 from inside the cabin, similar to what would be required for a spacecraft. This improved closure since samples did not require removal for analysis. The crew workload was high, and it became necessary to reduce labor time. Heat exchanger condensate provided drinking and hygienic water and system closure increased significantly with respect to food. This high level of closure allowed observation of autonomous dynamics of microflora without external contamination.

The first 180-day test of BIOS-3 was broken into three 2-month long phases, starting in 1972. *Phase I* studied gas and water exchange between higher plants and humans. The configuration included living quarters and two phytotrons growing wheat and vegetables. Plants provided gas exchange, water, grain, and vegetables. Additional freeze dried food was stocked. Solid and liquid human metabolic wastes were removed from the complex. Controlled parameters included light, CO<sub>2</sub>, and nutrient solution composition. Productivity exceeded expectations, providing 30% of crew food requirements. Operators controlled light intensity to regulate photosynthesis, and hence control atmospheric composition. *Phase II* included one phytotron with wheat and vegetables and another compartment with three *Chlorella* cultivators. The algal link gave a higher degree of closure. The algal reactors consumed human liquid waste and test operators removed solid dried wastes from the system. Each algal cultivator satisfied O<sub>2</sub>/CO<sub>2</sub> requirements for one person. The phytotron received twice the concentration of sewage than in *Phase I*, which weakened and eventually destroyed the wheat plants due to toxic proportions of ammonium nitrogen. In *Phase III*, the phytotron grew only vegetables, with no sewage influent.

The crew had to spend 20% of their time controlling the system, indicating the need for automation. Multiple crewmembers smoothed metabolic transients. System closure was 91% in phases II-III. Dynamic equilibrium of organic volatile compounds indicated balanced production and consumption rates. Two phytotrons provided three people with 26% of carbohydrate, 14% of protein, and 2.3% of fat requirements. As was learned in earlier BIOS-1 and BIOS-2 experiments, gas exchange between humans and autotrophic links needed to be balanced. Because the algal nitrogen source was fixed (human waste), alteration of the human diet was necessary to achieve the desired RQ for gas balance.

### *2. Four Month Experiment in BIOS-3 at the Institute of Biophysics in Krasnoyarsk, Siberia (1977)<sup>1</sup>*

The next BIOS-3 experiment consisted of two phytotrons to grow wheat, chufa, and vegetables; and a living compartment. Three crewmembers lived in the facility for the first 27 days, then two crew in the next phases. Operators removed liquid and solid human wastes. Higher plants grew for three months prior to the experiment to bring them to a steady state, in terms of daily CO<sub>2</sub> uptake, O<sub>2</sub> emission, and daily productivity of edible biomass. An incinerator burned vegetable inedible biomass, providing plants with needed extra CO<sub>2</sub> and oxidizing volatile organics, without contaminating the atmosphere. However, when operators burned wheat straw (high in nitrogen), jumps in atmospheric NO<sub>2</sub> occurred. Overall plants were able to regenerate the atmosphere for the humans and aid in trace contaminant stabilization. Total system closure for this experiment ranged from 78-81%. Plants provided the crew's drinking and hygiene water, vegetable food, and some grain. Plants had capacity to restore photosynthetic productivity after damage due to system faults or release of toxic oxides from straw incineration. Wheat growth slowed under elevated CO<sub>2</sub>, while chufa demonstrated high tolerance to elevated CO<sub>2</sub> and other toxic substances. Chufa also provided higher edible biomass per area than wheat. The crew exhibited no adverse effects

over the four-month test. Due to the problems with incinerating wheat straw (buildup of NO<sub>2</sub> levels toxic to plants), investigators identified the need for other methods of biomass oxidation (like wet oxidation or biological oxidation).

### 3. *CELSS Program, NASA*<sup>1</sup>

By the 1970's, the negative consequences of using hydrogen bacteria or green algae for food resulted in reduced support for research. However, in 1976, the National Research Council held a workshop to consider whether NASA should re-examine biological life support. The consensus was that biological approaches appeared reasonable for long-term missions and that a wider variety of organisms should be explored. In 1978, at the request of the NASA Exobiology Program, the Bodkin group (consisting primarily of ecologists) considered the role of ecology in regenerative life support systems, and they recommended that the principal research focus needed to be the interactions among organisms in the system. This resulted in redefinition of biological life support as an element of an ecological system. The term Controlled Ecological Life Support Systems (CELSS) was born. A new NASA CELSS program began, involving Johnson Space Center and Ames Research Center. The CELSS program approach proposed that a regenerative life support system is an ecosystem resembling a strictly controlled farm, with the goal of sustaining human beings, their primary component.

## C. 1980's

### 1. *Five Month Experiments in BIOS-3 at the Institute of Biophysics in Krasnoyarsk, Siberia ('83-84)*<sup>1</sup>

The next BIOS-3 experiment investigated the feasibility of full regeneration of vegetable food (75-80% of total crew diet) and utilization of human liquid waste by plants to bring the water cycle closure to 100%. Two crewmembers remained in the facility for 5 months. Three phytotrons provided 63 m<sup>2</sup> of plant growing area. A new incineration technology decreased nitrogen oxide release. Operators dried solid wastes and removed them from the system. The higher plant link had a higher quantity of grain and a more diverse and nutritionally optimal diet, while regenerating the atmosphere and water. Plants received water from transpiration, cabin condensate, hygiene wastewater, and human liquid waste. Operators found that a ratio of 70-75% urea nitrogen to total nitrogen in plant growth medium reduced urine toxicity. Prolonged use of unchanged nutrient solution brought about an increase in microflora in solution. Regenerative processes provided all human requirements for oxygen and water, all vegetable food, and about 70% of total caloric needs. Thermal oxidation recycled excess inedible biomass and oxygen. Technical imperfections in the thermo-catalytic converter for incineration led to formation of toxic contaminants (nitrogen oxides) that did not hurt the humans but reduced plant productivity. Overall system closure was 95.4%. The overall time humans spent in BIOS 3 totaled two years with a maximum stay of 6 months, with 1-3 occupants at a time. In all of that time, there were no recorded adverse effects on participants.

### 2. *Continuation of CELSS work, NASA*<sup>1</sup>

In the 1980s, life support research included both physicochemical and bioregenerative advocates. Research emerged related to system control using dynamic models to simulate stability. Researchers had difficulty attaining stable steady states but thought that control systems would be energy consuming and complicated. Investigators discovered that the human respiratory quotient was not the same as that of animals, indicating that the use of single organisms for life support would not work. The CELSS program turned to closed system cultivation of higher plants as agricultural crops. NASA funded several research proposals to address issues of growing higher plant food crops in a closed system at several universities. Investigators developed dwarf wheat cultivars with higher harvest indexes per unit of growth area. Studies showed that with continuous planting and harvesting, and with oxidation of inedible biomass, 13-m<sup>2</sup> growth area could provide calories for one person, absorb one person's CO<sub>2</sub>, and produce one person's O<sub>2</sub>. Many studies in the 1980s developed design concepts for plant growth systems. By the end of the decade, the CELSS program aimed to maximize plant productivity with efficient use of power and volume, to understand the link between environmental control and productivity, to increase material recycling, to oxidize organic waste materials, to develop water purification methods, and to develop experimental tools for use in the space environment. NASA built the *CELSS Test Facility* to test concepts for in-flight plant growth experiments. NASA also built the *Biomass Production Chamber* as part of the Breadboard Project at Kennedy Space Center in 1985. It is a large, closed plant growth chamber with 20 m<sup>2</sup> of growing area in a 113-m<sup>3</sup> volume, where scientists have conducted many controlled environment tests of crop plants. The Breadboard Project also explored biological waste treatment and resource recovery by treating inedible biomass to recover minerals and to look at alternative food production options like fungus. Other tests have added liquid waste streams to plant systems.

## D. 1990's

### 1. *Advanced Life Support System Test Bed (BIO-Plex) & Lunar-Mars Life Support Test Project, NASA*<sup>1</sup>

NASA initiated the Advanced Life Support System Test Bed, also known as BIO-Plex, at Johnson Space Center. This facility was the largest of NASA's life support test systems and the first US facility to involve humans. The



system utilized higher plants while optimizing volume, mass, energy, and labor with a goal of process closure. The Lunar-Mars Life Support Test Project (LMLSTP) was a 3-phase test of BIO-Plex. Phase I of this project started in 1995, with a crop of wheat to provide air revitalization for a human test subject for 15 days. This test showed that 11.2 m<sup>2</sup> of wheat crop could provide all CO<sub>2</sub> removal and O<sub>2</sub> production for one person for 15 days. The test demonstrated the ability to control crop photosynthetic rates. Phase II was a 30 day test with four crewmembers in 1996. Phase IIA was a similar 60 day test for a crew of four in 1997. Phase III was a 90 day test with four human subjects demonstrating an integrated biological and physicochemical life support system, obtaining data necessary to perform mass and energy balance of the system. The key biological component was wheat grown in the Variable Pressure Growth Chamber, providing air revitalization and food. The Breadboard Scale Aerobic Bioreactor at Kennedy Space Center processed wheat crop residues. The Waste Incineration for Resource Recovery system developed in Phase II increased closure. An integrated water recovery system used both biological and physicochemical processes to treat a combined wastewater stream. Microbial bioreactors provided organic material degradation and ammonium nitrification, while other physicochemical systems removed inorganic salts and provided water post-treatment. Crops used recycled nutrients from inedible biomass for growth. Physicochemical systems provided about 75% of air revitalization and wheat crops provided the remainder, demonstrating effective integration of the two systems. Staged cropping methods required intensive management of nutrient solution composition. The combined system continuously recovered 100% of water used by the crew. The incinerator, used to process human solid waste, demonstrated the feasibility of converting solid waste into CO<sub>2</sub> and ash for plant consumption.

## *2. Biosphere II Test Facility, Space Biosphere Ventures, Tucson, Arizona*

Biosphere II was a privately funded ecological research facility constructed between 1987 and 1991 by Space Biosphere Ventures in Tucson, Arizona. It was an artificial materially closed ecosystem, with a sealed glass and steel structure covering 3.15 acres. Biosphere II contained seven biomes, including an ocean, a desert, a savannah, a rainforest, a marsh, an area of intensive agriculture, and a human habitat. From 1991-1993, a crew of eight people lived inside. The goal of this project was to research the interaction of people and technology with natural ecosystems, while investigating the use of artificial biospheres to support human life for space colonization.

*Biosphere II Test Module:*<sup>16</sup> Prior to building Biosphere II, a smaller test module designed to support one person provided a platform to examine long-term atmospheric changes in a closed system of people, higher plants, and soil. Double laminated glass transmitted 65% of sunlight from outside. The module included a variable volume chamber, or lung, to minimize leakage and structural loads from atmospheric pressure deltas. Microbes and aquatic plants completely recycled wastewater, used in plant irrigation. A large diversity of plants provided high growth rates for air revitalization, water purification, and food provision. CO<sub>2</sub> levels depended upon people, plants, and soil metabolism. Soil bed reactors removed air toxins and trace gases, such as methane or ethylene. All wastes were recycled during the test module experiments. In 1989, a human lived inside for 21 days. By 1990, the facility operated under 100% closure for over 60 person days. The module was very sensitive to changes in solar energy and ambient air temperature. CO<sub>2</sub> levels behaved predictably with diurnal curves, maximizing just prior to sunrise and minimizing just prior to sunset. Trace gases were within acceptable limits and the system appeared to be self-regulating and homeostatic.

*Biosphere II Facility:*<sup>2</sup> Biosphere II was a hermetically sealed glass and steel structure, with 50% light transmission. To minimize atmospheric leakage, two large pressure-volume expansion chambers minimized pressure differentials as the atmosphere heated and cooled. Soil bed reactors removed trace gases (as in the test module). Heterotrophic respiration, plant photosynthesis, carbonate reactions in the ocean, and a sodium hydroxide scrubber balanced carbon dioxide. An agricultural area that included 18 plots, as well as a series of fish/rice paddies, an orchard, and planting containers, provided 100% of the food for the 8-person crew and animals. In addition to crops, three species of domestic animals were included (pigmy goat, feral swine, and chicken). Domestic animals ate crop residue that humans could not consume. Worm beds were in the soil and fed the chickens. Azolla, a water fern, was a high protein feed for Tilapia fish, chickens, and goats. Animals produced milk, eggs, and meat, giving the crew 50% of their fat intake, and helping to recycle nutrients. Food processing machines reduced labor. The water cycle consisted of evaporation from the ocean, fresh water rain (and condensation), irrigation collection in streams and marsh, and runoff back to the ocean. Condensate used for potable water passed through 2-stage filters and ultraviolet light for sterilization. Anaerobic and aerobic microbial and plant systems processed wastes through composting, anaerobic holding tanks, and aerobic “marsh” lagoons. The lagoons recirculated the water, while aquatic plants cleaned it further by taking up nutrients. Water from the marsh went to the irrigation supply for agriculture, utilizing any remaining nutrients.

*Results and Observations:*<sup>2</sup> In the first closure experiment, a crew of eight (4 men and 4 women) were sealed inside from 9/26/1991 – 9/26/1993, followed by a second 6 month experiment in 1994. Ecological self-organization

was observed. Some species became dominant and displaced others. One of the big challenges was managing shifting equilibria among the carbon, oxygen, nitrogen, and other element cycles within and between biomes. Due to dependence on sunlight, CO<sub>2</sub> levels fluctuated seasonally (1000-4000 ppm) and diurnally (by 600 ppm). A physicochemical precipitator provided additional CO<sub>2</sub> removal. Crewmembers implemented several strategies to increase photosynthesis and decrease respiration during low light months, such as lowering nighttime temperatures, discontinuing composting, minimizing soil disturbance, pruning dead vegetation, and dry storing cut biomass to slow decomposition rates. Oxygen unexpectedly decreased from 21% down to 15% in 1993 and was added from the outside. This was concurrent with an observable loss in active carbon, detected in isotopic analysis. Theories for the loss included the oxidation of soil organic material and subsequent deposition as calcium carbonate. Despite environmental controls in an effort to minimize pests, crop losses occurred due to fungus infection from excessive ground moisture. Atmospheric monitoring showed no dangerous trace gas levels. In fact, trace gas build up was low enough, due to natural (but slower) mixing of air through the soils, that the soil bed reactors were not needed.

### 3. MELiSSA (*Micro-Ecological Life Support Alternative*), *European Space Agency (ESA)*

The European Space Agency organized the MELiSSA (Micro-Ecological Life Support System Alternative) project to aid in understanding the behavior of artificial ecosystems, and to develop technology for future biological life support systems for long-term human space missions.<sup>1</sup> The goal of this project is to “construct autonomous habitats in deep space, supplying astronauts with fresh air, water, and food through continuous microbial recycling of human wastes.”<sup>24</sup> In contrast to Biosphere II, MELiSSA minimizes biodiversity as much as possible, including only the most basic microbiological processes to provide necessary ecological functions.<sup>24</sup> It is a human/microbe/plant association with microbial species specifically selected and engineered for reliable culturing and process rates.<sup>24</sup>

The MELiSSA ecosystem framework includes five compartments: a multi-species anaerobic composter to break down plant material and human feces; a photoheterotrophic bacteria compartment for the absorption of volatile organic acids; an aerobic nitrification compartment to oxidize ammonium; a photosynthetic link (microalgae and higher plants) to produce food, purify water, and revitalize the air; and finally a human crew compartment.<sup>24</sup> The higher plant compartment utilizes hydroponic, conveyer-style cultivation systems, with staggered planting to produce up to 20 plant varieties.<sup>24</sup> The byproducts of the other microbial compartments provide the higher plants with nutrients. These waste processing compartments contain pure microbial strains with the exception of the composting unit, which includes a mix of species introduced from the human gut.<sup>24</sup> The composting unit has proved the most technically demanding part of the MELiSSA infrastructure, and waste decomposition has proved the most difficult process to simplify and stabilize.<sup>24</sup> Experimental results suggest that feces mineralization through composting may be too slow and that a physicochemical link for mineralization of waste products is essential for a life support system.<sup>1</sup> A lot of work in the 1990’s on the MELiSSA project focused on technology development for individual compartments, mechanistic process modeling, and algorithm development for control.<sup>1,25</sup> The ESA developed the MELiSSA pilot plant (MPP) to demonstrate integration of the compartments and their long-term reliability. The MPP was relocated from ESA facilities to the Autonomous University of Barcelona in 1995,<sup>26</sup> and a new MPP was inaugurated in 2009.<sup>25</sup> This facility serves to integrate technologies, including control system and compartment interfaces; operationally test the complete MELiSSA loop; provide infrastructure for testing compartment technologies; and support integrated system level experiments.<sup>25</sup> Recent research priorities have included improved mechanistic process modeling of element flows and higher plant growth, development of process interfaces, preliminary flight experiments to study reduced gravity effects, ground demonstrations with animals, preparation for a human-rated facility, and knowledge management.<sup>25</sup>

## E. 2000’s

### 1. ALSSIT (*Advanced Life Support Systems Integration Test Bed*), *NASA*

As of 2003, the BIO-Plex facility at Johnson Space Center became ALSSIT. Its objective was to support large scale, long duration testing of integrated biological and physicochemical regenerative life support systems with human test subjects under closed, controlled conditions.<sup>1</sup> At a 1997 NASA meeting, attendees discussed candidate crops and research needs for Bio-Plex.<sup>27</sup> Issues included nutrient delivery systems, cultivation protocols, environmental effects on nutritional quality and growth, lighting methods and control, crop cultivar selection, biological-physicochemical system integration, reliability, and safety.<sup>27</sup> However, by 2005, NASA redirected research in bioregenerative life support towards the needs of the Constellation Program, and terminated research in the use of higher plants for producing food and oxygen.<sup>27</sup>

### 2. *Bioregenerative Life Support program CAB (Controllo Ambientale Biorigenerativo)*, *Italian Space Agency*<sup>28</sup>

In 2006, the Italian Space Agency began the CAB Bioregenerative Life Support program with the objective of defining and preparing “the technological, scientific and demonstration elements needed to setup a controlled

biological system, allowing the regeneration of resources and the production of food for life support in long duration missions.” CAB includes higher plants for food production, atmospheric revitalization, and water purification; and physicochemical systems for environmental control and support (e.g. power and data). Ref. 28 describes the proposed functional and physical architecture, with a targeted initial mass closure of 20-40% and a long-term goal of 80% closure. The CAB program plans incremental demonstration of functionality through laboratory testing, integrated ground-based controlled environment testing, and flight-testing.

### 3. *Closed Ecological Experimental Facility (CEEF), Japan*<sup>29,30</sup>

In the 1990s, the Japanese government supported development of the Closed Ecological Experimental Facility (CEEF) and the Institute of Environmental Sciences was founded.<sup>1</sup> The CEEF, designed to simulate and predict carbon transfer in a materially closed system, consists of a closed plantation facility, a closed animal breeding and human habitat experiment facility, and the geo-hydrosphere experiment facility. During material circulation experiments conducted during 2005-2007, the plant and animal/human facilities were connected and isolated from the outside, with the 2 human participants, 2 goats, and 23 hydroponic plant crops. Three closed one-week experiments occurred in 2005, followed by 2-week experiments in 2006, and up to 4-week experiments in 2007. In the 2005 experiments, crops produced 82% of the human diet and the entire goat feed. Gas exchange occurred between the crop and animal/human facilities with a gas separation and distribution system. Due to the lack of waste processing during the 2005 experiments, plants produced twice the oxygen consumed by the crew, and the crew produced half the CO<sub>2</sub> needed by the crops. In the 2006 experiments, crops provided >90% of human food requirements and 79% of the goat feed; and water was circulated with reverse osmosis filtering approaches, to achieve ~90% water loop closure. Pyrolysis provided waste processing for goat feces and urine, externally. In 2007, pyrolysis decomposed human and goat metabolic waste and inedible plant waste; while incineration oxidized ash/char from pyrolysis, recovering approximately 90% of the CO<sub>2</sub> consumed by crops. Some carbon was lost during cleaning of the pyrolysis system.

### 4. *Experimental Mini-Ecosystems (MES) and BIOS-3, Institute of Biophysics, Siberian Branch*<sup>31</sup>

The Institute of Biophysics, RAS Siberian Branch has continued studies of closed ecological systems with a mini-ecosystem (MES) facility. Recent experiments have investigated the dynamics of gas exchange processes in response to soil respiratory activity, using chickweed plants as the phototrophic link, grown on soil-like substrate. Other experiments have studied the temporary and long-term respiratory/photosynthetic response of the MES to temperature perturbations, demonstrating the facility’s value for the investigation of ecosystem resistance to changes in environmental parameters. To create a high degree of mass closure, scientists added a heterotrophic link, NaCl accumulating plants, and a physicochemical waste treatment process. The heterotrophic link was a soil-like substrate containing fungi, worms, and microorganisms to perform biological waste combustion. The physicochemical technology chosen was alternating current activated wet oxidation in a 30% hydrogen peroxide aqueous solution, which is environmentally friendly, energy efficient, and safer than other combustion methods. As of 2014, BIOS-3 was undergoing renovation to incorporate these additional technologies and to accommodate international human test subjects.

### 5. *Lunar Palace 1, Beihang University, Beijing, China*<sup>32</sup>

Lunar Palace 1 is a ground based integrated experimental facility for the “Permanent Astrobase Life-support Artificial Closed Ecosystem (PALACE)” research program, built in 2013. It includes a 105 m<sup>3</sup> crew cabin module and two 203 m<sup>3</sup> plant cultivation modules. The crew module has four bedrooms, a bathroom, living room, and waste treatment area. Each plant cultivation module has two culture rooms to allow for independently controlled environments. The hermetically sealed facility has a 0.04%/day leakage rate. Functionally, the facility provides for plant cultivation, water treatment, solid waste conversion, and animal rearing. Conveyer style plant cultivation produces oxygen and consumes CO<sub>2</sub> with a variety of food crops. Red and white LEDs provide crop illumination. Water treatment includes humidity condensate processing, hygiene water treatment (with biological activated carbon membrane reactors), and urine treatment (with low-pressure distillation). Urine brine leftover after distillation is collected, stored, and eventually removed from the system. A solid waste microbial bioconverter degrades inedible plant waste and human feces, and provides CO<sub>2</sub> to the plant modules. Operators can adjust heat in the bioconverter to regulate CO<sub>2</sub> production rates. Atmospheric composition, temperature, and humidity are continuously monitored and controlled. An air purification system removes trace gases, which operators monitor weekly.

In a 105-day mission from February to May 2014, 3 crewmembers lived inside the sealed facility. They grew wheat, chufa, peas, carrots, and green leafy vegetables, producing 60% of their food and 100% of their oxygen and water.<sup>33</sup> Yellow mealworms grown on inedible plant biomass provided supplemental protein. To maintain mass balance, the crew imported about 1.2 kg of food and salts for plant minerals.<sup>33</sup> The crew stored and periodically exported an equivalent mass of solid waste from the system.<sup>32</sup> The experiment achieved 100% water regeneration, stable oxygen concentrations between 19.5 and 21.5%, and CO<sub>2</sub> concentrations between 500 and 5500 ppm.<sup>32</sup>

#### IV. Areas for Future Research

When the text “Manmade Closed Ecological Systems” was published in 2003,<sup>1</sup> there were only six experimental facilities available in the world for full-scale, integrated CELSS investigations: Ground Experimental Complex at the Institute of Biomedical Problems in Moscow; BIOS-3 in Krasnoyarsk; BIO-Plex at Johnson Space Center; Biosphere 2 in Arizona; CEEF at Institute of Environmental Sciences in Japan; and the Pilot Plant constructed as part of MELISSA at Universidad Autonoma de Barcelona Spain. In 2013, the Lunar Palace 1 facility in Beijing joined this list, further advancing experimental CELSS research. The observations made over decades of investigations at these facilities have proven the feasibility of reliable and safe biological human life support systems. By building on lessons learned, we can identify the next steps in bio-regenerative life support system development and prioritize future research objectives. Areas for future research identified by the previously summarized BLSS experiments include:

- 1) *Mass Exchange Dynamics for Stability Analysis and Control*: Detailed models of mass exchange dynamics are necessary to understand the effects of residence time, buffering capacity, and cycling rates on system behavior and stability.<sup>34</sup> Incorporation of detailed process models into environmental control algorithms may improve system stability. Detailed process models also enable designers to understand and track buildup of trace contaminants through the system, such as volatile gases or pathogenic microorganisms that may pose a problem to system integrity over long durations.
- 2) *Ecosystem Carrying Capacity for a Human*: We have summarized herein three decades of controversy about what combination of biological components can sustain a human-containing ecosystem.<sup>16</sup> Unanswered questions include how much technology, biodiversity, hierarchy, and energy is needed.<sup>16</sup> The minimum area and resources required per person for a closed ecological system remains undefined. How much diversity will provide adequate stability and robustness? Ref. 34 states, “The science and engineering of closed ecological systems is still too new to determine how fully recycling systems can be implemented, and at what scale, mass and volume.”
- 3) *The Role of Microbial Communities in Plant Substrate*: BIOS-3 and Biosphere II experiments showed microbial communities in plant substrate to have significant effects on atmospheric dynamics, trace contaminant control, and plant disease control. The cost-benefit and mass-energy exchange dynamics of microbial communities in plant substrate is an important area for future research.
- 4) *Nutrient Cycling & Improved Closure*: Experiments thus far have not fully realized the balance of nutrient cycling between food production and waste mineralization. However, efforts are well underway at the MELISSA, CEEF, Lunar Palace 1, and BIOS-3 facilities. If plants are being supplied nutrients through a liquid waste stream, designers must have an understanding of the limits for waste nutrient composition and optimal dilutions. The need to mineralize inedible plant biomass in the scenario of 100% food production is very clear. The most effective means of doing so requires further investigation. Due to problems encountered when incinerating wheat straw (buildup of NO<sub>2</sub> levels toxic to plants), other methods of biomass oxidation should be developed (like wet oxidation or biological fermentation).
- 5) *Aquatic Plants as Food*: Studies conducted thus far make little mention of aquatic plants as a food source for human crew. Some scientists conducted laboratory and flight experiments with duckweed, but found it unsuitable for consumption due to oxalic acid concentrations. However, the potential of aquatic plants as a fast growing, high yield food source that can also recycle organic waste products is being realized on a global scale. It may be worthwhile to take another, closer look at aquatic plants like duckweed as a high yield, high protein, nutritious food supplement that can also directly purify waste water.
- 6) *Automation and Control Systems*: In all of the experiments conducted, system control required significant personnel time, pointing to the need for a higher degree of automation. Integrated control systems are needed that respond to off nominal or unexpected events and make adjustments to the system as necessary without human intervention. Model based adaptive control systems might accommodate changes in operating parameters or user requirements.
- 7) *Improved reliability of mechanical components and system robustness*: Reliability of biological components depends upon their physical response to the environment.<sup>35</sup> Much data exists on optimal conditions for plant growth, which can establish boundary conditions for operation. Biological systems are inherently reliable in their capability to self-repair.<sup>13</sup> Despite this inherent reliability of biological systems, the long-term behavior of components operating at increased cycling rates (such as bioreactors or plant cultivation systems) is little studied. In addition, improved component reliability is necessary for improving overall system robustness.
- 8) *Other bioregenerative research needs and challenges* include nutrient management for staged crops, processes for converting raw food to edible material, definition of the operational envelope for each link,

water/nutrient delivery in microgravity, improvements in lighting efficiency, and genetic engineering of small high yield crops.<sup>27</sup>

- 9) *THESEUS (Towards Human Exploration of Space: a European Strategy)*: A 2012 THESEUS report recognized several of the research needs listed above, such as model-based systems, improved autonomy via monitoring and control, and improved component reliability.<sup>36</sup> In addition, the THESEUS report recommended the development of common metrics for life support system architecture evaluation and improvement; improved system level robustness and availability for long-term operations; development of high performance materials; and capabilities to utilize in-situ resources for life support on other planets.<sup>36</sup>

Throughout all of the research conducted thus far, researchers repeatedly identified the need for autonomy through intelligent control systems, to reduce operation time and respond to off nominal events. In order to develop this capability, designers need a good understanding of the mass and energy exchange dynamics that determine stability and efficiency. Designers also need reliable models of system behavior for design optimization. Modeling is a tool that can help define, test, and analyze system dynamics in response to environmental or other design changes without large-scale experimental facilities. Models used as experimental tools can be either mathematical (based on theory or empirical data) or physical (e.g. microcosms).

## V. Mathematical Modeling for Design Evaluation and Performance Prediction

### A. Ecological Modeling Overview

Mathematical modeling can aid in predicting system behavior in response to changing conditions and outside influences once validated by experiments. Models can also reveal how we might force the system to behave the way we want. They allow us to size the components, figure out how much buffer or storage is needed, determine ideal initial conditions, and illuminate issues related to scheduling and operation (like planting frequency). Designers can evaluate several architectural options under a wide range of operating conditions. Because of the complexity of natural feedback mechanisms in biological systems and their inherent nonlinearity, parameter estimation is the most difficult and weakest aspect of ecological modeling. It would be impossible in our lifetimes to collect enough empirical data to mathematically describe a complex ecosystem with all of its feedback mechanisms in accurate detail.<sup>37</sup> Therefore, it becomes advantageous to describe system level properties in holistic, rather than reductionist, models.<sup>37</sup> Physical processes to consider in ecological modeling include space and time resolution, mass transport (advection, diffusion), mass balance, energetic factors like solar energy input, light extinction, temperature variation (heat transport), and settling and resuspension (moving matter).<sup>37</sup> Chemical processes to consider include chemical reactions (reaction kinetics, temperature effects, and enzymatic reactions), chemical equilibrium, hydrolysis (processes that occur with water), acid-base reactions, ion exchange, and volatilization (phase change from water to air).<sup>37</sup> Biological processes to consider include biogeochemical cycles (especially for N, P, O, H, and C), photosynthesis, population growth, and toxicological processes like bioaccumulation.<sup>37</sup>

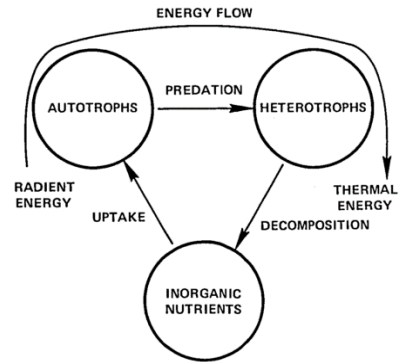
*Static versus Dynamic Models:* Assessment of system performance (outputs versus inputs) and steady state can incorporate static average values of component characteristics or use dynamic modeling and simulation to predict time dependent, dynamic behavior. Static estimates assume steady input/output rates to size components and do not account for effects of external disturbances, emergency scenarios, or perturbation in component performance. Static measures of performance can provide a rough approximation for preliminary design trades however. To understand system performance in more detail temporal variability must be analyzed (either in simulation with dynamic models or in testing), including behavior in emergency scenarios.<sup>13,38</sup> For example, switching between different modes of operation (e.g. changes in crew size) might result in temporary system instabilities. Both static and dynamic models can predict the system's ability to meet requirements, optimize functional interfaces between components, analyze flow distribution schemes, size components, test control methodology, compare alternate technologies, and identify interface issues or design deficiencies.<sup>2</sup>

### B. Dynamic Ecosystem Models

Network diagrams typically depict ecosystem models, in which bins represent a component or storage, and connections represent flows between components, or external forcing functions. The size of a compartment may grow, but, in a closed ecosystem, the total mass must stay constant. Since an ecosystem in space is ideally closed to mass, a mass balance technique appears best suited to describe it.<sup>39</sup> Figure 2 depicts a very high-level mass balance model of an ecosystem. In this approach, the processes of polymer synthesis through photosynthesis, consumption and subsequent matter oxidation, and then decomposition back into inorganic minerals determine the flow and storage of elemental mass in an ecosystem. The mass balance model tracks the flow of individual elements (e.g. C, H, O, N) through functional compartments. Differential equations (usually non-linear) define mass flow rates, very

similar in form to Lotka-Volterra predator-prey equations. Given stoichiometric composition of different materials flowing in and out of each compartment, the flow of each individual element (C, O, H, N, S, P) between compartments is then modeled at steady state with differential equations, and the instantaneous level of any element can be determined through integration.<sup>39</sup>

Ref. 40 developed the basic biochemical stoichiometry for typical processes in a biological life support system, focusing on the elements (CHON) that constitute most of the system mass. Assuming mass conservation, this simplified stoichiometry allows formation of differential equations to represent balanced matter transformations (for growth of food, consumption, respiration, and waste recycling). Analysts have commonly applied this approach to compare design configurations and analyze mass balance in experimental systems. A priori rate constants from published data are typically used, which may be constant or functions of environmental conditions (e.g. plant growth as a function of light or CO<sub>2</sub>). Ref. 41 describes a model similar in form to that described above to investigate design issues for the ALSSITB (Advanced Life Support Systems Integrated Test Bed) at Johnson Space Center.



**Figure 2. Flow of Mass in a Small Closed Ecosystem<sup>39</sup>**

### C. Structurally Dynamic Ecosystem Modeling<sup>37</sup>

Due to the high number of feedbacks, living organisms can survive and proliferate despite changing external conditions. This is what makes an ecosystem *robust*. For example, phytoplankton regulate chlorophyll concentration according to incoming radiation. Even the feedback mechanisms themselves adapt to change. Ref. 42 refers to models with constant rate coefficients as “rigid metabolism models”. Stability of rigid models decreases with increased system complexity, which is not usually the case in natural ecosystems.<sup>42</sup> Ref. 42 theorizes that contradictions between ecosystem models and observational data lies in rigid stoichiometric coefficients; and that more realistic models would allow process rates (like consumption) to change depending on conditions.

Since ecosystem structures can adapt, so must our models adapt. These characteristics create a need for *structurally dynamic models*.<sup>37</sup> Models with rigid structures and parameters extrapolate outside of conditions in which they were created. Structurally dynamic models allow for changes in species as well as changes in species’ properties.<sup>37</sup> In this technique, parameters are chosen (within an allowable range) that optimize a goal function. Goal functions might be to maximize useful power (or energy flow); minimize entropy; maximize exergy; maximize biomass, etc.<sup>37</sup> Ref. 37 suggests exergy optimization to predict changes in model parameters over the life cycle of the system. Exergy is available work energy that flows through the system and keeps it functioning. It measures the distance of the system from “inorganic soup”.<sup>37</sup> An exergy goal function offers a possible way to generate adaptable models to predict shifts in process rates and even changes in species composition that move the system away from thermodynamic equilibrium.<sup>37</sup> Ref. 37 also suggests a method to compute exergy from the ecosystem composition: chemical energy of dead organic matter weighted by species complexity (represented by number of genes).

## VI. Dimensional Analysis & Reduced Scale Prototypes

### A. The Need for Reduced Scale Prototypes

For any system, the validity of mathematical performance predictions depends upon available data. Dynamic model development and verification for a complex system requires a large number of experiments to test over a wide range of variables with replication. Empirical assessment of reliability and robustness also requires replication, across a wide state space, as well as long duration experiments in order to capture true failure rates over the system lifespan. Furthermore, design optimization requires characterization of performance over a large design space (i.e. many possible architectures). Collecting the large amount of experiment data needed for model verification, robustness estimation, or design optimization is very difficult with integrated systems testing. It is not practically feasible to build and test all feasible architectures. Tests of full size integrated systems with human operators are hardware and time intensive. Typically, analysts use simulations to verify models and predict system behavior, using experimental data on individual component behavior. Component level laboratory tests provide a wealth of useful information, like plant growth rates in a growth chamber. However, because of complex feedback mechanisms, ecosystems are greater than the sum of their parts. Component level experiments may not tell you very much about how the components will interact with one another.

There have only been seven facilities worldwide capable of integrated full-scale life support system tests. These full-scale integrated facilities are large, expensive, and take a lot of time and labor to operate. Accelerated testing is one way to cover a wide state space and to induce failures by decreasing test time and inducing environmental stress. A reduced scale CELSS can achieve accelerated process rates, due to decreased organism and buffer sizes. For decades, ecologists have used small-scale physical models (microcosms or mesocosms) as a way to test ecosystem function in a controlled environment. In ecology, "informative experiments have been conducted at scales ranging from test tubes and liter-sized containers to whole lakes and streams."<sup>43</sup> Small-scale models of life support system architectures might also provide a means of testing complex system behavior on a budget. Microcosms are small, controllable, replicable, low cost experimental ecosystems. Small ECLSS prototypes, much like microcosms, would enable faster cycling times, reducing both size and cost of conducting multiple experiments. For example, the generation time for wheat is about 30 times longer than that of *Chlorella*, such that 10-day observation of *Chlorella* based dynamics in a microcosm might be equivalent to 300 days of full-scale life support system experiments with wheat.<sup>44</sup>

### **B. The Problem of Scaling Experiments**

Small prototypes can only serve as analogs of a full-scale system if we trust that the scaled model is a valid representation and that the scaling has not affected system dynamics. Since processes change as scale increases, it might not always be possible to apply findings from an experimental system directly to a large-scale one, challenging researchers to develop quantitative and systematic approaches for extrapolation.<sup>45</sup> The dynamics observed on the experimental system must be applicable and transferrable to other systems. An *invariant description* of CELSS must exist to compare architectures and transfer results from one scale to another. Ref. 45 notes two aspects of scale: grain (smallest scale that is observable) and extent (the largest scale detected). The scaling problem is the need to extrapolate beyond experimental extent.<sup>45</sup> The goal in experiment design should be to preserve functional similarity in ecological relationships while minimizing effects of experimental artifacts.<sup>45</sup> In a book titled "*Enclosed Experimental Ecosystems and Scale*", Ref. 45 provides a detailed summary of quantitative and systematic approaches to extrapolate findings across a range of scales in microcosm studies. In the Multiscale Experimental Ecosystem Research Center (MEERC), scientists used habitat mesocosms to assess scale as an independent variable driving dynamics and response to perturbation.

Obvious scaling factors are extensive variables like space and time that constrain the system.<sup>43</sup> Experiments must be long enough to observe the dynamic processes at work, but not so long as to amplify the effect of experimental artifacts.<sup>43</sup> Other temporal factors include residence time of elements and reaction rates inside compartments.<sup>43</sup> Spatial effects (such as enclosure volume) affect physical conditions and constrain the behavior of mobile organisms.<sup>43</sup> Spatial scaling factors discussed by Ref. 45 include container geometry, water column depth, temporal effects, mixing and flow characteristics, water exchange, material residence time, light attenuation, and ecological complexity. For example, the walls of a container exaggerate the influence of attached organisms over the physical conditions within the enclosed volume.<sup>43</sup> Enclosure size can affect fluid mixing, diffusion of material and energy, light penetration and concentration gradients.<sup>45</sup> Limited spatial extent of enclosures can also interrupt feedback loops if the enclosure excludes an important element of that loop.<sup>46</sup> Intensive variables might also have a great impact on system dynamics. Intensive variables might include container shape and geometric ratios, nutrient concentrations, spatial heterogeneity of the habitat or environment.<sup>43</sup> For example, high surface area to volume ratios will exaggerate the importance of benthic communities.<sup>43</sup> Bioreactors with the same volume but different surface area will have different productivity in a light limited system, whereas in a nutrient limited system, productivity will be constant in constant volume, independent of surface area.

### **C. Ecological Similarity for Scaling Experiments**

Ref. 1, 45, and 47 suggest *dimensional analysis* as a tool for scaling experimental ecosystems while conserving key ecological relationships that apply regardless of the system dimensions. In dimensional analysis, model terms are combined such that units are cancelled resulting in dimensionless percentages and ratios. For example, one might characterize metabolic processes as a photosynthesis to respiration ratio (P/R). By defining dimensionless properties for an invariant system, one can describe and predict system behavior no matter its size. Ref. 1 gives a mathematical example of an "invariant description" or dimensionless version of a simple ecological system that is closed with respect to the atmosphere. Using dimensionless model parameters, small-accelerated closed ecological systems (CES) can be built with analogs for human and plant components that have the same dynamics with respect to mass flow. If the small systems are cheap and fast to build, many experiments are possible that cover a variety of conditions. This would be useful if designers want to scale up a design for more crew, without having to build a larger test facility.

Ref. 47 proposes an *ecological similarity* approach to scale experimental CELSS correctly, based on the principle of *similitude*. Fluid flow tests with scaled models for aircraft or ship design incorporate this concept. Ref. 48 explains that the *method of similitude* consists of two basic steps: 1) Innumerate forces thought to be independent in a problem and express them in terms of physical or dimensional parameters; and 2) construct pertinent non-dimensional groups by forming ratios of the forces, while including length ratios to insure geometric similarity. Ref. 48 suggests the following postulate for the method of similitude,

*“If two systems obey the same set of governing equations and conditions and if the values of all parameters in these equations and conditions are made the same, then the two systems must exhibit similar behavior provided only that a unique solution to the set of equations and conditions exists.”*

The difficulty in creating experimental CELSS that are similitudes of the full-scale system lies in the complexity of ecological system dynamics. There is no (as of yet) “general equation (or system of equations) which describes ecological systems exactly as equations of hydro- and aerodynamics do,” hence ecological similarity measures require mathematical system descriptions of reduced complexity.<sup>44,47</sup>

Ref. 45 suggests that two challenges of dimensional analysis are deciding what needs to be conserved among several system attributes and simultaneously conserving those attributes within both biological and experimental limitations. Ref. 47 suggests ecosystems might be comparable based on metabolic similarity; similarity in closure or equilibrium, which the author uses as an analog for stability; and similarity of relaxation processes, i.e. local stability against small disturbances based on similarity of eigenvalues for linearized state space models.

Ecologists have called for the development of a *science of scale* and Ref. 49 suggests that it should be a primary focus of research efforts. A similar science of scale would benefit spacecraft ECLSS design, especially for biological systems will scaling effects that may be misunderstood and often overlooked. The method of similitude is an established mathematical framework that would provide a strong foundation for a CELSS science of scale. The definition of ecological parameters defining similarity between biological life support systems of different dimensions in time and space is a critical area of research to enable the modeling, validation, and prediction of mass and energy exchange dynamics for a variety of life support system configurations. We propose that this research is a critical step to accomplish all the other objectives described in *Section IV*. Scalable CELSS not only allow small prototypes for model verification and performance assessment, but they also allow scaling in response to operational changes. Designers could develop a larger system to support a larger crew, with minimal redesign and testing.

## VII. Conclusion

Since the beginning of the 20<sup>th</sup> century, even before the Wright Brother’s first flew, K.E. Tsiolkvsky was imagining the possibility of biological life support systems for people in space. The science of ecology has come a long way since then, as has life support system technology and our understanding of what it takes to enable a human crew to live and work in space. Though researchers have opposing opinions about the feasibility of creating closed biological systems for life support in space, most cannot deny their potential benefit. Biological life support systems by definition are ecosystems (communities of organisms within their environment), and hence it is reasonable to conclude that such systems should be designed and evaluated according to ecological principles and theory. In order to study, understand, and even control the material and energy exchange processes between biological entities and non-biological components, one must combine the basic principles of ecology and ecosystem theory with the knowledge gained through BLSS research to date. Observations made over decades of research at integrated test facilities have proven the feasibility of reliable and safe biological human life support systems. By building on the lessons learned, we can identify next steps for development and prioritize future research objectives.

Ref. 1 classifies research objectives into technical and biological challenges, including 1) improved reliability of mechanical components; 2) improved closed ecosystem control; 3) improved closure of the carbon cycle with improved nutrient recycling; 4) improved maintenance of biological stability (including trace elements, potential viruses, and bacteria); and 5) definition of the minimal ecosystem that can support a human being. All of these research objectives require detailed reliable models of system behavior, especially mass exchange dynamics. The validity of complex ECLSS models, used for stability and closure analysis, reliability assessment, control algorithms, and design optimization depend upon available data. This necessitates a large number of long duration experiments over a wide state space. Full scale integrated life support system test facilities are large, expensive, and take a lot of time and labor to operate. Component level laboratory experiments contribute to model development, but do not allow observation of emergent system properties, e.g. how the components will interact with one another in feedback loops. Small-scale CELSS prototypes (microcosms and mesocosms) can enable accelerated testing to



cover a wide state space and shorten cycle times, with reduced size and cost. Of course, design verification will ultimately require full-scale experimental systems. However, those experiments can be selective with refined designs based on the knowledge gained from miniature models. Designers must scale these models properly to represent the true dynamics of larger systems. Thus, the definition of ecological parameters defining similarity between biological life support systems of different dimensions in time and space is a critical area of research. The definition of ecological similarity might enable the modeling, validation, and prediction of mass and energy exchange dynamics for a variety of life support system configurations. Therefore, a science of scale is a critical step for future CELSS research, design, and development.

### Declaration of Conflicting Interest

In accordance with University of Colorado policies and procedures, and our ethical obligation as researchers, we are reporting that one of the authors, Christine Escobar, has a financial and business interest in a company that may be affected by the research reported in the enclosed paper. She has disclosed those interests fully to the University of Colorado, and she has in place an approved plan for managing any potential conflicts arising from the business interest.

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