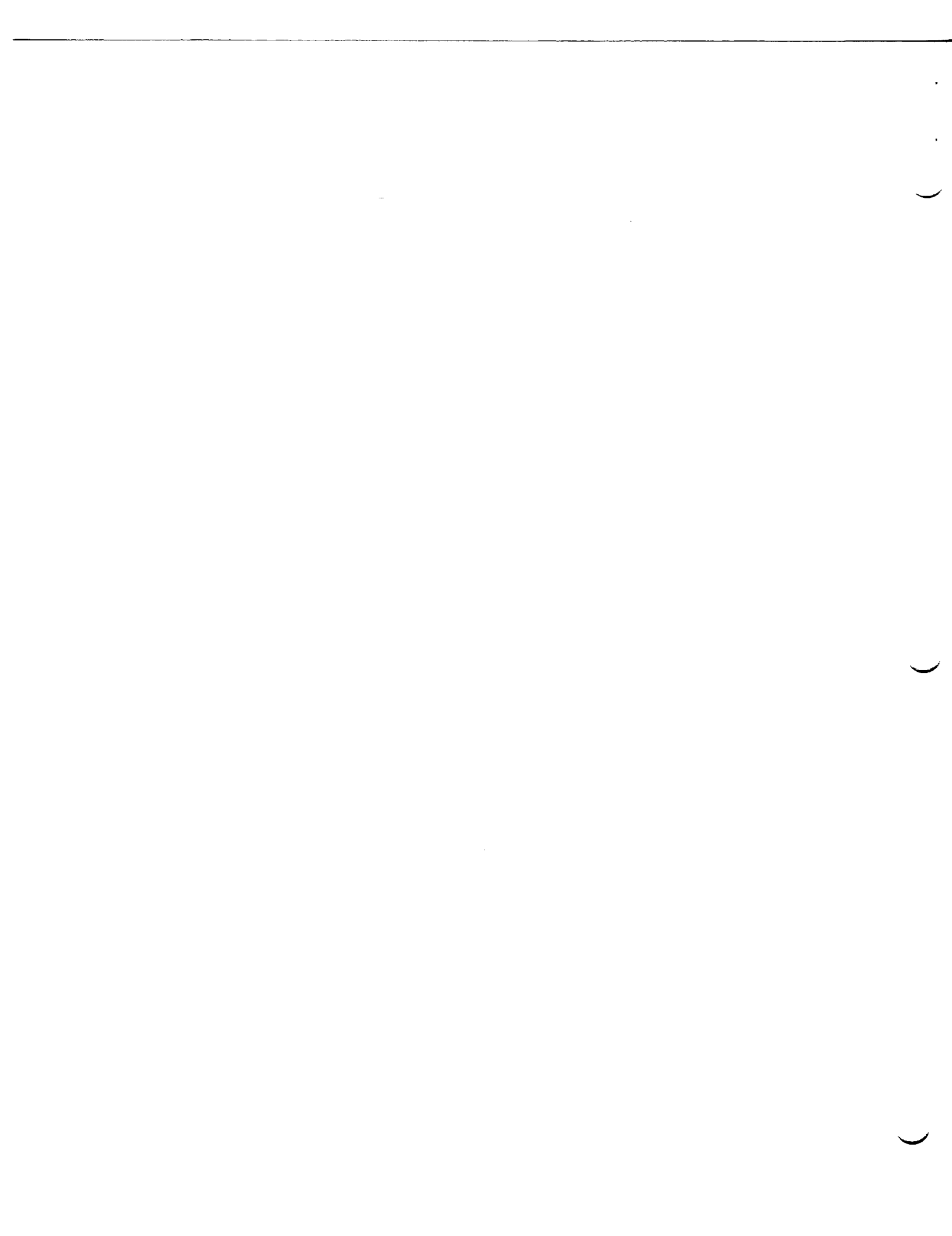


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# Use of Bioregenerative Technologies for Advanced Life Support: Some Considerations for BIO-Plex and Related Testbeds

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**USE OF BIOREGENERATIVE TECHNOLOGIES FOR  
ADVANCED LIFE SUPPORT: SOME CONSIDERATIONS  
FOR BIO-PLEX AND RELATED TESTBEDS**

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## BACKGROUND

The concept of using biological systems for life support in space has been studied since the 1950s, and most likely discussed long before this (Myers, 1954; Krall and Kok, 1960; Golueke and Oswald, 1964). Early studies centered on the use of algae (e.g., *Chlorella*) for photosynthetic atmospheric regeneration (Krall and Kok, 1960, Golueke and Oswald, 1964; Eley and Myers, 1964). Testing was expanded in the late 1960s and 1970s by Russian researchers to also include higher plants (Gitelson et al., 1988). In the late 1970s, NASA created the Controlled Ecological Life Support System (CELSS) Program to continue research on bioregenerative life support, with much of the effort focused on controlled environment production of higher plant species (MacElroy and Brecht, 1985). These studies were conducted at several universities and NASA field centers throughout the 1980s and early 1990s, after which the program was merged with physical / chemical life support research efforts under the Advanced Life Support Program (Averner, 1993).

In the fall of 1995, various members of the Advanced Life Support (ALS) community from NASA centers met at Johnson Space Center (JSC) to develop research tasks for ALS, including bioregenerative technologies for food production and resource recovery. This was followed by a series of teleconferences between NASA's Ames Research Center (ARC), JSC, and Kennedy Space Center (KSC), with the resulting task lists published as a preliminary program plan (Henninger, 1996). In September of 1996, a follow-up meeting of ALS food/plant research groups from different NASA centers and universities was held at JSC to provide up-dates on the past year's activities and to identify issues for development of the fully integrated, life support system test facility, BIO-Plex (Bioregenerative Life Support System Complex). In October of 1996, a Memorandum of Understanding was signed between NASA's Johnson Space Center and Kennedy Space Center designating KSC with the responsibility to conduct and coordinate bioregenerative research in support of the ALS Program. A second meeting was held at JSC in October 1996 to review a draft of program-wide requirements for ALS. Plant researchers from the ALS community met most recently at Kennedy Space Center to discuss plant and food production issues specifically for the BIO-Plex project. (Note: A preprint of this document was distributed to the participants of that meeting and the minutes from the meeting are attached as Appendix A).

The following document provides an overview of some issues facing bioregenerative research and development for ALS, along with suggestions for the design and operation of plant production and resource recovery subsystems for BIO-Plex and related ground-based testbeds. Concerns related to development of habitats for space missions (e.g., Lunar, Mars transit, and Martian scenarios) will not be addressed in this document but clearly must be considered for ALS planning.

## ISSUES

Issues for bioregenerative research and development for BIO-Plex might be categorized into following general areas: 1) crop selection; 2) environmental management and horticulture, including lighting/energy and nutrient delivery concerns; 3) incorporation of biological resource recovery concepts,

and 4) microbiological and chemical characterization of bioregenerative systems. The following discussion offers a status of these areas along with recommendations to address each. The comments and positions presented are based largely on the bioregenerative research and testing from the KSC Breadboard Project (Appendix B) and fundamental findings from university investigators.

### ***Crop Selection***

Several reviews of crops that might be considered for ALS have been published (Tibbitts and Alford, 1982; Hoff et al., 1982; Salisbury, 1991; Langhans et al., 1995; Salisbury and Clark, 1996; Mitchell et al., 1996). Criteria considered in generating these crop lists included: crop yield, nutritional value, harvest index, horticultural requirements, and processing requirements (Tibbitts and Alford, 1982; Hoff et al., 1982). An initial list of crops was suggested for early testing in BIO-Plex as a result of the meetings of 1995 and 1996; this included: wheat, potato, rice, peanut, soybean, and salad crops (e.g., lettuce and tomato). An updated list was recently (12-12-96) distributed from the ALS program management office at JSC based on the recommendations of Hoff et al. (1982) and Tibbitts and Alford (1982) and included: wheat, potato, soybean, rice, peanut, carrot, chard, cabbage, lettuce, and tomato.

Wheat has been studied in numerous growth chamber experiments for ALS (Guerra et al., 1985; Goyal and Huffaker, 1986; Bugbee and Salisbury, 1988; Gerbaud et al., 1988; Volk and Bugbee, 1991; Barnes and Bugbee, 1992; Wheeler et al., 1993a), as have potato (Wheeler et al., 1986; Wheeler et al., 1990; Tibbitts et al., 1994), soybean (Tolley-Henry and Raper, 1986; Vessey et al., 1990; Raper et al., 1991; Wheeler et al., 1993b), lettuce (Knight and Mitchell, 1983, 1988a, 1988b; Wheeler et al., 1994), and sweetpotato (Hill et al., 1989, 1992; Mortley et al., 1993). Some other crops studied for ALS include: tomato (McAvoy et al., 1988; Janes, 1994), cowpea (Ohler and Mitchell, 1996), rice (Bugbee et al., 1994; Volk and Mitchell, 1995), radish (Mackowiak et al., 1994), *Brassica* (Frick et al., 1993), peanut (Mackowiak et al., 1997; Mortley et al., 1997), spinach (Both et al., 1996), strawberry (Stutte, per. com.), and quinoa (Schlick and Bubenheim, 1993). In addition to growth chamber studies, large scale (20 m<sup>2</sup>), closed environment studies with wheat, potato, soybean, lettuce, and tomato have been conducted in NASA's Biomass Production Chamber at KSC (Wheeler et al., 1996).

Obviously the list of crops for ALS could be expanded, particularly for future ALS applications (e.g., Mars colonies). But the first BIO-Plex test will attempt to provide only 40% of the food; thus, it is perhaps prudent to keep the list limited for BIO-Plex (e.g., 5 to 10 spp.) to assure timely acquisition of needed data. The following discussion will focus on the most recent crop list developed for BIO-Plex.

### ***Environmental Management***

Each crop species has optimal environmental conditions for producing high yields and acceptable canopy height. Among the important environmental conditions are photosynthetic photon flux (PPF), photoperiod, spectral quality, CO<sub>2</sub>, temperature, humidity, and mineral nutrition. With the assistance of physical/chemical environmental control systems, near optimal levels of CO<sub>2</sub> and humidity

should be achievable throughout the plant production module for most species, e.g., 1000-1500 ppm for CO<sub>2</sub> and 60-80% RH. But if CO<sub>2</sub> levels cannot be controlled rigorously, than studies on the effects of sub- and superoptimal CO<sub>2</sub> should be considered for BIO-Plex (Wheeler et al., 1993; Bugbee et al., 1994; Mackowiak and Wheeler, 1996). In contrast to gas partial pressures, photoperiod and temperature will likely require some partitioning if multiple species are to be grown in the same module (see below).

Photoperiod. With the possible exceptions of wheat and lettuce, long photoperiods are not optimal for most of the crops discussed for BIO-Plex. A 12-h photoperiod has worked well for many of the suggested species (Wheeler et al., 1996) and would allow alternative switching between growing areas to maintain uniform electrical power draw with relatively constant rates of O<sub>2</sub> production and CO<sub>2</sub> removal. Dim daylength extensions of 5 μmol m<sup>-2</sup> s<sup>-1</sup> PPF effectively block tuber development potatoes (Wheeler and Tibbitts, 1986), and recent BPC tests have demonstrated that light leakage even as low as 0.2 μmol m<sup>-2</sup> s<sup>-1</sup> during a “dark” period can provide an undesirable long day stimulus (Yorio, unpub.); thus if different photoperiods are used in BIO-Plex, areas should be partitioned to prevent light leakage.

Several photoperiod management scenarios can be envisioned:

1) The entire chamber is synchronized on one photoperiod, which would avoid the need for light partitions. If the chamber were maintained under a long photoperiod (e.g. 24-h), this would be acceptable for wheat but not for short-day crops (e.g., soybean), and potentially be deleterious for some tomato and potato cvs. (Hillman, 1956; Wheeler and Tibbitts, 1986). On the other hand, if short photoperiods are used, this would not utilize wheat to its maximum advantage (Bugbee and Salisbury, 1988; Bugbee 1992). Moreover, anything other than continuous light would unevenly load the electrical power and the CO<sub>2</sub> and O<sub>2</sub> control systems.

2) The chamber could be divided into two sections, with the lamps alternately illuminated (12-h cycles). This would require a light partition but electrical power draw and gas exchange could be maintained at relatively constant rates. This would accommodate most species but limit wheat's productive potential (i.e., g m<sup>-2</sup> day<sup>-1</sup>) by limiting daily total PPF levels.

3) The chamber could be divided into three (or more) parts; one section could be maintained under continuous light, while the other two are alternately illuminated to provide two short (12-h) photoperiod zones. This would require at least two light partitions but it could fully utilize wheat's productive potential and allow uniform power draw. In addition, the partition between the short photoperiod zones may facilitate temperature zones (see below). Another variation of a 3-partitioned environment might be the use of overlapping 16-h photoperiods with two sections illuminated at any one time, but this may compromise the use of more obligate short-day species or cultivars (Salisbury, 1981).

PPF. Grasses such as wheat and rice, with their highly inclined leaves, are able to distribute light over a large leaf area; hence intensive lamping for high PPF (1500 μmol m<sup>-2</sup> s<sup>-1</sup>) should be beneficial for grasses. On the other hand, the broad leaf crops (e.g., potato, sweetpotato, and soybean) cannot distribute high irradiance throughout the canopy as well as grasses, so productivities and photosynthetic rates saturate at lower levels (Stutte et al., 1995). On the basis of BPC studies and recent studies with

soybean at Utah State (Dougher and Bugbee, 1997), PPF levels of 750 to 1000  $\mu\text{mol m}^{-2} \text{s}^{-1}$  should be sufficient for most broad-leaved species, particularly in terms of radiation conversion efficiency (Norman and Arkebauer, 1991). An additional consideration is that some species are susceptible to physiological disorders at higher PPFs, such as leaf tipburn of lettuce (Collier and Tibbitts, 1981; Barta and Tibbitts, 1991) and leaf chlorosis from HPS lamps (Barker et al., 1989).

If power requirements for BIO-Plex must be minimized, more efficient lighting scenarios should be considered, including the use of sub-saturating PPF levels. Lamp output efficiency might be increased by the use of vertical (base-up) and/or high-powered lamps, e.g., 1000-W lamps, which are more efficient than 400-W lamps (Sylvania, 1980). However, such modifications will require more vertical room in the chamber for a given growing area, thereby reducing the area of crops that can be supported. Innovative lighting technologies such as light-emitting diodes (Bula et al., 1991), microwave lamps (MacLennan et al., 1994), or lamps mounted remotely with light delivered via conduits or optical fibers might be considered (Mori et al., 1987). In the latter case, the light might even be distributed to the sides and/or within the canopies (Tibbitts et al., 1993). However, little plant research has been conducted with such systems and their use would involve a degree of risk. Use of available sunlight might also be considered, both to reduce power requirements and heat rejection; however, this would require light capture and tracking systems, as well as conduit and diffusing technologies. This approach would also require some supplemental electrical lighting used to maintain desired photoperiods and compensate for ambient weather conditions.

Temperature. Rice, peanut, soybean, and sweetpotato tend to prefer warmer temperatures (e.g. 26/22 C light/dark) (Thomas and Raper, 1978; Hill et al., 1992), whereas potato prefers cooler temperatures (20/16 C) (Ewing, 1978; Wheeler et al., 1986). Wheat yields are high at cool temperatures (< 20 C), but cooler temperatures promote taller shoot growth and prolong the life cycle (Bugbee, 1992; Bugbee and Koerner, 1997). Lettuce and tomato do well at intermediate temperatures, e.g. 23 C (Hicklenton and Wolynetz, 1987; Janes, 1994). Cabbage, chard, and carrot production from the field is typically best at cooler temperatures (e.g. 16 to 18 C) (Yamaguchi, 1983), but this would need verification in controlled environment studies.

Based on BPC and Early Human-Rated Test (EHT) experiments (D. Barta, personal communication), areas for high temperatures should be avoided in BIO-Plex, since high temperatures can have adverse effects on pollination (e.g. wheat and tomato) and promote vegetative growth (e.g. potato). At a minimum, a partitioned warm and cool zone in the short photoperiod area would seem advisable to accommodate a wider range of crops.

General comments. A crop production protocol or "handbook" is needed to define environmental set-points and acceptable ranges for the different species considered for BIO-Plex and future ALS applications. Such a handbook could be developed from a controlled environment crop production database currently being compiled by Frank Salisbury and Mary Ann Clark (Utah State). Among the issues covered in the protocol should be suggestions on cultivar selection (e.g., need for dwarf cvs.) ,



use of environmental response surface approaches to define acceptable ranges for crop growth, guidelines for testing with recirculating nutrient cultures, and considerations for sustained crop production and potential system failures.

### **Horticulture**

Lettuce. Lettuce is amenable to variety of hydroponic methods (Davis, 1985) and was perhaps the easiest crop to harvest in the BPC studies (Wheeler et al., 1994). Seed germination and seedling establishment were critical phases in BPC studies using an NFT approach with a wicking support. Germination covers and daily (manual) misting were used to maintain high humidity around the seedlings for the first few days. A fixed spacing was used in BPC studies but a seedling nursery might be considered for BIO-Plex to maintain seedlings for about 10 to 15 days prior to transplanting to a final spacing (Wheeler et al., 1994). Alternatively, variable spacing systems might be used to save system volume (Prince and Bartok, 1978; Knox, 1986). Lettuce is susceptible to leaf tipburn when plants are grown rapidly through a heading stage (Collier and Tibbitts, 1981; Barta and Tibbitts, 1991). Mild tipburn was observed in BPC studies with 'Waldmann's Green' lettuce grown 1000 ppm CO<sub>2</sub> and 300  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPF with a 16-h photoperiod ( $\sim 17 \text{ mol m}^{-2} \text{d}^{-1}$ ); thus high PPF is probably not needed for lettuce in BIO-Plex. If lettuce is not grown to heading, higher PPF might be acceptable (Knight and Mitchell, 1988b).

Wheat. Wheat was arguably the most labor intensive crop to grow in the BPC studies (Wheeler et al., 1993, 1996). Planting and harvesting were done manually, and mechanization of these procedures should be considered. In addition, harvesting and threshing create a lot of dust and debris. As with lettuce, seedling establishment with a wicking system was a critical phase, and germination covers and daily misting were used for the first few days to maintain high humidity around the seedlings. Wheat stands in controlled environments are susceptible to lodging, and canopy supports should be considered. For BPC studies, wire grids were positioned about 30 cm above and parallel to the tray surface to support the stand. However, use of any canopy support systems will affect harvesting and materials handling and this should be considered in BIO-Plex planning. Wheat produced substantial amounts of ethylene gas during rapid vegetative growth (Wheeler et al., 1996b; Barta et al., personal communication) and this biogenic ethylene can adversely affect seed set (Rowell and Miller, 1971); thus, effective ethylene removal systems will be required for acceptable wheat yields. The recent release of the cv. Apogee represents the most extensive effort to date in developing a crop cultivar specifically for the use in ALS (Bugbee and Koerner, 1997), and recent tests in the BPC have shown that Apogee significantly out-yields cv. Yecora Rojo.

Soybean. Soybeans have grown well in the recirculating hydroponic systems of the BPC, and recent tests with cv. Hoyt soybean have shown it to be an excellent dwarf cultivar for ALS applications, with a harvest index of nearly 40% (Dougher and Bugbee, 1997; Mackowiak, unpublished). Shoots of cv. Hoyt plants in BPC studies typically stopped growing at 40 cm and no canopy support system was

required. However, taller, less determinate cvs., such as McCall, will require canopy support systems similar to wheat (Wheeler et al., 1993, 1996a). Soybean leaves can become chlorotic under high PPF from HPS lamps (KSC and Utah State findings, unpublished); despite this, photosynthetic rates and productivities seem to remain high. In BPC studies, lower canopy leaves of soybean tended to abscise during maturation and periodic removal of leaf litter was useful to provide good air circulation and tray surface reflection. As with wheat, harvesting of soybean was labor intensive and dusty, and mechanization of harvesting and threshing should be tested for BIO-Plex.

Potato. Potatoes have produced the highest yields of any crop grown in the BPC (Wheeler et al., 1996). Harvest indices can reach 70 to 80% when tuber induction is strong. Potatoes are amenable to NFT culture, but tuberization can be suppressed when stolons are submerged in solution cultures (Wheeler et al., 1990; Tibbitts et al., 1994). The use of NFT greatly simplifies harvesting in comparison to using solid media. Potato studies at the University of Wisconsin and KSC have used in-vitro-grown plantlets for starting materials and this would require a tissue-culture support system for propagation. Alternative methods for propagating potatoes (e.g., use of mini tubers) should be explored to eliminate the need for in vitro plants for BIO-Plex. Recent studies at KSC have shown that using the same recirculated nutrient solution for successive plantings causes a premature tuber initiation (Wheeler et al., 1995; Stutte and Sager, 1995). This results in stunted shoot growth, which can reduce yields if the stand ground cover is incomplete. The effect is likely caused by a growth regulating factor(s) that builds up in the solution. This factor can be removed by passing the water through a charcoal filter, but management of recirculating systems with potatoes will require further testing to develop strategies for consistent yields. As with lettuce and soybean, a single transplant management scheme should be considered to optimize space utilization.

Tomato. BPC tests with the tomato cv. Reimann Philippe have produced high yields without assisted pollination, and harvest indices ranged from 45 to 55%. Shoots of the tomatoes tended to sprawl but growth was determinate with the heavy fruit set. The fruit-laden stems caused the canopy to collapse, and use of shoot supports may be beneficial. Tomato plants for BPC studies were started from seed at the final spacing, but use of a nursery system and transplanting should be considered for BIO-Plex to optimize space utilization. Harvesting was carried out manually, and use of mechanization for harvesting would seem to have little advantage unless large areas were allocated to tomato plantings. Comparisons of Reimann Philippe with other cvs. studied for ALS (Janes, 1994) should be conducted.

Peanut. Controlled environment production studies with peanut have been limited. Studies at KSC have demonstrated that peanut can be grown successfully in recirculating NFT (Mackowiak et al., 1997a), but yields were similar to field yields and harvest index was low (~20%). Shoot growth can be excessive in hydroponic culture with continuous EC and pH control, suggesting that control of nutrient levels may be important to control shoot growth and harvest index (see sweetpotato comments below). Peanut seems particularly important to ALS diet planning because of its high oil and protein content

(Mitchell et al., 1996) and baseline horticultural tests and cultivar selections are currently underway at Tuskegee University (Mortley et al., 1997).

Rice. Some controlled environment testing has been conducted with rice at Purdue and Utah State for the ALS program, and the cultivar Ai-Nan-Tsao was shown to be a relatively short (~60 cm), high yielding variety (Bugbee et al., 1994; Volk and Mitchell, 1995). However, harvest index values for rice have been mediocre (30%) and dehulling of grain may present a challenge for harvesting / processing. Additional screening for dwarf cultivars should be considered and large scale production tests should be conducted to assess whole stand performance, management, and harvesting requirements.

Sweetpotato. Extensive studies on controlled environment production of sweetpotato have been conducted at Tuskegee University (Hill et al., 1989, 1992; Mortley et al., 1993). Results from these tests have produced good yields in NFT systems when nutrient levels were allowed to deplete prior to replenishment (e.g., weekly intervals). However, studies in which nutrient solution EC and pH were controlled continuously have produced extensive vegetative growth with reduced storage root yields (Mackowiak et al., unpublished; P. Loretan et al., unpublished). Collectively, the findings suggest that management of nutrient availability, particularly N and K, may be critical for sweetpotato storage root production, and studies of this nature should be pursued. High PPF ( $>800 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) from HPS lamps can cause chlorosis and bleaching of leaves of young sweetpotato plants, but these effects seem to be transient (Mackowiak, unpublished). Because of the vining nature and potential for large shoot growth, continued screening of sweetpotato selections for dwarf or determinate cultivars is needed.

Spinach. Spinach has been grown commercially in controlled environment settings (Davis, 1985) and is amenable to nutrient film technique systems of production (Resh, 1989; Both et al., 1996). Spinach is a long day plant for flowering (Yamaguchi, 1983), but can be grown successfully under long days if harvested prior to bolting. The edible leaves of spinach, as with lettuce, chard and beets, may provide a good means for recycling NaCl from system wastes back to the human diet (i.e., the sodium would accumulate in the edible tissues, which is not the case for seed and storage root / tuber crops) (Richards, 1969). Recent studies at Kennedy Space Center have shown that spinach can grow well in NFT systems with up to 50 mM NaCl in solution, but analyses are still underway to determine how much Na and Cl accumulate in the tissue (C.L. Mackowiak, per. com.).

Strawberry. Strawberries have been grown in controlled environment settings (Stutte and Darnell, 1987; Resh, 1989; Chow et al., 1992) and generally require short photoperiods and cool temperatures for flowering and fruit set (Durner and Poling, 1988). Initial results from growth chamber studies at KSC suggest that manual pollination of strawberry flowers enhances both fruit quality and total yield; thus methods for assisting pollination or increased crew time may be required for strawberry production. In addition, a consistent means of propagating the plants is needed for sustained fruit production in ALS.

*Cabbage, carrot, and chard.* We are not aware of any significant controlled environment efforts in producing these species. Cabbage is not photoperiod sensitive for leaf production and does best at cool temperatures, with growth being slowed substantially > 25 C (Hackett and Carolane, 1982; Yamaguchi, 1983). Chard and carrots are also cool temperature crops, with the optimal field temperatures for carrot averaging 16 to 18 C (Yamaguchi, 1983). At higher temperatures, carrot roots tend to be more strongly flavored (Yamaguchi, 1983). If cabbage, carrot, and chard are scheduled for use in BIO-Plex and future ALS efforts, baseline controlled environment studies and cultivar selection should be initiated as soon as possible. The taproot growth of carrot may present some horticultural challenges for the relatively shallow NFT trays used in some ALS studies, although there are several cultivars that produce short, more rounded roots. Chard, like its "chenopod" relative spinach, may be a good "sodium return" source for human diet (Richards, 1969). Cabbage might require adaptations to hydroponic culture trays because of the weight of large heads.

*General horticultural comments.* To date, tests in support of bioregenerative technologies for ALS have utilized a range of nutrient/water delivery concepts (e.g., solution culture--Bugbee and Salisbury; Raper et al., 1991; solid media--Tibbitts et al., 1993; Allen et al., 1995; NFT--Wheeler et al., 1990; Mackowiak et al., 1997), and a discussion of the physiological consequences of the different approaches can be found in Bugbee and Salisbury (1989). Prior to the final design of BIO-Plex, there is a need to determine whether a common nutrient delivery scheme can be used for all crops, or whether different approaches will be required for different crops. In addition, there is a need to develop effective seed / seedling support concepts that minimize or eliminate the need for wicking systems and their associated clean-up. Mechanizing certain planting and harvesting steps seems critical to minimize crew time requirements for species that require dense planting and substantial threshing / cleaning steps at harvest. BIO-Plex designs also should carefully consider storage and space requirements for horticultural activities, including: storage for chemicals, seeds, and biomass; nutrient solution mixing and testing; and a general planting, harvesting, and clean-up area.

### ***Crop Readiness Assessment***

Based on the available ALS literature and large-scale verification tests conducted at the KSC Breadboard Facility, the following readiness levels were assigned for some crops considered for ALS. Ratings refer to the technology readiness scale described in the ALS program plan (Henninger, 1996):

**Table 1.** Readiness levels of different plant species for BIO-Plex, where 3 = ready for testing; 2 = intermediate readiness; 1 = low readiness; 0 = significant lack of test data.

|         |     |             |     |            |     |         |     |
|---------|-----|-------------|-----|------------|-----|---------|-----|
| Lettuce | 3.0 | Tomato      | 2.0 | Cowpea     | 1.5 | Cabbage | 0.5 |
| Wheat   | 3.0 | Spinach     | 2.0 | Strawberry | 1.5 | Chard   | 0.5 |
| Soybean | 2.5 | Sweetpotato | 2.0 | Brassica   | 1.0 | Carrot  | 0.5 |
| Potato  | 2.5 | Rice        | 1.5 | Peanut     | 1.0 |         |     |

A readiness level of 0.0 indicates there is little or no knowledge of the crop in controlled environments; 1.0 indicates some controlled environment and / or horticultural testing have been conducted and results have been published; a level of 2.0 indicates that extensive testing has been conducted with published results, and that cultivar selection trials have been conducted; and a level of 3.0 indicates thorough laboratory-level testing and cultivar comparisons have been conducted, and that successful scale-up tests have been conducted in a closed system such as the Biomass Production Chamber. Although the ratings are somewhat subjective, they provide a starting point for assessing where research is needed to support upcoming BIO-Plex testing.

### ***Incorporation of Biological Resource Recovery***

*Resource recovery and waste processing.* Development of an optimal resource recovery approach for bioregenerative life support systems should depend on a cost/benefit analysis. Cost can be measured in terms of the system resource requirements (i.e., mass, volume, energy, heat rejection, and manpower; Drysdale, 1995), and benefits can be quantified by the percentage of nutrient content of waste material that can be recycled for plant growth, by the percentage reduction in noxious compounds and human pathogens, by the degree of stabilization of processed output for storage, and by reduction in logistics requirements. Nutrients include CO<sub>2</sub> produced from the oxidation of organic material and the inorganic minerals associated with the waste material.

Biological processors have been proposed as an integral component in a hybrid ALS resource recovery system (Finger and Strayer, 1994; Finger and Alazraki, 1995; Strayer and Cook, 1995). Biological processing of inedible plant biomass has been shown to be an efficient, reliable method for recycling up to 80% of inorganic nutrients contained in plant biomass, but relatively inefficient at degrading recalcitrant waste materials such as cellulose, hemicellulose, and lignin (30% at short retention times of 5 days, 80% at long retention times of 48 days).

Use of minerals recovered from inedible plant biomass would reduce the need for resupplying those nutrients from outside a life support system. Mackowiak et al. (1994a,b) have estimated that the mass of reagent-grade salts used in place of nutrient recycling could be equivalent to approximately 30% of the food requirement mass, although salts would also supply additional elements to the system, e.g., oxygen. Nutrient recovery has been shown to be a function of rapid, abiotic solubilization of inorganic minerals from inedible plant material when extracted with water (Garland and Mackowiak, 1990; Garland, 1992a; Garland, 1992b). However, plant growth was depressed when extracts were used directly, which may have been related to the high total organic carbon in the leachate (Garland and Mackowiak, 1990; Garland, 1991; Garland et al., 1993; Mackowiak, et al., 1996). A 48-h microbiological pretreatment eliminated most of the negative effects (Garland and Mackowiak, 1990; Garland, 1991; Garland et al., 1993; Mackowiak et al., 1996). This work led to the development of aerobic bioreactors to hasten the microbial degradation of TOC from plant residues (Finger and Strayer, 1994; Finger and Alazraki, 1995;

Strayer and Cook, 1995). Full-term, hydroponic production tests have been performed with lettuce (Mackowiak et al., 1994b), wheat (Garland et al., 1993), and potato (Mackowiak et al., 1996) using nutrients recovered from aerobic bioreactor effluents.

Various physical/chemical techniques, including wet oxidation (Jacquez, 1990; Modell, 1986), dry incineration (Dreschel et al., 1991; Bubenheim et al., 1993), and low temperature plasma reaction (Ness et al., 1992), have been proposed for waste oxidation in an ALS. These physical-chemical systems would have the benefit of rapid conversion of organic matter into CO<sub>2</sub>. However, significant difficulty and costs would be associated with operating the systems and with reconstituting the oxidized mineral residuals into forms suitable for incorporation into plant growth systems. For example, potassium is an easily extracted, water-soluble nutrient, but it produces low relative melting point ash that would require a reduction of incinerator operating temperature to prevent slagging. A lower operating temperature could cause incomplete oxidation, leading to contaminants in the incinerator exhaust gas, which would then require a second combustion stage. The presence of another easily extracted, water-soluble nutrient--nitrogen--in the waste can lead to incinerator production of noxious products such as NO<sub>x</sub> gases upon combustion.

These observations suggest that a hybrid, integrated physical-chemical /biological system could be a more efficient approach for waste processing in an advanced life support system such as BIO-Plex. Biological processing could allow for effective removal of soluble nutrients and phytotoxic soluble organics, after which physical/chemical processing could oxidize recalcitrant residuals. Prior removal of the water-soluble nutrients from the waste stream could enhance the performance of physical/chemical systems proposed for use in ALS, such as the fluidized bed incinerator [Under development (J.S. Lighty, Univ. of Utah)].

Because initial BIO-Plex tests will include some food stowage, considerations might also be given to incomplete conversion (oxidation) or stabilization of some waste biomass to achieve a better balance of carbon and oxygen entering and leaving the system (Wheeler, 1996).

Estimated quantities of ALS solid and liquid wastes. Several studies have attempted to estimate the weight and volume of wastes likely to be generated in a life support system. Crop residues would be a large portion of solid waste generated from a fully sustaining life support system. For example, based on a diet totally of wheat, potato, soybean and lettuce, inedible plant materials would be 640 g DW person<sup>-1</sup> day<sup>-1</sup> (Wheeler et. al 1996). Human solid wastes would be significantly lower (20 to 32 g DW person<sup>-1</sup> day<sup>-1</sup> ; Parker and Gallagher, 1988; Shubert et al., 1984; Diem and Lentner, 1970), even when combined with other waste solids dissolved or suspended in urine (ca. 59 g DW person<sup>-1</sup> day<sup>-1</sup> ; Parker and Gallagher, 1988) and graywater (ca. 11 g DW person<sup>-1</sup> day<sup>-1</sup> ; from Wydeven and Golub, 1990). Even at 90% closure, recycling of nutrients from crew feces could be ignored unless there is a need to demonstrate technology for processing this biohazardous waste. We recommend that for the initial mission, BIO-Plex not attempt to recover minerals from human solid waste. This approach minimizes

crew contact with those human pathogens associated with feces. Instead, feces could be collected and stored, removed, or incinerated without biological processing.

In addition to solid wastes, an integrated, hybrid waste treatment system will need to process urine and graywater in addition to the solids. Because these wastes are comprised mostly of water, the estimated weights and volumes of the urine and graywater are significantly greater than for the solids. Urine values range from 1.3 to 2.1 L (kg) person<sup>-1</sup> day<sup>-1</sup> and graywater (i.e., laundry water [54%], dish water [23%], and shower/hand-wash water [23%]) is estimated to be in the range of 25 L person<sup>-1</sup> day<sup>-1</sup> (Wydeven and Golub, 1990). Bioreactor technologies under consideration for inclusion in an ALS will require liquid/water inputs. It makes sense to use urine and graywater to supply bioreactors rather than relatively clean condensate from crop production water recovery systems or, worse, potable or de-ionized water. Use of crops that partition Na and Cl to edible structures (e.g., spinach leaves; Richards, 1969) should be explored to prevent NaCl build-up in the waste recycling streams. Alternatively, if the NaCl could be separated from the urine (or conversely the urea separated from the urine), nitrogen from the urine could be processed by the bioreactors and/or plants with little concern for salt build-up.

Background to three basic bioreactor types. Biological processes have long been used for treatment of municipal, agricultural, and industrial organic wastes (Tchobanoglous and Burton, 1991), with a primary aim of removing soluble organic carbon (Gaudy and Gaudy, 1972; Tchobanoglous and Burton, 1991). Biological treatment processes almost exclusively employ mixed microbial populations cultured under aerobic conditions. Three fundamentally different approaches to aerobic microbiological processing of wastes are: well-mixed, suspension culture (i.e., Continuous Stirred Tank Reactor--CSTR); composting with high particle/low moisture content (i.e., solid-state fermentation--SSF); and immobilized microbial biofilms attached to hardware surfaces (e.g., fixed-film bioreactors--FFB). All three bioreactor technologies have been considered for processing of ALS solid crop residues and human wastes. Relevant cost/benefit parameters (resources and recycling efficiency) still need to be determined for each technology to provide necessary information for the selection of components for a hybrid, integrated biological and physical/chemical system for BIO-Plex.

CSTRs utilize a suspension of microorganisms to degrade soluble and/or particulate organic compounds. Substrate addition is usually continuous, so maintenance of microbial metabolic activities is relatively constant with time. The stabilized particulate end product--sludge--consists of microbial cells, extracellular microbial matrix material, and undegraded crop residues and human waste solids. The design, fabrication, and successful operation of CSTRs have been underway at KSC for several years (Strayer, 1993; Finger and Strayer, 1994; Finger and Alazraki, 1995; and Strayer and Cook, 1995). These bioreactors ranged in size from 4 to 120 L working volumes and were used to conduct studies at both an intermediate-laboratory scale and at a larger, breadboard scale. The bioreactors were fully instrumented for computer monitoring and control, and were used to process inedible ALS crop residues to accomplish multiple objectives: 1) Biological decomposition of crop residue lignocellulose--Depending on crop type and retention time, fiber degradation ranged between 35 - 40% (short retention times) and

65 - 80 % (24 to 48 day retention times). The longer retention times are considered impractical for utilization in a mass-limited life support system; 2) Reduction of soluble organic compounds that were readily leached from crop residues and accounted for nearly 25% of crop residue carbon. Without biodegradation of these compounds, crop growth on the resulting solution was reduced. Over a wide range of bioreactor retention times (1.3 to 48 days), microbial decomposition contributed to a 75% reduction in soluble organic compounds; (3) Most important, we have shown that CSTR can be used to recycle inorganic nutrients from the crop residues back to hydroponic crop production systems. Over a wide range of retention times (1 to 48 days), upwards of 80% of the inorganic nutrient mass contained in crop residues was recovered in bioreactor effluent. These effluents were used successfully at both laboratory (3-month studies) and breadboard (418-day study) scales to replenish at least 50% of nutrients for crop hydroponic solutions (Garland et al., 1993; Mackowiak et al. 1996b).

SSFs for composting solid wastes have lower requirements for water, volume, and energy than CSTRs or FFBs. The major cost savings for SSFs come from reduced moisture requirements, which would lead to lower system mass requirements. Compost ranges from 50 to 60% moisture, with the optimal content varying with the type of waste (Cook, 1993; Atkinson et al., 1996a,b). Short-term composting effectively removes readily degradable organic materials (Bono et al., 1992) and inorganic nutrients can be readily extracted from composts (Villar et al., 1993; Forster et al., 1993; Levi-Minzi et al., 1992). A variety of solid wastes--sewage solids, poultry litter, municipal solid wastes, pulp and paper-mill primary solids, and pine sawdust--have been successfully composted in laboratory-scale bioreactors (Atkinson, 1995; Atkinson et al., 1996 a,b). SSF is the only option with the ability to kill human enteric pathogens in the bioreactor itself as a result of heat generated through microbial metabolic activities (Lau et al., 1992). Preliminary data from SSFs being operated at KSC indicate they would allow crop residue bioprocessing on a scale that would be competitive with the other biological processes and be appropriate for space-based applications.

As with the other two options, FFBs are a mature technology with widespread uses. The use of FFBs in space ALS has been proposed and studied. Allied Signal Corp., in conjunction with JSC, has designed, built, and operated an immobilized cell graywater processor (Nacheff-Benedict et al., 1994; Edeen et al., 1995). Miller et al. (1991) showed that a packed bed, fixed film bioreactor, operating at a 27-h retention time, could remove up to 99.8% of phenol in a 100 ppm phenol-laced waste stream. KSC has utilized FFBs for the dual use of TOC reduction and nitrification (Strayer et al., 1997b) as a final polishing step in an anaerobic-based, inedible plant biomass digestion experiment. Steady-state TOC reduction and nitrification rates of 80% and 98%, respectively, were demonstrated during 180 days of continuous operation. Effluent from the bioreactor was successfully used in a concurrent plant growth experiment (Strayer et al., 1997b; Mackowiak et al., 1997b).

The major advantage of fixed-film designs is retention of high concentrations of decomposer microbes in the attached biofilms. This advantage could enable the reactor to have lower volume, mass, and possibly power requirements than CSTR. Disadvantages relate to diffusion-limited transfer of



dissolved oxygen into biofilms with a resulting potential for denitrification (Stutte, 1996). Also, fixed film bioreactors are generally designed to process soluble, or low particulate waste streams, thus upstream particle/liquid separation would be needed. Because bacterial or greater sized pathogens would be removed by the particle/liquid separation step, operation of the fixed-film bioreactor will not have to be concerned with exposure to these pathogens.

### ***Microbiological and Chemical Characterization***

Microbial community stability will be an important component of overall system stability in closed bioregenerative life support systems. Biomass production systems will harbor a significant microbial load due to the release of organic material from plant roots (Garland et al., in press, Garland 1994, Strayer 1991, 1994). The growth of human-associated microorganisms within these systems could lead to human health problems, and the growth of plant pathogens could affect the production of the life support commodities by the plants. Consistent recycling of elements through bioregenerative resource recovery process also will depend on the long-term stability of microbial communities within the bioreactors.

Research to date indicates that the microbial communities associated with prototype plant production systems and bioreactors are stable during long periods of operation (Cook and Garland, 1997; Garland 1996, Garland et al., 1997 a,b). Further characterization of the resistance and resiliency of these microbial communities to stress, including potential invasion by deleterious microorganisms is necessary. The recycling of human waste streams, and the concomitant introduction of human-associated microorganisms into bioreactors or plant growth systems is of particular concern. Preliminary experiments suggest that the complexity of the microbial inoculum to the closed system is an important element in the community stability (Morales et al. 1996), and emphasize the need to define what types of "beneficial" microorganisms need to be introduced into the system.

Some common practices in non-regenerative life support systems are likely to give rise to problems with biological systems. In particular, the widespread use of chemical antibacterials and/or preservatives can pose problems for plant and microbial systems. Iodine and silver are used in the US and Russian space programs, respectively, to ensure that potable water is safe to drink. If a similar approach is used in BIO-Plex, then these ions must be removed before introduction of waste or excess quantities of water to either the plant systems or the bioreactors. Sulfuric acid and oxone have been used to chemically stabilize and prevent breakdown of urea in urine, but these would be deleterious to biological waste treatment systems and very likely make biological recovery of the nitrogen more difficult. Other contaminants could be introduced by the crew flushing unsuitable materials down the toilet or adding unsuitable materials to the incinerator. For example, glues used to hold air filter frames together were found to be a potential source of boron contamination in BPC studies (Stutte, unpub.).

With respect to the atmospheric system, any potential sources of ethylene or other phytotoxic gases should be considered carefully, just as any potentially toxic gases from the bioregenerative components must be considered for their effects on the crew. A listing of organic volatiles measured

during Biomass Production Chamber studies can be found in Batten et al., (1995, 1996), Wheeler et al. (1996b), and Stutte and Wheeler (1997) and additional measurements are needed as new species of crops are tested. Use of certain types of foam materials can be toxic to plants (Wheeler et al., 1985), and recent evidence showed that volatiles likely from pyrel foam, which is cleared for human use and has been used in EHT tests, were toxic to slime mold organisms on STS-69 (I. Block, unpublished).

## RECOMMENDATIONS

### *Immediate Issues for BIO-Plex Design:*

- Partition the plant production module to provide separate photoperiod and temperature zones.
- Reduce the lighting requirements from 1500 to ~ 750  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (tray level) for most of the crop production areas except for wheat and rice to reduce power consumption and heat rejection requirements.
- Utilize a biological reactor to process inedible plant biomass and food processing waste for: 1) recovery of inorganic nutrients in an aqueous stream; 2) reduction of soluble organic compounds to a level that does not inhibit crop production; 3) partial mineralization of lignocellulose components of crop residues to carbon dioxide.
- Determine area requirements for seed storage, biomass handling and storage, chemical storage, solution mixing, and general work areas for planting, harvesting, resource recovery, and clean-up.
- Consider more energy efficient lighting technologies if power requirements are more critical than total growing area for BIO-Plex (e.g., higher powered HID lamps, LEDs, microwave lamps, or remote lighting with light conduits).

### *Supporting Research, Food / Plant Production:*

- Develop a crop task handbook and include a list of candidate crops, the crop environmental and horticultural requirements, and a standard protocol for testing candidate crops for ALS.
- Conduct baseline horticultural and environmental studies with peanut, carrot, chard, and cabbage and select short (dwarf), high yielding cultivars; studies should include tests utilizing a standard range of environmental conditions and nutrient delivery approach pertinent to BIO-Plex (note, additional species may be added as the result of annual meetings of the ALS food/plant research community).
- Resolve issues pertaining to controlling 1) potato tuberization and 2) sweetpotato storage root formation in continuous production hydroponic systems.
- Conduct comparison of nutrient delivery systems (NDS) for core crops. Comparisons should include solution, thin film, and solid media systems (e.g., use of nutrient-rich, zeolite/apatite media) and address constraints applicable to BIO-Plex and general NDS management issues.
- Determine if bioprocessed solids can be utilized as a starting and/or rooting medium for crops and explore systems that minimize clean-up after harvest.
- Conduct large-scale (e.g.  $\geq 10 \text{ m}^2$  area) verification tests with untested crops (e.g., rice, peanut, chard, cabbage, carrot, sweetpotato, strawberry, tomato) prior to incorporation into BIO-Plex; tests should include the use of nursery/transplant schemes and mechanization technologies where appropriate.
- Conduct mixed crops studies with environmentally matched species to verify compatibility, particularly with regard to photoperiod and temperature.
- Develop mechanized systems for planting and harvesting (e.g., wheat, soybean, and rice), and materials handling and processing for resource recovery.

**Supporting Research, Resource Recovery and Waste Processing:**

- Determine if the microbial flora present in the crop nutrient delivery system (NDS) can be utilized to process gray water *in situ*. If, gray water cannot be introduced directly into the NDS due to health issues, utilize a biological reactor to pretreat the graywater prior to addition.
- Determine bioprocessing conditions that reduce human-associated microorganisms from crew wastes (graywater) to acceptable levels. Define acceptable levels.
- Find ways to minimize sodium in ALS wastes (e.g., potassium soaps) Develop a biological (salt tolerant and/or accumulating plants) or physical-chemical method for removing sodium from the waste processing system.
- Define the advantages and disadvantages of fundamental bioprocessing approaches (continuous stirred tank reactors [CSTR], fixed-film bioreactors [FFB], and solid state fermentation [SSF, or composting]). These evaluations should be made with regard to a cost-benefit analysis for each approach: Costs are volume, mass, energy, and manpower. Benefits are reliability, carbon mineralization, nutrient recovery, and a product stream that contains acceptable levels of soluble TOC (eliminate problems in hydroponic NDS), pathogen reduction, and trace organic contaminant control, without introducing hazards such as high temperatures and pressures.
- Explore use of physical/chemical waste processing outputs (e.g., combustion ash and gas) for sustaining food/plant production systems.
- Define microbial inocula necessary for reliable and stable (over time) operation of resource recovery and plant production systems. Determine effects of inocula on survival of contaminants, including plant and human pathogens.
- Evaluate resource recovery bioprocesses with regard to mission scenarios, including rate of input-- (e.g., the crop harvest interval for inedible biomass), degree of system closure, storage of waste system inputs and outputs, etc.
- Determine the stability of bioprocessed liquids and solids under storage conditions.

These recommendations focus on the issues for development of BIO-Plex and draw largely on published results from the KSC Breadboard Project, which has been operation for nearly 10 years (Appendix A), as well as university investigations throughout the 1980s and 1990s. Achieving more far-reaching ALS objectives, including a flight testing program, will require updated NASA Research Announcements (NRAs), to sustain the needed bioregenerative research and technology development. Priorities will need to be assigned for developing both bioregenerative and physical / chemical system flight support hardware. These priorities, along with schedules, costs, and responsible organizations, should be agreed to by all members of the ALS program team in order to maximize the progress.

It is recommended that representatives of the bioregenerative community participate in preliminary and critical design reviews for BIO-Plex, and that members of the food/plant production and waste treatment/resource recovery working groups meet on an annual basis to formally discuss issues regarding BIO-Plex development, as well as future ALS mission needs. These meetings will provide the basis for re-prioritizing program research and development needs.

## REFERENCES

- Allen, E.R., D.W. Ming, L.R. Hossner, D.L. Henninger, and C. Galindo. 1995. Growth and nutrient uptake of wheat in clinoptilolite-phosphate rock substrates. *Agron. J.* 87:1052-1059.
- Atkinson C.F. 1995. Biodegradabilities and microbial activities during composting of solid wastes. Doctoral Dissertation, University of Alabama at Birmingham, Birmingham, Alabama.
- Atkinson, C.F., D.D. Jones, and J.J. Gauthier. 1996a. Biodegradabilities and microbial activities during composting of oxidation ditch sludge. *Compost Science and Utilization* 4:84-96.
- Atkinson, C.F., D.D. Jones, and J.J. Gauthier. 1996b. Biodegradability and microbial activities during composting of poultry litter. *Poultry Science* (In press).
- Averner, M. 1993. NASA Advanced Life Support Program Plan. Office of Life and Microgravity Sciences and Applications Division, NASA Headquarters, Washington, DC.
- Barker, A.V., K.A. Corey, and L.E. Craker. 1989. Nutritional stresses in tomato genotypes grown under high-pressure sodium vapor lamps. *HortScience*, 24:255-258.
- Barnes, C. and B. Bugbee. 1992. Morphological responses of wheat to blue light. *J. Plant Physiol.* 139:339-342.
- Barta, D.J. and T.W. Tibbitts. 1991. Calcium localization in lettuce leaves with and without tipburn: Comparison of controlled environment and field grown plants. *J. Amer. Soc. Hort. Sci.* 116:870-875.
- Batten, J.H., G.W. Stutte, and R.M. Wheeler 1995. Effect of crop development on biogenic emissions from plant populations grown in a closed plant growth chambers. *Phytochemistry* 39:1351-1357.
- Batten, J.H., G.W. Stutte, and R.M. Wheeler. 1996. Volatile organic compounds detected in the atmosphere of NASA's Biomass Production Chamber. *Adv. Space Res.* 18(4/5)189-192.
- Bono, J.J., N. Chalaux, and B. Chabbert. 1992. Bench-scale composting of two agricultural wastes. *Bioresource Technology* 40:119-124.
- Both, A.J., A.R. Leed, E. Goto, L.D. Albright, and R.W. Langhans. 1996. Greenhouse spinach production in a NFT system. *Acta Hort.* 440:187-192.
- Bubenheim, D.L., W. Kanapathipillai, and T. Wydeven. 1993. Incineration in a Controlled Ecological Life Support System: a method for resource recovery from inedible biomass. SAE Tech. Paper 932249.
- Bugbee, B.G. and F.B. Salisbury. 1988. Exploring the limits of crop productivity. Photosynthetic efficiency of wheat in high irradiance environments. *Plant Physiol.* 88:869-878.
- Bugbee, B.G. and F.B. Salisbury. 1989. Controlled environment crop production: Hydroponic vs. lunar regolith. In: D.W. Ming and D.L. Henninger (eds.) *Lunar Base Agriculture: Soils for Plant Growth*. Amer. Soc. Agronomy, Madison, WI, USA.
- Bugbee, B. 1992. Determining the potential productivity of food crops in controlled environments. *Adv. Space Res.* 12:85-95.
- Bugbee, B., B. Spanarkel, S. Johnson, O. Monje, and G. Koerner. 1994. CO<sub>2</sub> crop growth enhancement and toxicity in wheat and rice. *Adv. Space Res.* 14:257-267.
- Bugbee, B. and G. Koerner. 1997. Yield comparisons and unique characteristics of the dwarf wheat cultivar 'USU Apogee'. Submitted to *Adv. Space Res.*
- Bula, R.J., R.C. Morrow, T.W. Tibbitts, D.J. Barta, R.W. Ignatius, and T.S. Martin. 1992. Light-emitting diodes as a radiation source for plants. *HortScience* 26:203-205.
- Chow, K.K., T.V. Price, and B.C. Hanger. 1992. Nutritional requirements for growth and yield of strawberry in deep flow hydroponic systems. *Scientia Hort.* 52:95-104.
- Collier, G.F. and T.W. Tibbitts. 1982. Tipburn of lettuce. *Horticultural Reviews* 4:49-65.
- Cook, K.L. 1993. Microbial ecology of composting under various physical conditions. Master's Thesis, University of Alabama at Birmingham, Birmingham, Alabama.
- Cook, K.L. and J.L. Garland. 1997. The relationship between electron transport activity as measured by CTC reduction and CO<sub>2</sub> production in mixed microbial communities. *Microbial Ecology* (in press).
- Davis, N. 1985. Controlled-environment agriculture – Past, present, and future. *Food Technology* 39:124-126.
- Diem, K., and C. Lentner (eds.). 1970. *Scientific tables*. 7th Ed. Ciba-Geigy, Basle.
- Dougher, T.A.O. and B.G. Bugbee. 1997. Blue light and temperature effects on internode elongation, growth and yield. Submitted to *Adv. Space Res.*
- Dreschel, T.W., R.M. Wheeler, C.R. Hinkle, J.C. Sager, and W.M. Knott. 1991. Investigating combustion as a method of processing inedible biomass produced in NASA's biomass production chamber. *NASA Tech. Mem.* 103821.
- Drysdale, A.E. 1995. The effect of resource cost on life support selection SAE Tech. Paper 951492.
- Durner, E.F. and E.B. Poling. 1988. Strawberry developmental responses to photoperiod and temperature: A review. *Adv. Strawberry Prod.* 7:6-14.
- Edeen, M.A., G.H. Kumagai, L. N. Dittner, L. M. Landau, R.W. Schweickert, C.D. McFadden. 1995. Advances in development of bioreactor technology for a regenerative life support primary water processor. SAE Tech. Paper 951740.

- Ewing, E.E. 1978. Heat stress and the tuberization stimulus. *Amer. Potato J.* 58:31-49.
- Finger, B.W. and R.F. Strayer. 1994. Development of an intermediate-scale aerobic bioreactor to regenerate nutrients from inedible crop residues. SAE Tech. Pap. 941501.
- Finger, B.W., and M.P. Alazraki. 1995. Development and Integration of a Breadboard-scale aerobic bioreactor to regenerate nutrients from inedible crop residues. SAE Tech. Paper 951498.
- Forster, J.C., W. Zech, and E. Würdinger. 1993. Comparison of chemical and microbiological methods for the characterization of the maturity of composts from contrasting sources. *Biol. and Fertility of Soils* 16:93-99.
- Frick, J., S. Nielsen, and C.A. Mitchell. 1994. Yield and seed oil content response of dwarf, rapid-cycling *Brassica* to nitrogen treatments, planting density and CO<sub>2</sub> enrichment. *J. Amer. Soc. Hort. Sci.* 119:1137-1143.
- Garland, J.L. and C.L. Mackowiak. 1990. Utilization of the water soluble fraction of wheat straw as a plant nutrient source. NASA Tech. Mem. 107544.
- Garland, J.L. 1991. Carbon flux within hydroponically-based plant growth systems: Analysis of microbial community structure and function. Ph.D. Dissertation, Dept. Environmental Science, University of Virginia.
- Garland, J.L. 1992a. Coupling plant growth and waste recycling systems in a controlled life support system (CELSS). NASA Tech. Mem. 107544.
- Garland, J.L. 1992b. Characterization of the water soluble component of inedible residue from candidate CELSS crops. NASA Tech. Mem. 107557.
- Garland, J.L., C.L. Mackowiak, and J.C. Sager. 1993. Hydroponic crop production using recycling nutrients from inedible crop residues. SAE Tech. Paper 932173.
- Garland, J.L. 1994. The structure and function of microbial communities in recirculating hydroponic systems. *Adv. Space Res.*, 14:383-386.
- Garland, J.L. 1996. Patterns of potential carbon source utilization by rhizosphere communities. *Soil Biol. Biochem.* 28:223-230.
- Garland, J.L., K.L. Cook, M. Johnson, R. Sumner, and N. Fields. 1997a. Density and composition of microorganisms during long-term (418 day) growth of potato using biologically reclaimed nutrients from inedible plant biomass. Submitted to *Adv. Space Res.*
- Garland, J.L., K.L. Cook, C.A. Loader, and B.A. Hungate. 1997b. The influence of microbial community structure and function on community-level physiological profiles. In: Insam, H. and A. Rangger (eds.) *Microbial Communities: Functional Versus Structural Approaches*, Springer, Hiedelberg. (in press).
- Gaudy Jr., A.F., and E.T. Gaudy. 1972. Mixed microbial populations. In: Ghose, Fiechter, and Blakebrough (eds.) *Advances in Biochemical Engineering* 2. Springer-Verlag, New York. pp.97-143.
- Gerbaud, A. M. Andre, and C. Richaud. 1988. Gas exchange and nutrition patterns during the life cycle of an artificial wheat crop. *Physiol. Plant.* 73:471-478.
- Gitelson, J.I., I.A. Terskov, B.G. Kovrov, G.M. Lisoviskii, Yu. N. Okladnikov, F. Ya. Sid'ko, I.N. Tuubachev, M.P. Shilenko, S.S. Alekseev, I.M. Pan'kova, and L.S. Tirranen. 1989. Long-term experiments on man's stay in biological life-support system. NASA. Conf. Pub. 10040, Ames Research Center, Moffett Field, CA.
- Golueke, C.G. and W.J. Oswald. 1964. Role of plants in closed systems. *Ann. Rev. Plant Physiol.* 15:387-408.
- Goyal, S. and R.C. Huffaker. 1986. The uptake of NO<sub>3</sub>, NO<sub>2</sub> and NH<sub>4</sub> by intact wheat (*Triticum aestivum*) seedlings. I. Introduction and kinetics of transport systems. *Plant Physiol.* 82:1051-1056.
- Guerra, D., A.J. Anderson, and F.B. Salisbury. 1985. Reduced phenylalanine ammonia-lyase and tyrosine ammonia-lyase activities and lignin synthesis in wheat grown under low pressure sodium lamps. *Plant Physiol.* 78:126-130.
- Hackett, C. and J. Carolane. 1982. Edible horticultural crops. A compendium of information of fruit, vegetable, spice, and nut species. Academic Press, Sydney.
- Henninger, D.L. 1996. Advanced Life Support Program Plan. (draft). Life Sciences Division, Office of Life and Microgravity Sciences and Applications, NASA, Washington, DC.
- Hill, W.A., P.A. Loretan, C.K. Bonsi, C.E. Morris, J.Y. Lu, and C. Ogbuehi. 1989. Utilization of sweetpotatoes in controlled ecological life support systems. *Adv. Space Res.* 9:29-41.
- Hill, W.A., D.G. Mortley, C.L. Mackowiak, P.A. Loretan, T.W. Tibbitts, R.M. Wheeler, C.K. Bonsi, and C.E. Morris. 1992. Growing root, tuber and nut crops hydroponically for CELSS. *Adv. Space Res.* 12(5):125-131.
- Hillman, W.S. 1956. Injury of tomato plants by continuous light and unfavorable photoperiodic cycles. *Amer. J. Bot.* 43:89-96.
- Hicklenton, P.R., and M.S. Wolynetz. 1987. Influence of light- and dark-period air temperatures and root temperature on growth of lettuce in nutrient flow systems. *J. Amer. Soc. Hort. Sci.* 112:932-935.
- Hoff, J.E., J.M. Howe, and C.A. Mitchell. 1982. Nutritional and cultural aspects of plant species selection for a regenerative life Support system. Report to NASA Ames Research Center, NSG2401 and NSG 2404.
- Jacquez, R.B. 1990. Preliminary evaluation of waste processing in a CELSS. NASA Tech. Mem. 102277.
- Janes, H.W. 1994. Controlled environment intercropping of lettuce and tomatoes. Final Report, NASA Ames-University Consortium Agreement.

- Knight, S.L. and C.A. Mitchell. 1983. Enhancement of lettuce yield by manipulation of light and nitrogen nutrition. *J. Amer. Soc. Hort. Sci.* 108:750-754.
- Knight, S.L. and C.A. Mitchell. 1988a. Growth and yield characteristics of 'Waldmann's Green' leaf lettuce under different photon fluxes from metal halide or incandescent + fluorescent radiation. *Scient. Hort.* 35:51-61.
- Knight, S.L. and C.A. Mitchell. 1988b. Effects of CO<sub>2</sub> and photosynthetic photon flux on yield, gas exchange and growth rate of *Lactuca sativa* L. 'Waldmann's Green'. *J. Exp. Bot.* 39:317-328.
- Knox, J. 1986. A method of variable spacing for controlled plant growth systems for spaceflight and terrestrial agriculture applications. NASA Contract Report 177447, Ames Research Center, Moffett Field, CA.
- Krall, A.R. and B. Kok. 1960. Studies on algal gas exchangers with reference to space flight. *Dev. Indust. Microbiol.* 1:33-44.
- Lau, A.K., K.V. Lo, P.H. Liao, and J.C. Yu. 1992. Aeration experiments for swine waste composting. *Bioresource Technology* 41:145-152.
- Levi-Minzi, R., A. Saviozzi, and R. Riffaldi. 1992. Evaluating garbage compost: Nutritive elements. *BioCycle* 33:75-77.
- MacElroy, R.D. and J. Bredt. 1985. Current concepts and future directions of CELSS. NASA Conf. Publ. 2378.
- MacLennan, D.A., B.P. Turner, J.T. Dolan, M.G. Ury, and P. Gustafson. 1994. Efficient, full-spectrum, long-lived, non-toxic microwave lamp for plant growth. In: T.W. Tibbitts (ed.) International Lighting in Controlled Environments Workshop. Madison, WI, March 27-30. NASA-CP-95-3309, Kennedy Space Center, FL.
- Mackowiak, C.L., R.M. Wheeler, W.L. Berry, and J.L. Garland. 1994a. Nutrient mass balances and recovery strategies for growing plants in a CELSS. *HortSci.* 29:464.
- Mackowiak, C.L., J.L. Garland, and G.W. Stutte. 1994b. Growth regulator effects of water soluble materials from crop residues for use in plant hydroponic culture. Proc. 21st Annual Mtg. PGRSA. Portland OR.
- Mackowiak, C.L., L.M. Ruffe, N.C. Yorio, and R.M. Wheeler. 1994c. Effect of carbon dioxide enrichment on radish production using nutrient film technique (NFT). NASA Tech. Mem. 109198.
- Mackowiak, C.L., J.L. Garland, R.F. Strayer, B.W. Finger, and R.M. Wheeler. 1996a. Comparison of aerobically-treated and untreated crop residue as a source of recycled nutrients in a recirculating hydroponic system. *Adv. Space Res.* 18:281-287.
- Mackowiak, C.L., J.L. Garland, and J.C. Sager. 1996b. Recycling crop residues for use in recirculating hydroponic crop production *Acta Hort* 440:19-24.
- Mackowiak, C.L. and R.M. Wheeler. 1996. Growth and stomatal behavior of hydroponically cultured potato (*Solanum tuberosum* L.) at elevated and super-elevated CO<sub>2</sub>. *J. Plant Physiol.* 149:205-210.
- Mackowiak, C.L., R.M. Wheeler, G.W. Stutte, N.C. Yorio, and L.M. Ruffe. 1997a. A recirculating hydroponic system for studying peanut (*Arachis hypogaea* L.). Submitted to HortTechnology.
- Mackowiak, C.L., R.M. Wheeler, G.W. Stutte, N.C. Yorio, and J.C. Sager. 1997b. Use of biological reclaimed minerals for continuous hydroponic potato production in a CELSS. Submitted to Adv. Space Res.
- McAvoy, R.J., H.W. Janes, and G.A. Giacomelli. 1989. Development of a plant factory model: I The organizational and operational model. II. A plant growth model: The single truss tomato crop. *Acta Hort.* 248:85-94.
- Miller, G.P., R.J. Portier, D.P. Dickey, and H.L. Sleeper. 1991. Using biological reactors to remove trace hydrocarbon contaminants from recycled water. SAE Tech. Paper 911504.
- Miller, R.L. and C.H. Ward. 1966. Algal bioregenerative systems. In: E. Kammermeyer (ed.) Atmosphere in space cabins and closed environments. Appleton-Century-Croft Pub., New York.
- Mitchell, C.A., T.A.O. Dougher, S.S. Nielsen, M.A. Belury, and R.M. Wheeler. 1996. Costs of providing edible biomass for a balanced vegetation diet in a controlled ecological life support system. In: H. Suge (ed.), Plant in Space Biology. Inst. Genetic Ecology, Tohoku Univ.
- Modell, M. 1986. Super critical waste oxidation of aqueous wastes. NASA Tech. Mem. 88215.
- Morales, A., J.L. Garland, and D.V. Lim. 1996. Survival of potentially pathogenic human-associated bacteria in the rhizosphere of hydroponically grown wheat. *FEMS Microb. Ecol.* 20:155-162.
- Mori, K., H. Ohya, K. Matsumoto, and H. Furune. 1987. Sunlight supply and gas exchange systems in microalgal bioreactor. In: R.D. MacElroy and D.T. Smernoff (eds.) Controlled Ecological Life Support Systems, Regenerative Life Support System in Space. NASA Conf. Publ. 2480, Ames Research Center, CA.
- Mortley, D.G., C.K. Bonsi, W.A. Hill, P.A. Loretan, and C.E. Morris. 1993. Irradiance and nitrogen to potassium ratio influences sweetpotato yield in nutrient film technique. *Crop Sci.* 33:782-784.
- Mortley, D.G., P.A. Loretan, J.H. Hill, and J. Seminara. 1997. CO<sub>2</sub> enrichment influences the yields of 'Florunner', 'Georgia Red', and 'New Mexico' peanut cultivars. Submitted to Advances Space Research.
- Myers, J. 1954. Basic remarks on the use of plants as abiological gas exchangers in a closed system. *J. Aviation Med.* 25:407-411.
- Nacheff-Benedict, M.S., G.H. Kumagai, G.E. Petrie, R.W. Schweickert, C.D. McFadden, N.J.C. Packham, and M.A. Edeen. 1994. An integrated approach to development of bioreactor technology for an advanced life support primary water processor. SAE Tech. Paper 941397.

- Ness, R.O., J.R. Rindt, and S.R. Ness. 1992. Plasma reactor waste management systems. In: W.W. Mendell (ed.) Second Conference of Lunar Bases and Space Activities of the 21<sup>st</sup> Century. NASA Conf. Publ. 2166 .
- Norman, J.M. and T.J. Arkebauer. 1991. Predicting canopy photosynthesis and light-use efficiency from leaf characteristics. In: K.J. Boote and R.S. Loomis (eds.) Modeling crop photosynthesis—From biochemistry to canopy . Crop Sci. Soc. Amer. Madison, WI, USA.
- Ohler, T.A., and C.A. Mitchell. 1996. Identifying yield-optimizing environments for two cowpea breeding lines by manipulating photoperiod and harvest scenario. J. Amer. Soc. Hort. Sci. 121:576-581.
- Parker, D.B., and S.K. Gallagher. 1988. Distribution of human waste samples in relation to sizing waste processing in space. Paper LBS-88-107, Symposium on lunar bases & space activities in the 21<sup>st</sup> century, Houston, Texas, 1988.
- Prince, R.P. and J.W. Bartok. 1978. Plant spacing for controlled environment plant growth. Transactions of ASAE 21:332-336.
- Raper, C.D. Jr., J. K. Vessey, and L.T. Henry. 1991. Increase in nitrate uptake by soybean plant during interruption of the dark period with low intensity light. *Physiol. Plant.* 81:183-189.
- Resh, H.M. 1989. Hydroponic food production. 4<sup>th</sup> ed. Woodbridge Press Pub. Comp. Santa Barbara, CA, USA.
- Richards, L.A. (ed.). 1969. Diagnosis and improvement of saline and alkali soils. Agricultural Handbook No. 60, USDA, Washington, DC.
- Rowell, P.L. and D.G. Miller. 1971. Induction of male sterility in wheat with 2-chloroethylphosphonic acid (Ethrel). *Crop Science* 11:629-632.
- Salisbury, F.B. 1981. Responses to photoperiod. In: O.L. Lange, P.S. Nobel, C.B. Osmond, and H. Ziegler (eds.), *Physiological Plant Ecology I. Ency. Plant Physiol.* 12A: 135-167.
- Salisbury, F.B. 1991. Lunar farming: Achieving maximum yield for the exploration of space. *HortSci.* 26:827-833.
- Salisbury, F.B. and M.A.Z. Clark. 1996a. Suggestions for crops grown in controlled ecological life-support systems, based on attractive vegetarian diets. *Adv. Space Res.* 18:33-39.
- Salisbury, F.B. and M.A.Z. Clark. 1996b. Choosing plant to be grown in a controlled environment life support system (CELSS) based upon attractive vegetarian diets. *Life Supp. Biosphere Sci.* 2:169-179.
- Schlick, G. and D.L. Bubenheim. 1993. Quinoa: An emerging 'new' crop with potential for CELSS. NASA Tech. Paper 3422.
- Shubert, F.H., R.A. Wydeven, and P.D. Quattrone. 1984. Advanced regenerative environmental control and life support systems: Air and water regeneration. *Adv. Space Res.* 4:279-288.
- Strayer, R.F. 1991. Microbiological characterization of the Biomass Production Chamber during hydroponic growth of crops at the CELSS Breadboard Facility, SAE Tech. Paper 911427.
- Strayer, R.F. 1993. Evaluation of enzymatic hydrolysis of CELSS wheat residue cellulose at a scale equivalent to NASA's KSC Breadboard Project. SAE Tech. Paper 932253.
- Strayer, R.F., 1994. Dynamics of microorganism populations in recirculating nutrient solutions. *Adv. Space Res.* 14:357-366.
- Strayer, R.F., and K.L. Cook. 1995. Recycling plant nutrients at NASA's KSC-CELSS Breadboard-scale aerobic bioreactor during two runs. SAE Tech. Paper 951708.
- Strayer, R.F. and C.F. Atkinson. 1997a. Recycling nutrient from crop residues for space applications. Submitted to *Compost Science and Utilization*.
- Strayer, R.F., B. W. Finger, and M. P. Alazraki. 1997b. Evaluation of an anaerobic digestion system for processing CELSS crop residues for resource recovery. Submitted to *Adv. Space Res.*
- Stutte, G.W. and R.L. Darnell. 1987. A nondestructive development index for strawberry. *HortScience* 22:218-221.
- Stutte, G.W., and J.C. Sager. 1995. Biological consideration in the design of continuous potato production systems. ASAE Paper No. 955654.
- Stutte, G.W., N.C. Yorio, and R.M. Wheeler. 1996. Interacting effects of photoperiod and photosynthetic photon flux on net carbon assimilation and starch accumulation in potato leaves. *J. Amer. Soc. Hort. Sci.* 121:264-268.
- Stutte, G.W. 1996. Nitrogen dynamics in the CELSS Breadboard Facility at Kennedy Space Center. *Life Support and Biosphere Science* 3:67-74.
- Stutte, G.W. and R.M. Wheeler. 1997. Accumulation and effects of volatile organic compounds in closed life support systems. Submitted to *Advances in Space Research*.
- Sylvania/GTE. 1980. Designers handbook. Light source applications. GTE Products Corp., Danvers, MA.
- Tchobabanoglous, G., and F.L. Burton. 1991. Biological unit processes. In: *Wastewater engineering: treatment, disposal, and reuse.* 3<sup>rd</sup> ed. McGraw-Hill, Inc. pp. 359-943.
- Thomas, J.F. and C.D. Raper Jr. 1978. Effect of day and night temperatures during floral induction on morphology of soybeans. *Agron. J.* 70:893-898.
- Tibbitts, T.W. and D.K. Alford. 1982. Controlled ecological life support system. Use of higher plants. NASA Conf. Publ. 2231.

- Tibbitts, T.W., W. Cao, and R.M. Wheeler. 1994. Growth of potatoes for CELSS. NASA Cont. Report 177646. Ames Research Center, Moffett Field, CA.
- Tolley-Henry, L. and C.D. Raper. Jr. 1986. Utilization of ammonium as a nitrogen source. Effects of ambient acidity on growth and nitrogen accumulation by soybean. *Plant Physiol* 82:54-60.
- Vessey, J.D., C.D. Raper. Jr., and L. Tolley Henry. 1990. Cyclic variations in nitrogen uptake rate in soybean plants: Uptake during reproductive growth. *J. Exp. Bot.* 41:233-1579-1584.
- Villar, M.C., M.C. Beloso, M.J. Acea, A. Cabaneiro, S.J. Gonzalez-Prieto, M. Carballas, M. Diaz-Ravina, and T. Carballas. 1993. Physiological and chemical characterization of four composted urban refuses. *Bioresource Technology* 45:105-113.
- Volk, G.M. and C.A. Mitchell. 1995. Photoperiod shift effects on yield characteristics of rice. *Crop Sci.* 35:1631-1635.
- Volk, T. and B. Bugbee. 1991. Modeling light and temperature effects on leaf emergence in wheat and barley. *Crop. Sci.* 31:1218-1224.
- Wheeler, R.M., S.H. Schwartzkopf, T.W. Tibbitts, and R.W. Langhans. 1985. Elimination of toxicity from polyurethane foam plugs used for plant culture. *HortScience* 20:448-449.
- Wheeler, R.M. and T.W. Tibbitts. 1986. Growth and tuberization of potato (*Solanum tuberosum* L) under continuous light. *Plant Physiol.* 801-804.
- Wheeler, R.M., K.L. Steffen, T.W. Tibbitts, and J.P. Palta. 1986. Utilization of potato for life support systems. II. The effects of temperature under 24-h and 12-h photoperiod. *Am. Potato J.* 63:639-647.
- Wheeler, R.M., K.A. Corey, J.C. Sager, and W.M. Knott. 1993a. Gas exchange rates of wheat stands grown in a sealed chamber. *Crop Sci.* 33:161-168.
- Wheeler, R.M., C.L. Mackowiak, L.M. Siegriest, and J.C. Sager. 1993b. Supraoptimal carbon dioxide effects on growth of soybean (*Glycine max* (L.) Merr.). *J. Plant Physiol.* 142:173-178.
- Wheeler, R.M., C.L. Mackowiak, J.C. Sager, N.C. Yorio, W.M. Knott, and W.L. Berry. 1994. Growth and gas exchange by lettuce stands in a closed, controlled environment. *J. Amer. Soc. Hort. Sci.* 119:610-615.
- Wheeler, R.M., G.W. Stutte, C.L. Mackowiak, N.C. Yorio, and L.M. Ruffe. 1995. Accumulation of possible potato tuber-inducing factor in continuous use recirculating NFT systems. *HortSci.* 30:790 (#262)
- Wheeler, R.M. 1996. Gas balance in a plant-based CELSS. In: H. Suge (ed.), *Plants in Space Biology*, Inst. Genetic Ecology, Tohoku Univ.
- Wheeler, R.M., C.L. Mackowiak, G.W. Stutte, J.C. Sager, N.C. Yorio, L.M. Ruffe, R.E. Fortson, T.W. Dreschel, W.M. Knott, and K.A. Corey. 1996a. NASA's Biomass Production Chamber: A testbed for bioregenerative life support studies. *Adv. Space Res.* 18(4/5):215-224.
- Wheeler, R.M., B.V. Peterson, J.C. Sager, and W.M. Knott. 1996b. Ethylene production by plants in a closed environment. *Adv. Space Res.* 18(4/5):193-196.
- Wignarajah, K, D. Bubenheim, and T. Wydeven. 1992. Performance of lettuce in gray-water streams. Ames Research Center Research and Technology Report, pp. 165-66.
- Yamaguchi, M. 1983. *World vegetables. Principles, production and nutritive values.* AVI Books, Van Nostrand Reinhold Comp. Inc., New York, NY.



## Appendix A: Minutes from ALS Plant/Food Production Research Meeting, May 1997.

Date: 24 June 1997  
To: Advanced Life Support (ALS) Program Management and Attendees  
From: R.M. Wheeler, Kennedy Space Center, FL  
Re: Minutes from ALS Plant/Food Production Meeting

On May 5 and 6, 1997, a meeting was convened at Kennedy Space Center (Hangar L) to discuss issues related to Food and Plant Production activities for NASA's Advanced Life Support (ALS) Program. Two primary topics were addressed: 1) development and maintenance of an ALS plant production database, and 2) candidate crops and support research for BioPlex I.

Questionnaires on the growth of crops for bioregenerative life support were mailed out to most of the attendees prior to the meeting by Frank Salisbury (Utah State Univ.). Frank along with Mary Ann Clark (Utah State) are currently compiling inputs from these questionnaires to develop a database on controlled environment crop production for ALS. During the Monday morning session, the structure of the questionnaire and the types of data needed for ALS were discussed, and manipulations with the software were demonstrated. Frank also presented an overview of rules and proper usage of SI units for ALS research and reporting.

The Monday afternoon session included a discussion of systems-level considerations for food/plant production by Alan Drysdale of Mc Donnell Douglas Corp. Following this, Ted Tibbitts (Univ. of Wisconsin) and Ray Wheeler (KSC) led discussions on the types of testing and environmental measurement needed for thorough assessment of ALS crops. Key questions included: Has the crop been grown in recirculating nutrient delivery system? Are there short or dwarf cultivars available? Has the crop been grown under high-pressure sodium lighting? Cheryl Mackowiak (Kennedy Space Center) then presented the concept of developing workbooks or experiment protocols from the database; such protocols would be used to provide guidelines for ALS projects and mission planning involving plants.

Frank Salisbury is retiring from Utah State in July of 1997 and maintenance of the database following his retirement was discussed. The group thought it worthwhile for the program to maintain the database (current funding is provided by a contract through JSC). Options for maintaining the database were discussed. A preliminary recommendation was for Ted Tibbitts to act as the database curator through funding to the University of Wisconsin. Protocols for different crop studies for the program could be developed utilizing the database as needed.

On Tuesday morning, Dan Barta (JSC) provided an overview of the plant activities associated with the Lunar/Mars Life Support Test Project (formerly EHT) phase III and plans for the BioPlex project. Dan noted that the first phase of BioPlex would strive for a 90% crop-derived diet (crew of four), with 45% produced within the system, and 45% stowed. The remaining 10% would be non-crop related foods. The BioPlex food production module would provide about 80 m<sup>2</sup> growing area in a 185 m<sup>3</sup> volume and use horizontally mounted 400-W HPS lamps providing up to 1500  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPF. Plants would be grown in shallow trays on shelves 9.8 m long by 1.4 m wide with a nutrient solution reservoir for each shelf. For the "staple" crops requiring large growing areas, an entire shelf would be planted at once (i.e., no mixed ages of plants on a shelf).

Yael Vodovotz (JSC) followed this with an overview of food concerns for BioPlex as they might relate to plants. Some of the issues discussed included: finding a lipoxygenase-free soybean cultivar (to reduce processing requirements), considering adding dry beans (as a protein and Ca source), adding fruits and other salad crop varieties (for nutrition and dietary diversity), and continuing to search for dwarf rice and sweetpotato cultivars (to include them in as staple crop selections). Useful measurements to consider for ALS plant research include: edible tissue moisture content, proximate composition, protein and non-amino acid N content, micronutrient content, presence of anti-nutritive factors (e.g., tyrosin inhibitors, phytic acid), and the presence of any human-associated organisms in the foods.

On Tuesday afternoon, the initial crop list for BioPlex I and other possible crops were discussed (Hoff et al., 1982; Tibbitts and Alford, 1982; Salisbury and Clark, 1996). The crops fell into two general categories: 1) staple crops, which could supply significant amounts of carbohydrate, protein, and/or fat, but could require substantial processing, and 2) supplemental crops (vegetables and fruits), which are generally perishable but would add dietary variety. The following criteria were used to assign readiness levels of 0 - 3 for the use of the crops in BioPlex: 0 = little knowledge of the crop in controlled environment conditions; 1) limited testing of the crop in controlled environment conditions and limited published results; 2) extensive testing in controlled environment conditions with several papers published in the scientific literature; 3) extensive controlled environment testing, published results, and large scale (> 10 m<sup>2</sup>), closed system (i.e., pre-integration) testing conducted.

The crops and their current readiness ratings for use in BioPlex I:

| Staple         |                  | Vegetables and Fruits |                  |          |     |
|----------------|------------------|-----------------------|------------------|----------|-----|
| Wheat          | 3.0              | Lettuce               | 3.0              | Kale     | 1.0 |
| Soybean        | 2.5              | Tomato                | 2.0              | Quinoa   | 1.0 |
| Potato         | 2.5              | Spinach               | 2.0              | Onion    | 1.0 |
| Sweetpotato    | 2.0              | Radish                | 2.0              | Carrot   | 0.5 |
| Peanut         | 1.5              | Strawberry            | 1.5              | Broccoli | 0.5 |
| Rice           | 1.0              | Chard/Beet            | 1.0              | Cabbage  | 0.5 |
| Dry Bean / Pea | 1.0              | Chufa                 | 1.0 <sup>b</sup> | Melon    | 0.5 |
| Cowpea         | 1.0 <sup>a</sup> |                       |                  |          |     |
| Sugar Beet     | 0.5              |                       |                  |          |     |

<sup>a</sup> not discussed but used in previous CELSS studies; <sup>b</sup> nutsedge.

Because time was limited, thorough assessment of readiness levels was not possible. Assessment should continue in conjunction with the database development to refine ratings for BioPlex and related efforts. There was a general agreement that staple crops, which require a large planted area, reach a readiness level of ~ 3.0 for inclusion in BioPlex. Supplemental crops might be expanded to include other salad crops or cvs., and readiness levels might be relaxed because the area investment would be substantially less for these species. Food / diet development staff should provide approval of the tentative crop list prior to integration testing. If possible, the crew might also be canvassed for supplemental crop suggestions.

Following the crop discussions, an outline of pressing research needs for BioPlex I was discussed briefly. Issues fell into four general categories: 1) nutrient delivery systems (e.g., growing mixed species and ages of crops on one solution, allelopathy, recycling of minerals from treated waste products, sodium chloride build-up, iodine removal, phytopathogens, and sanitation procedures); 2) cultivation and environmental effects (e.g., propagation, starting media for seedlings, transplanting, spacing, mixed crops in the same environment, volatile organic contaminants, automated planting and harvesting); 3) lighting (e.g., acceptability of HPS lamps for crops, light leakage during dark cycles, improved thermal management, use of LEDs and microwave lamps, and use of native sunlight), and 4) crop and cultivar selection (e.g., dwarf cultivars, lipoxygenase-free soybeans, and dry bean cvs.).

Following discussions of BioPlex issues, Yuri Syniak presented an overview of life support related activities at the Moscow Institute for Biomedical Problems (IMBP). IMBP activities include the development of flight hardware for producing salad crops ("Vitamin Greenhouse") for the Russian module of the International Space Station. Following this, Bernie Grodzinski presented an overview of bioregenerative research at the University of Guelph, Ontario. Guelph activities are currently focused on carbon metabolism of whole crop stands using tightly closed chambers. The chambers utilize microwave lighting systems and have no plastic components, which allows tracking of volatile organic compounds. The group is also investigating the use of water/biological filtration approaches for removing atmospheric contaminants in closed buildings and has a working system in the Canada Life Insurance Building in Toronto.

The last topic scheduled was a discussion of inputs on plant/food production research for NASA Research Announcements (NRAs), but time did not permit a dialogue on this topic.

The meeting was adjourned at about 5:00 pm on Tuesday afternoon, May 6, 1997.

Attendees:

|   |   |
|---|---|
| Mike Alzaraki / Kennedy Space Center    | Bob Langhans / Cornell                    |
| Dan Barta / Johnson Space Center        | Colleen Loader / Kennedy Space Center     |
| Maynard Bates / Ames Research Center    | Howard Levine / Kennedy Space Center      |
| Yuli Berkovitch / IMBP                  | Bill Little / Kennedy Space Center        |
| Doug Britt / Kennedy Space Center       | Phil Loretan / Tuskegee University        |
| Dave Bubenheim / Ames Research Cen.     | John Lu / Tuskegee University             |
| Bruce Bugbee / Utah State Univ.         | Cheryl Mackowiak / Kennedy Space Center   |
| Peter Chetirkin / Kennedy Space Cen.    | Desmond Mortley / Tuskegee University     |
| Mary Ann Clark / Utah State Univ.       | John Sager / Kennedy Space Center         |
| Dave De Villiers / Cornell Univ.        | Frank Salisbury / Utah State University   |
| Mike Dixon / University of Guelph       | Greg Schlick / Ames Research Center       |
| Tom Dreschel / Kennedy Space Cen.       | Lisa Siegriest-Ruffe / Kennedy Space Cen. |
| Alan Drysdale / McDonnell Douglas (KSC) | Gary Stutte / Kennedy Space Center        |
| Barry Finger / Kennedy Space Center     | Yuri Syniak / IMBP                        |
| Gene Giacomelli / Rutgers Univ.         | Ted Tibbitts / Univ. of Wisconsin         |
| Greg Goins / Kennedy Space Center       | Yael Vodovotz / Johnson Space Center      |
| Bernie Grodzinski / Univ. of Guelph     | Ray Wheeler / Kennedy Space Center        |
| Jill Hill / Tuskegee University         | Neil Yorio / Kennedy Space Center         |
| Ross Hinkle / Kennedy Space Center      | Scott Young / Kennedy Space Center        |
| Bill Knott / Kennedy Space Center       |   |

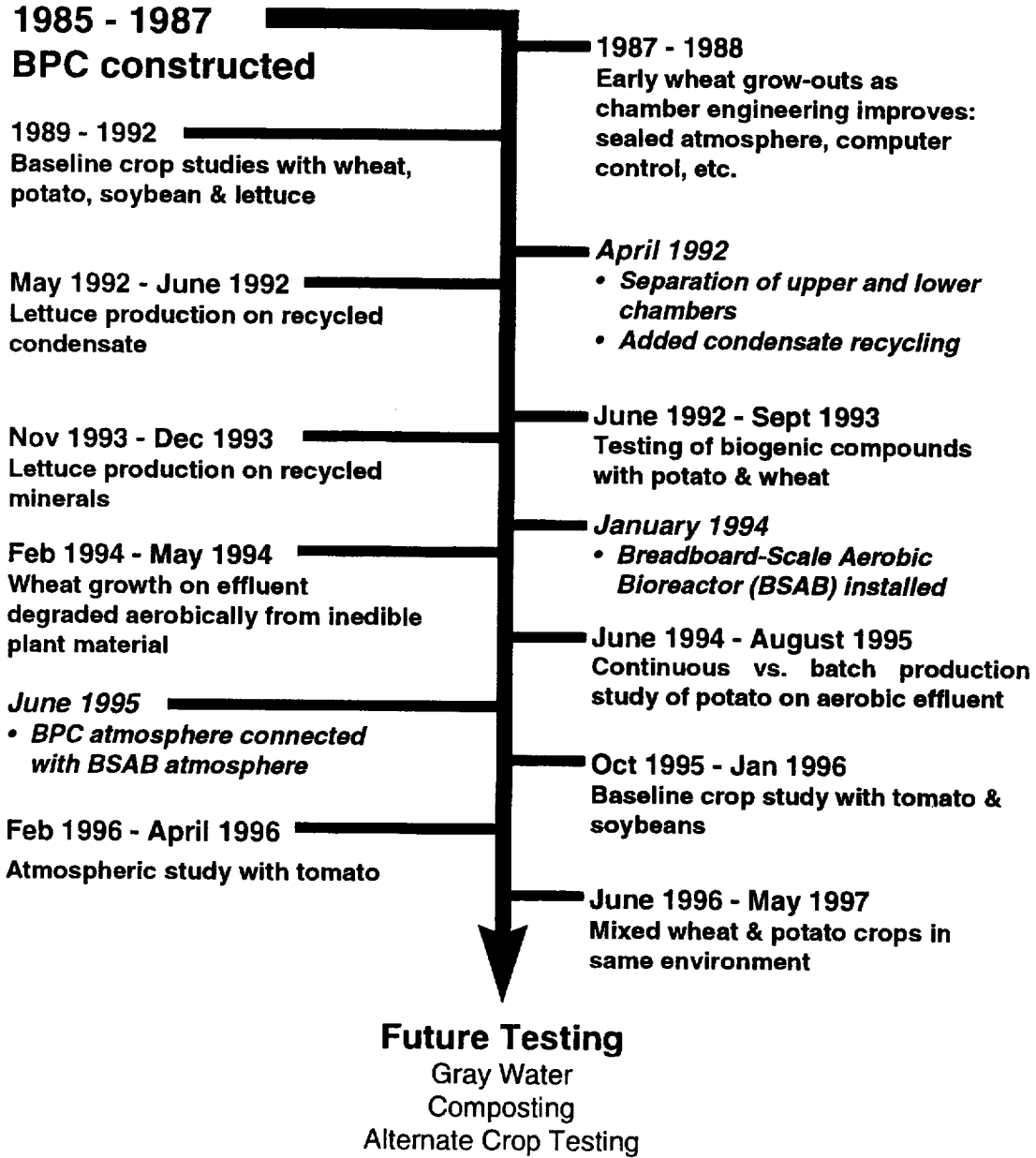
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Hoff, J.E., J.M. Howe, and C.A. Mitchell. 1982. Nutritional and cultural aspects of plant species selection for a regenerative life support system. NASA Contract Report for Grants NSG 2401 and 2404.

Salisbury, F.B. and M.A. Clark. 1996. Choosing plants to be grown in a controlled environment life support system (CELSS) based upon attractive vegetarian diets. *Life Sup. And Biospher. Sci.* 2:1699-179.

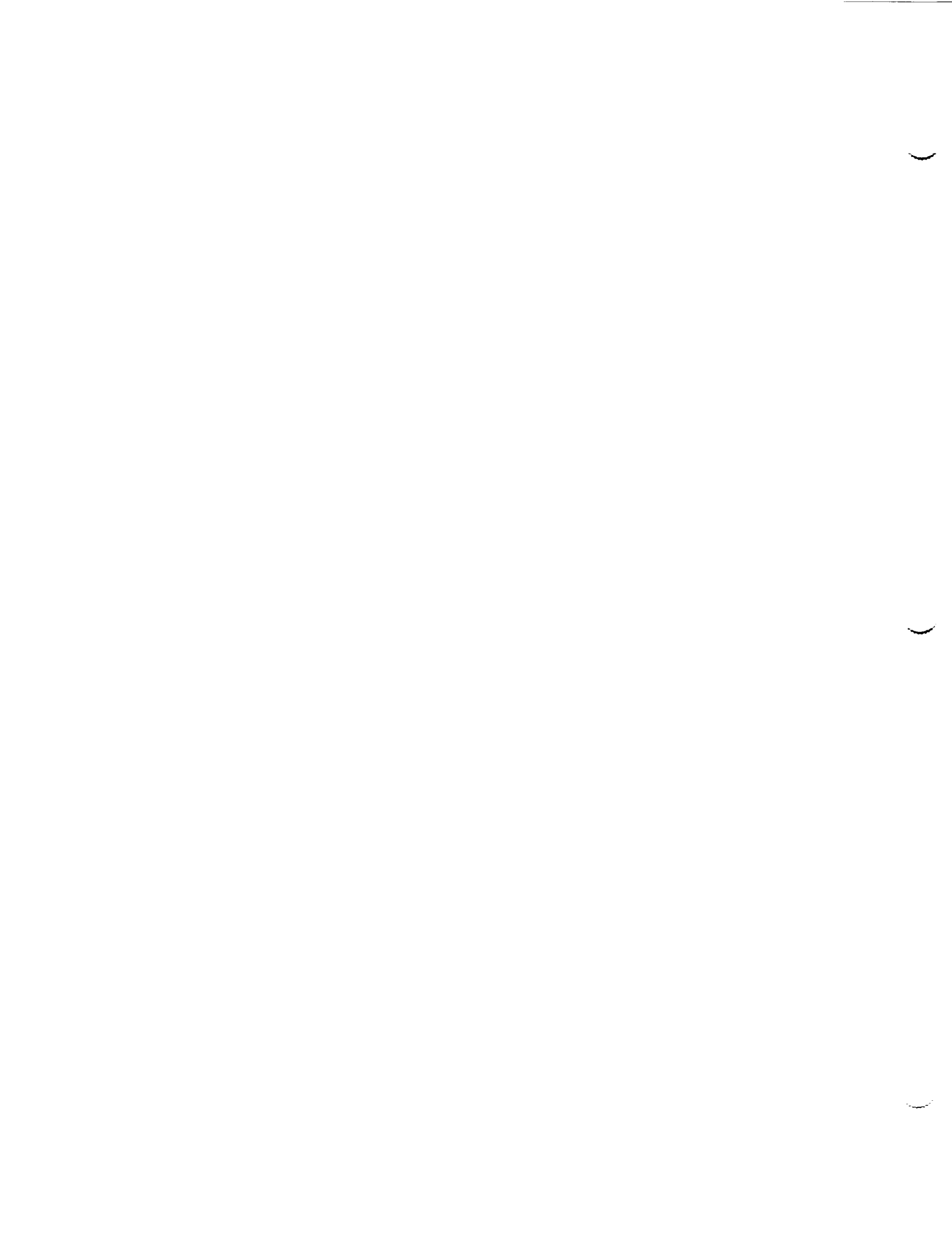
Tibbitts, T.W. and D.K. Alford. 1982. Controlled Ecological Life Support System. Use of higher plants. NASA Conf. Pub. 2231. Ames Research Center, Moffett Field, CA.

**Appendix B: Timeline of KSC Breadboard Project Activities**



**Appendix C: Abbreviations**

|          |  |
|----------|--|
| ALS      | Advanced Life Support                        |
| ARC      | Ames Research Center                         |
| BIO-Plex | Bioregenerative Life Support Systems Complex |
| BPC      | Biomass Production Chamber                   |
| CELSS    | Controlled Ecological Life Support System    |
| CSTR     | Continuous Stirred Tank Reactor              |
| EC       | Electrical Conductivity                      |
| EHT      | Early Human-Rated Tests                      |
| FFB      | Fixed-Film Bioreactor                        |
| HID      | High Intensity Discharge (lamp)              |
| HPS      | High Pressure Sodium                         |
| JSC      | Johnson Space Center                         |
| KSC      | Kennedy Space Center                         |
| LED      | Light-Emitting Diode                         |
| NDS      | Nutrient Delivery System                     |
| NFT      | Nutrient Film Technique                      |
| NRA      | NASA Research Announcement                   |
| PPF      | Photosynthetic Photon Flux                   |
| STS      | Space Transportation System                  |
| TOC      | Total Organic Carbon                         |



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