



EARTH BENEFITS OF INTERDISCIPLINARY CELSS-RELATED RESEARCH BY THE NSCORT IN BIOGENERATIVE LIFE SUPPORT

C. Mitchell,* L. Sherman,** S. Nielsen,** P. Nelson,**
P. Trumbo,** T. Hodges,** P. Hasegawa,** R. Bressan,**
M. Ladisch** and D. Auslander***

* *NASA Specialized Center of Research and Training in Bioregenerative Life Support, Purdue University, 1165 Horticulture Building, West Lafayette, IN 47907-1165, U.S.A.*

** *Purdue University, West Lafayette, IN 47907, U.S.A.*

*** *Department of Mechanical Engineering, University of California, Berkeley, CA 94720, U.S.A.*

ABSTRACT

Earth benefits of research from the NSCORT in Bioregenerative Life Support will include the following: development of active control mechanisms for light, CO₂, and temperature to maximize photosynthesis of crop plants during important phases of crop development; automation of crop culture systems; creation of novel culture systems for optimum productivity; creation of value-added crops with superior nutritional, yield, and waste-process characteristics; environmental control of food and toxicant composition of crops; new process technologies and novel food products for safe, nutritious, palatable vegetarian diets; creation of menus for healthful vegetarian diets with psychological acceptability; enzymatic procedures to degrade recalcitrant crop residues occurring in municipal waste; control-system strategies to ensure sustainability of a CELSS that will enable management of diverse complex systems on Earth.

NSCORT IN BIOGENERATIVE LIFE SUPPORT

The National Aeronautics and Space Administration (NASA) established a Research and Training Center specializing in Bioregenerative Life Support at Purdue University in November, 1990. The mission of this NASA Specialized Center of Research and Training (NSCORT) is to define the requirements for a Controlled Ecological Life-Support System (CELSS) to be self-sustaining in space without resupply from Earth (Fig. 1). Sustainability of life support in space will mean that resource demand by humans can be met by photosynthetic organisms and by complete recovery of renewable resources by recycling wastes within a system closed with respect to mass but open with respect to energy [1]. Such a system must operate stably for a long period of time without significant resupply from Earth and with reasonable cost of energy. The basic principles of CELSS operation are completely analogous to those occurring within Earth's biosphere. Cycles of carbon, oxygen, nitrogen, and mineral elements are similar in both complex systems, but the implementation and rates of

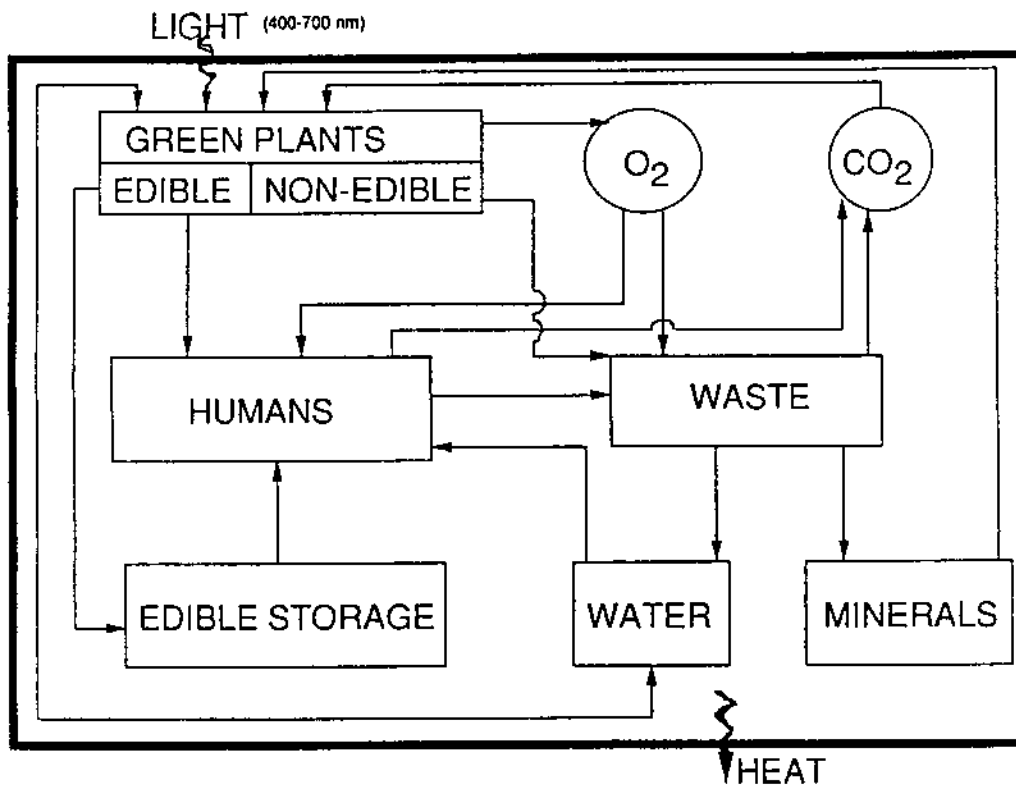


Figure 1. Schematic representation of a CELSS

element recycling must be faster in CELSS than in nature. A combination of biological and physico-chemical processes will cycle biomass through its various forms in a timely manner that ensures sustainability of the overall system /2/. Key elements for the success of a CELSS will be high harvest index (proportion of edible biomass) of the food crops selected, reasonable recycling times and power requirements for resource recovery, and creation of safe, nutritious, and palatable vegetarian diets for the crew /3/.

The NSCORT in Bioregenerative Life Support conducts ground-based, interdisciplinary research both to prove the feasibility of CELSS sustainability as well as to identify limiting factors in different scenarios of CELSS operation (Table 1). Research projects in the Center fall into the following topic categories: optimizing photosynthetic productivity and yield of higher plant food crops and blue-green algae; using biotechnology to add nutritional value to the staple crops of a vegetarian diet; analyzing the edible and non-edible parts of crop plants grown hydroponically in controlled environments for nutrient, antinutrient, and toxic constituents; studying bioavailability issues of strict vegetarian diets; developing food-process technologies and food products for CELSS diets; devising methods for hydrolyzing nonedible plant residues into metabolizable substrates for intermediate organisms in the food chain or which can be consumed directly by humans; modeling various CELSS subsystems and incorporating data from NSCORT and other laboratories into working dynamic models of CELSS.

A second mission of the NSCORT in Bioregenerative Life Support is to train undergraduate, graduate, and postdoctoral students who will help develop the first CELSS deployed in space. Thus, one of the first benefits of Center operations will be a cadre of cross-trained space life scientists and engineers with interest and expertise in Bioregenerative Life-Support Systems. This growing pool of human talent ultimately will contribute at least as much to Earth benefits of CELSS research as to the creation of a space-deployed CELSS *per se*.

Table 1. Research Projects and Investigators in the Purdue NSCORT, 1994

Topic	Investigator	Department
Higher Plant Biomass Productivity/Yield	Cary A. Mitchell	Horticulture
Cyanobacteria Productivity/ N ₂ Fixation	Louis A. Sherman	Biological Sciences
Genetic Improvement of Rice Protein/Lignin Reduction	Thomas K. Hodges	Botany & Plant Pathology
Genetic Improvement of Cowpea Protein	Paul M. Hasegawa and Ray A. Bressan	Horticulture
Composition of Edible and Non-edible Biomass	S. Suzanne Nielsen	Food Science
Food Products and Technologies for CELSS	Philip E. Nelson	Food Science
Bioavailability Issues in Vegetarian Diets	Paula R. Trumbo	Foods & Nutrition
Enzymatic/Microbial Degradation of Plant Cell Wall Wastes	Michael R. Ladisch	Agricultural Engineering/ LORRE
Systems Integration and Modeling of CELSS	David M. Auslander	Mechanical Engineering UC Berkeley

PRODUCTIVITY OF CROP PLANTS

Research in the topic area of higher plant biomass productivity involves optimizing yield for closed canopies of food crops grown in controlled environment chambers (Table 2). The approach typically involves hydroponic culture of crops under carbon dioxide enrichment and elevated irradiance with photosynthetically active radiation. Prior to NASA's CELSS program, there were few sources of funding for research to optimize growth environments for food crops. Controlled environment agriculture (CEA) is a fledgling growth industry limited from realizing profitability by costs of power and labor, and because automation is not well developed for planting and harvest /4/. One presently feasible approach is to minimize the total energy needed to produce a given crop, by identifying time periods during the cropping cycle when use of certain costly resources could be minimized /5/. Combining dynamic optimization of environmental factors by computers with "phasic control" of crop growth could provide only the minimum amounts of light, heat or cooling, CO₂, and nutrients required for a positive growth response at each stage of crop development. Net photosynthetic rate is an obvious parameter that could be automatically optimized by computer-assisted control of environmental variables. Large controlled environment rooms could be used to provide environmental

Table 2. Earth Benefits of Biomass Productivity Research

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- Dynamic optimization of crop yield for CEA
 - Active control of light/CO₂/temperature for maximizing real-time photosynthetic rate
 - Identify factors needed for phasic control of crop growth
 - Develop direct-seed/non-transplant hydroponic methods for small-seeded species
 - Automating crop culture systems
 - planting
 - spacing
 - within-canopy lighting
 - environmental monitoring/control
 - harvest
 - New crop identification/selection/breeding/engineering
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selection pressure when screening for or against genetic variants generated by plant breeding or genetic engineering. Selections based upon crop performance in controlled environments may not reflect performance of those genotypes in the field.

During NSCORT research to avoid the handling and transplant shock associated with establishing dense stands of cereal and oilseed crops, direct-seed/non-transplant hydroponic methods were developed for small-seeded plant species /6/. Such methods could be scaled up and perfected for commercial crop production systems in controlled environments.

Research to automate crop production sub-systems for CELSS should minimize manual labor for time-consuming cultural procedures (e.g., planting and harvest), maximize interception of photosynthetically active radiation by plants at all stages of crop production (e.g., variable spacing), explore new techniques for efficient use of expensive resources (e.g., develop within-canopy lighting systems for planophile foliar canopies), and develop stable, remote sensors to monitor the status of important environmental and cultural factors (e.g., specific ions, microbial contaminants, gaseous emissions).

Each advance in technology or protocol developed for the CELSS program will immediately benefit the developing CEA industry and will help bring it a step closer to profitability.

CYANOBACTERIA

Cyanobacteria (blue-green algae) are under investigation by the Purdue NSCORT to provide a photosynthetic backup to higher plants in cases of emergency (Table 3). Blue-green algae fix atmospheric nitrogen, which may prove to be essential in a CELSS. Mass culture of cyanobacteria in laboratory vats produces edible biomass to supplement the diets of growing rats in nutritional studies. These studies indicate that *Cyanothece* spp. are nontoxic and nutritionally beneficial. Potential exists for mixed hydroponic culture of cyanobacteria with higher plant food crops, such as already occurs naturally in paddy culture of rice. Algal biomass might be used as a soil conditioner in agriculture because its extracellular polysaccharide immobilizes water, thereby preventing water loss from soil and possibly improving crop yield. The hygroscopic nature of this polysaccharide matrix also may

Table 3. Earth Benefits of Cyanobacteria Research

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- Better understanding of the use of cyanobacterial N₂ fixation for fertilization of crops, such as rice. The extracellular polysaccharide (EPS) immobilizes water, protects the soil and increases harvests
 - Use of cyanobacteria for revitalizing arid or desert areas
 - Inexpensive source of protein (supplemental) in agriculturally impoverished areas
 - Extracellular polysaccharide (EPS) may have applications in food processing
 - Cyanothece may be a good model system for the analysis of circadian rhythms in higher organisms, including humans
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have useful food-processing applications. Individual cells of these microalgae are extremely rich in protein and could be used as a dietary supplement in agriculturally impoverished areas of the world. The cyanobacterium *Cyanothece* sp. strain ATCC51142 under investigation at Purdue also may be an excellent model system with which to advance the study of circadian rhythms in higher organisms.

MOLECULAR BIOLOGY

The NSCORT molecular biology group is using recombinant DNA to improve the protein nutritional value of cereal and legume crops (Table 4). In one approach, rice protoplasts are electroporated with a storage protein gene, glutelin, that has been mutated to contain more residues of lysine, tryptophan, and histidine. Transformed rice protoplasts are selected in the presence of antibiotics or herbicides and then multiplied in liquid suspension cultures from which sterile plantlets can be differentiated and grown to maturity in a growth chamber. The presence of the modified glutelin gene is determined by its unique sensitivity to specific nuclease enzymes. Seeds will be analyzed for the presence of higher levels of the essential amino acids listed above. The long-term goal of this project is to enhance the content of the amino acids lysine, tryptophan, and histidine in the storage protein of rice seeds. Since rice is a staple crop for more than 40% of the world's population /7/, this accomplishment will indeed be an important spinoff.

Table 4. Earth Benefits of Molecular Biology Research

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- Enhance cereal protein quality with lysine and tryptophan
 - Enhance legume protein quality with sulfur-containing amino acids
 - Extend crop harvest index by expressing seed storage protein in leaves/stems/roots
 - Enhance forage value of crops by blocking lignin biosynthesis with anti-sense RNA
 - Enhance seed size/number/sink strength
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A different approach using the biolistic gun to "shoot" DNA-coated particles into meristematic regions of cowpea shoots or morphogenically competent cells is being used in effort to ultimately transform the seed storage protein of this legume into one with higher content of sulfur-containing amino acids. Cowpea is an important food crop to many people in Africa and Asia /8/.

Other genetic engineering goals on the horizon include extending the harvest index of crops by using cell/tissue specific gene promoters other than those that express in seeds only; using anti-sense or co-suppression technologies /9/ to reduce the lignin content of crops grown in controlled environments, thereby reducing a major waste-producing component of crop residue. Ultimately, recombinant DNA will be used to enhance sink strength of reproductive tissues, leading to more or larger seeds per unit growth area and per unit plant weight, thereby increasing yield rate and harvest index. Each biotechnology advance will have immediate spinoff value for terrestrial agriculture.

FOOD SCIENCE AND NUTRITION

Earth benefits of research in the areas of compositional analysis, food processing, and human nutrition are summarized in Table 5. In the compositional analysis area, we find that crops grown in controlled environments may have tissue composition very different from that of field-grown crops, and that it is possible to manipulate that composition environmentally /10, 11, 12/. NSCORT food technologists have created a pasta with the right mix of wheat flour and cowpea meal to create an amino acid balance similar to that found in animal protein. Nutritionists are using animal models in feeding studies to detect bioavailability problems of vegetarian diets, and to determine how much unprocessed cyanobacteria can be mixed directly into the diet. The traditional food guide pyramid for balanced nutrition already lists the majority of needed foods supplied by plant sources: cereals make up the base of the pyramid, and vegetables and fruits build upon it. Dairy and meat products, fats, and sweets ideally should occupy only the tip of the food guide pyramid. In a CELSS, dairy and meat products will disappear from the food guide; vegetable oils would substitute for animal fat, and it would be possible to grow only a few small fruits. Fruits would not be needed for nutritional purposes, but would be desirable for variety and for psychological augmentation of the diet. Both environmental manipulation as well as special food-process techniques will be developed to reduce or eliminate anti-nutrients or toxins that may result from hydroponic culture in modified controlled environments.

Table 5. Earth Benefits of Food Science/Nutrition Research

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- Environmental control of nutrient/anti-nutrient/toxin content of edible biomass
 - Food-process procedures to remove/destroy anti-nutrients from biomass
 - Develop food-process technologies without use of polluting chemicals/solvents
 - Develop no-cholesterol vegetarian cheeses/tofus from fiber-hydrolyzed oilseed presscakes
 - Develop non-dairy yogurt/ice cream with cellulose hydrolysates
 - Develop cereal/legume pasta with nutritionally balanced protein in the noodle
 - Develop novel, new vegetarian diets/dishes
 - Extend crop harvest index by processing traditionally non-consumed biomass into safe, edible food products
 - Improve bioavailability of micronutrients in vegetarian diets by food processing
 - Supplementation of vegetarian diets with cyanobacteria-derived foods
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The food process and technology development area is one area of NSCORT research with many possibilities for ground application. Due to the sealed environments within space habitats, it will not be desirable to use non-recyclable chemicals or volatile solvents for extraction of food components, as often is done on Earth /13/. Oil from seeds may have to be expressed rather than extracted. The resulting proteinaceous press cakes might be acidified and fermented microbially to create vegetarian cheeses or tofu-like products. Parts of crops that are not traditionally eaten also might be processed for food, thereby extending the harvest index of the crop and result in less biomass to be degraded as waste. Sugars recovered from hydrolysis of plant polymers such as cellulose might be used to create a variety of specialty food products, including non-dairy yogurt or "ice cream". Potential markets for novel, engineered vegetarian food products include third-world countries where meat is scarce, vegetarian and health-food markets, and quite possibly the fast-food industry, from which the consuming public is beginning to demand low cholesterol, low sodium, and low saturated fat, and which tends to be receptive to novel ideas.

In the human nutrition area, development of novel vegetarian diets for CELSS will have direct spinoff value for diets on Earth. In developed countries, the proportion of plant material in diets is increasing, as is vegetarianism *per se*. Research with laboratory animals indicates that nutrient status is improved by incorporating as many different plant species into the diet as possible /14/. The bioavailability of mineral nutrients such as calcium, zinc, and iron appears to be limiting in a strict vegetarian diet, and we are destined to revisit this issue on Earth as well as in space. For CELSS, nutritionists also are interested in the content and availability of macro- and micro-nutrients from plant materials that traditionally are not eaten, such as roots, stems, and leaves. Revealing the composition of this material, including potential toxins and antinutrients, will have important implications for extending crop harvest index on Earth as well as in space. The successful incorporation of small amounts of novel cyanobacteria species into the diet of experimental laboratory animals also has potentially important ramifications for the mass feeding of humans on a global scale. Nutritive algae such as *Spirulina* already are eaten in many cultures for protein or as a health-food supplement /15/. The extracellular polysaccharide produced by this cyanobacteria strain is potentially useful in many industries, including shipping, food processing, and biotechnology. Owing to the high protein content and potential productivity of micro-algae, their spinoff value from the space program is promising.

WASTE MANAGEMENT

The waste-management engineers in our NSCORT have been developing both enzymatic /16/ and microbial /17/ methods for the degradation of cellulose and lignin to the level of metabolizable substrates or digestible foods (Table 6). Extracellular cellulases from the fungus *Trichoderma* convert cellulose to glucose; hemicellulases hydrolyze hemicellulose to hexoses and pentoses; mycelial mats of the fungus *Pleurotus* degrade lignin, which is a 3-dimensional polymer that is closely associated with hemicellulose and cellulose in stems and leaves. Such fungal cultures convert the nonedible plant polymers into edible fruiting bodies we know as oyster mushrooms.

One obvious spinoff value of developing these technologies includes the bioprocessing of yard waste, which has become a major component of landfills. Environmentally friendly, water-based methods for breaking apart the recalcitrant lignocellulose complex of plant waste are being developed that do not require volatile organic solvents, strong acid, or caustic alkali. These methods also could be of interest in pulp and paper production plants. The sugars produced by hydrolysis of cellulose and hemicellulose could be used as growth substrates for yeasts, mushrooms, and higher plant cells, all of which are candidate foods for human consumption in a CELSS. Furthermore, the press cakes remaining from expression of canola oil are high in protein with an excellent amino acid profile, but have too high a fiber content to be fed to monogastric animals. Reducing the fiber content by enzymatic or microbial action would add value to the meal of oilseed crops and possibly prove beneficial in formulating feeds for use in the poultry and swine production industries. Bioreactors will

Table 6. Earth Benefits of Waste Management Research

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- Develop non-polluting, inexpensive methods for degrading organic wastes (e.g., yard waste)
 - Produce metabolizable substrates from bioprocessing of plant polymer wastes
 - Produce edible mushrooms using inedible plant residues as growth substrates
 - Develop environmentally friendly methods for waste degradation based upon naturally occurring microbial and enzymatic biocatalysts
 - Improve digestibility of forages used as animal feeds by further bioprocess treatments
 - Develop bioreactors to enhance efficiency and speed of converting renewable resources to intermediate products
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be developed to enhance the efficiency and speed of processing plant polymer wastes for CELSS, and this advance will make scale-up and commercialization on Earth even more attractive.

SYSTEMS INTEGRATION

The NSCORT systems integration group incorporates CELSS subsystem data, estimates inputs and outputs, and determines bottlenecks to the overall process (Table 7). Since CELSS is a microcosm of Earth in some ways, predictive models could emerge from this work to help estimate impacts of pollution, etc. Also, unique strategies likely will be developed that will have direct application for the control of future man-made systems of complex nature, insights into weather forecasting, etc. Maturing models may be useful in helping to develop culture practices that will help approach a sustainable agriculture.

Table 7. Earth Benefits of Systems Integration Research

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- Develop predictive models for gauging global impact of insults to the environment
 - Develop control system strategy for complex systems with chaotic behavior
 - Predict sustainability potential of agro-ecosystems
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Core research within the NSCORT for Bioregenerative Life Support is sponsored by NASA grant NAGW-2329. The Center additionally has initiated a development mission to supplement mainline CELSS research with additional Earth benefit projects on topic areas identified in this article to be sponsored by foundations and corporations.

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