PRELIMINARY REPORT ON THE PHOTOSYNTHETIC GAS EXCHANGE POTENTIALITIES OF THE FAMILY LEMNACEAE (DUCKWEED)

S. S. Wilks

USAF SCHOOL OF AEROSPACE MEDICINE

The Development of Current Concepts of Biological Regenerative Systems in Closed Ecological Environments

The success of long-term space residence depends upon the capabilities of space technicians, scientists, and engineers for providing a living environment for the human cargo in space vehicles and/or space colonies. To accomplish these extraterrestrial "sorties", we must be able to compound an environment capable of sustaining human life for such a period as dictated by the flight profile. We must not exceed the irreversible limits of such factors as acceleration, cosmic radiation, temperature, nutritional requirements, accumulation of toxic environmental components, pressure and composition of environmental gaseous components, "physiological rhythmicity", and many others. With any space vehicle, the items of volume and mass of the cargo are of utmost importance.

For short-period orbital flights, many of the above problems are nearing solution; and it seems likely that space flights, designed to be completed within a time of 30 days or less, will probably be accomplished with nonregenerative systems supplying man's physiological requirements. The performance period of a nonregenerative system as a space-exploring vehicle would be determined by its load-carrying capabilities. To achieve any degree of success, a manned, space-exploring vehicle, without temporal limitations, will have to be a multiorganismal system capable of maintaining a flux equilibrum of total energies and a never-ending cycle of chemical reactions involving a multitude of organic compounds. Such a system, on a large scale, has evolved on the earth through countless eons of ages. To accomplish a small packaged ecology will tax to the utmost man's ingenuity and will rank in importance with the fission and fusion of the atom.

There appear two possible avenues of approach to the problem of regenerative systems: (1) proper selection and utilization of the minimal components of a multiorganismal system, including man, to give a miniature closed ecology, and/or, (2) decipher nature's secret of photoreception with the subsequent photo and chemosynthesis of the basic bioentities. Man has been intrigued for centures by the latter but still appears to be a long way from its solution. Concerning the former, there has been very limited effort in the development of sealed or isolated ecological systems, but with the advent of rocketry and nuclear-powered submarines, the need for studies on closed ecology is becoming more acute.

One of the prime considerations of a closed ecology is that the environmental gases shall remain physiologically tolerable to all of the ecologic components. Ideally, a photosynthetic gas exchange organism should possess the following characteristics: (1) highest gas/total mass exchange ratio (considering all paraphanalia incidental to growth, harvesting, processing, and final utilization), (2) amenable to confining quarters which may be imposed by inflexibility of rocket and/or space station design, (3) genetically and physiologically stable and highly resistant to all anticipated stresses, (4) edible and capable of supplying most or all of human nutritional requirements, (5) capable of utilizing the end products of raw or appropriately treated organic wastes, (6) controllable assimilatory quotient to maintain steady-state gas composition, (7) amenable to water recycling as demanded by other components of the ecosystem

Within recent years, most of the investigative work relating to photosynthetic gas exchange in closed ecologies has been carried out using the unicellular green algae: Myers (4,5,6), Bates (1), Zuraw (10). Most of these studies have been carried out in small units and the data obtained have been used as a basis for extrapolating logistic values relative to the use of these organisms as photosynthetic gas exchangers in manned space vehicles. Myers has shown that the quantity of algae necessary to support man (with an assumed O_2 requirement of 625 liters per day) would be in the order of 600-700 grams dry weight per day. If algal growth in mass cultures could be maintained in a steady-state concentration of 2.5 gm dry weight/liter and maintain such a growth rate as to yield 10 gm dry weight/liter/day, the volume of algal culture would be 60-65 liters and the total mass of the algal culture would approximate 200-250 pounds. Using an 8-liter system, investigators in our laboratory (Ward 9) have produced algal concentration of 5-7 gm/dry algae/liter with a high temperature algal strain (Sorokin #TX 71107). The maximum growth rate observed with the culture was .375 gm dry wt/l/hr. This was accomplished by using thin layers of culture (1 cm.) and a high light intensity (8,000 foot-candles).

Efforts to utilize multicellular plants as photosynthetic gas exchangers have been somewhat neglected. It has been assumed by many that the unicellular forms of green plants would be more efficient as gas exchangers than the multicellular plants. To determine the validity of this assumption, a series of investigations were initiated using small aquatic plants of the family Lemnaceae (duckweeds). These plants are small, primitive plants with a minimum of tissue differentiation. Practically all of the cells of the plant are chlorophyllated and apparently capable of photosynthetic activity. All species reproduce principally by a sexual budding of parent leaf-like fronds. Most all species are found floating on the surface of water and, by virtue of this, present no complexities in harvesting. Most species can be grown readily on almost any media suitable for the growth of autotrophic plants. The plants can be grown on surfaces kept moist with nutrient medium, Ney (8), thus greatly simplifying the problem of gaseous exchange. The fluid volume can be greatly decreased as compared with algae so that we attain a higher value of $\frac{\text{dry plant weight}}{\text{culture weight}}$. It is not difficult, using specially

designed chambers, to obtain 15–20 gm dry weight/liter of culture medium.

METHODS

Isolation and Cultivation of the Lemnaceae

Specimens of Spirodela, Lemna and Wolffia were obtained from their natural habitat (ponds and streams) within a radius of 50 miles of San Antonio, Texas. In addition to these cultures, specimens of Lemna and Spirodela were provided through the courtesy of William S. Hillman of Yale University, New Haven, Connecticut. The native cultures were carefully washed free of debris and treated with chlorox (10% of commercial solution) to kill the algae and bacteria. After submersion in the chlorox solution, the fronds were removed to small flasks containing Hoagland's (2) medium supplemented with 0.2% sucrose and 600 mg/L trytone. After immersion in the chlorox, some of the fronds will survive and be asceptic. These surviving specimens provided pure clones for subsequent studies. Permanent stock cultures were maintained on media enriched with sugar and organic nitrogen sources. Cultures for experimental purposes were obtained by growing some of the stock specimens under General Electric power groove, cool-white fluorescent lights at intensities from 50 to 400 foot-candles of illumination. The temperature at the beginning of the investigation fluctuated from 25°C to 40°C. Most observers, Landolt (3), find the temperature optima for the Lemnaceae to lie between 25 and 30°C.

Gas Exchange Studies

Separate cultures of Wolffia papulifers, Lemna minor and Spirodela polyhrrhiza were grown in a specially designed cylindrical plastic chamber 11 inches in diameter by 50 inches in length (Fig. 1). The tank, with accessory pumps, tubing and other ancillary components, was sterilized with ethylene oxide (5–8% in CO_2) for a period of 48 hours and subsequently flushed with decontaminated air for 24 hours to remove all of the sterilizing gas. Subsequent to sterilization, Hoagland's nutrient solution (6–8 liters) was introduced along with CO_2 (to a 3–5% level), the lights turned on and the drum set into rotation. The duration of this base-line period of 5–7 days made it possible to determine any diffusional losses as well as sterility of the system. The cylinder was rotated very slowly - 1 RPM - for at this speed, it had been previously established that the duckweeds, growing on the surface of the liquid medium, would be picked up on the inner surface of the cylinder and become surrounded by a thin layer of the nutrient medium. A design of this type can provide an extensive plant surface with a relatively small volume of nutrient fluid. During the "dry run", samples of gas were removed at intervals of one to four hours and analyzed for O_2, CO_2 and CO. The percent of CO_2 was determined with a Beckman LB-1 analyzer (sensitivity-full scale at two percent CO_2 in N_2). Oxygen percentage was determined with a Beckman F3 oxygen analyzer (sensitivity full scale at 25% O_2 in N_2). The CO concentration was measured with a Beckman LB-15A infrared analyzer and Brown electronik recorder (sensitivity full scale

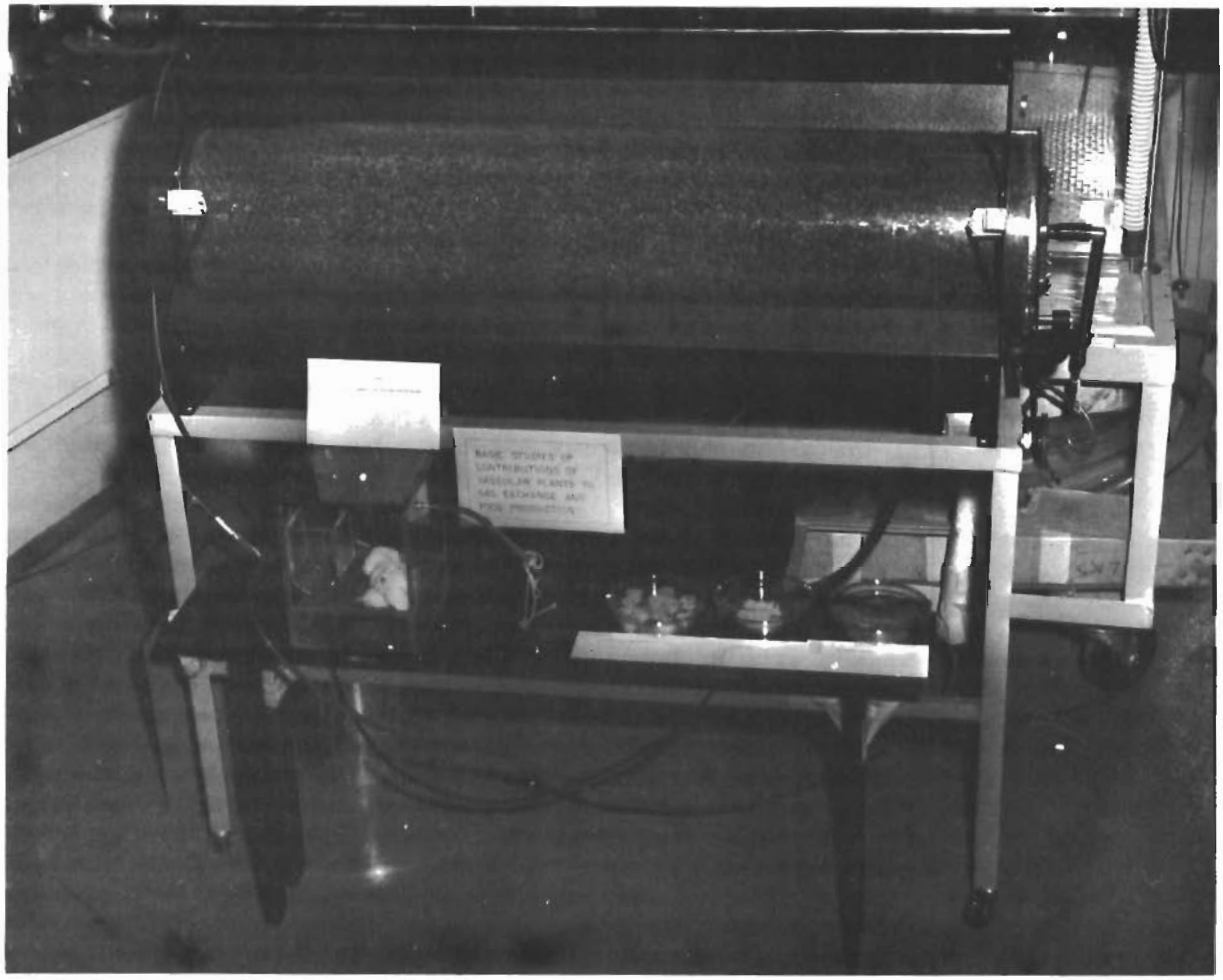

Figure 1. Cylindrical growth chamber used in cultivating duckweed plants. Two lights have been removed to show plants inside the chamber.

at 200 p.p.m. CO in nitrogen). The chamber gas was circulated by means of three dyna-vac pumps in parallel (capacity: 8 liters/min/pump). Gas leaving the tank was passed through a cooling coil to remove most of the water vapor and prevent condensation within the pump and the plastic tubes carrying the recirculated gas.

After establishing base-line data, the plants were introduced into the chamber through a door at the end. Sufficient CO_2 was introduced to give a 3-5 percent concentration. When the plants had grown sufficiently to cover the surface of the nutrient fluid, the cylinder was set into rotation. At this stage of the experiment, a 100 liter Douglas bag was inserted into the effluent gas line and pure CO_2 admitted to the influent line at a rate approximating its rate of uptake by the plants. Samples of gas were taken from the effluent line at intervals of one to two hours and analyzed for O_2, CO_2 and CO. At intervals of 12 to 16 hours, the gas volume in the bag was measured and the gas analyzed for its constituent gas composition. It was thus possible to determine the hourly gas exchange rate as well as the daily gaseous exchange. These data could later be correlated with growth rates as determined by plant frond area, wet plant weight or dry plant weight. The internal surface of the chamber which served as a plant surface (cylinder walls, ends of cylinder and surface of nutrient fluid at bottom of cylinder) approximated 12.5 $feet^2$ (1.16m^2). Measurement with Lemna, Spirodela and Wolffia gave values of approximately 0.125 gm wet weight or 8.75 mg dry weight/cm^2 of surface when the fronds formed a complete layer. Thus, a layer of plants covering the entire internal surface of the cylinder would have a dry weight mass of approximately 100 grams. With light intensity and temperature at optimum levels, it might be conceivable to have several layers of fronds on the illuminated surface. The limiting physical factor would likely be the inability of large masses of the plants to adhere to the surface of the cylinder under earth "G". Under a "G" factor of lower intensity, such as may be anticipated in a space or lunar station, it may be possible to accomplish this layering to greater thicknesses.

In the earlier phases of this investigation, attempts to measure gas exchange rates with Wolffia, Lemna and Spirodela were not entirely satisfactory because of a number of factors such as lack of adequate temperature controls, low intensity of illumination (occasioned primarily through lack of temperature control), bacterial contamination, occasional algal contamination, and occasional mechanical failure of the rotating mechanism.

The Light Factor

1. Growth rates of the Lemnaceae under continuous illumination:

Cultures of Spirodela, Lemna and Wolffia were grown in the laboratory in rectangular plastic petri dishes at a light intensity of approximately 200 foot-candles (cool-white fluorescent). The nutrient medium was Hoagland's solution with iron sequestrine as the chelating agent. In making the count, all detectable frond buds as well as mature

fronds were included. The objective of this study was to concentrate future activity on those specimens which appeared to grow best under average laboratory conditions.

2. Growth rates of Lemnaceae with varying periods of illumination:

Single fronds of Wolffia, Lemna and Spirodela were placed in plastic dishes containing Hoagland's medium and exposed to cool-white light of 200 foot-candle intensity (temp. 24-40°C.) and exposed to periods of illumination of 24, 15 and 9 hours daily. Plants placed in direct sunlight died, probably through overheating since there was no control of temperature.

3. Effect of light of different wave lengths on the growth of Wolffia:

Light-opaque wooden boxes were constructed and 6" x 6" narrow band Corning filter windows installed to control the wave length of light transmitted to the compartments containing the plants which were inclosed in plastic petri dishes. The fronds were counted at 1-2 day intervals and the increase in number used as a relative index of growth response. No attempt was made to maintain equal intensities of the light transmitted by the various filters used. Most of the filters possessed relatively high transmissivity in their respective transmission bands.

NUTRITIONAL STUDIES

Three hundred grams of dried duckweed (Wolffia) were ground in a ball mill to a fine flour and subsequently pressed into 10 gram cylindrical cakes with a hydraulic press set at 25,000 psi. These cakes were fed ad libitum to four adult, male, white mice for a period of 31 days as the sole source of food. The drinking water contained NaCl in the concentration of physiological saline. The mice were weighed at two-day intervals during the experiment.

RESULTS

Photosynthetic Gas Exchange Values

The changes in the concentration of oxygen and carbon dioxide are shown in Fig. 2. The total gas volume in the system was approximately 75 L and this gas was kept in continuous circulation at the rate of 20-25 liters per minute. Analysis of the effluent gas reflected the composition of the gas within the chamber. One of the recognized shortcomings of these experiments was the lack of temperature control. During the "runs", the temperature often rose to values of 35-39°C., well above the recognized optimum for the plants. It was also difficult to keep the large system free of bacteria and, occasionally, algal contaminations. It will be noticed from these figures that the gaseous exchange rates of Wolffia and Spirodela are almost identical whereas

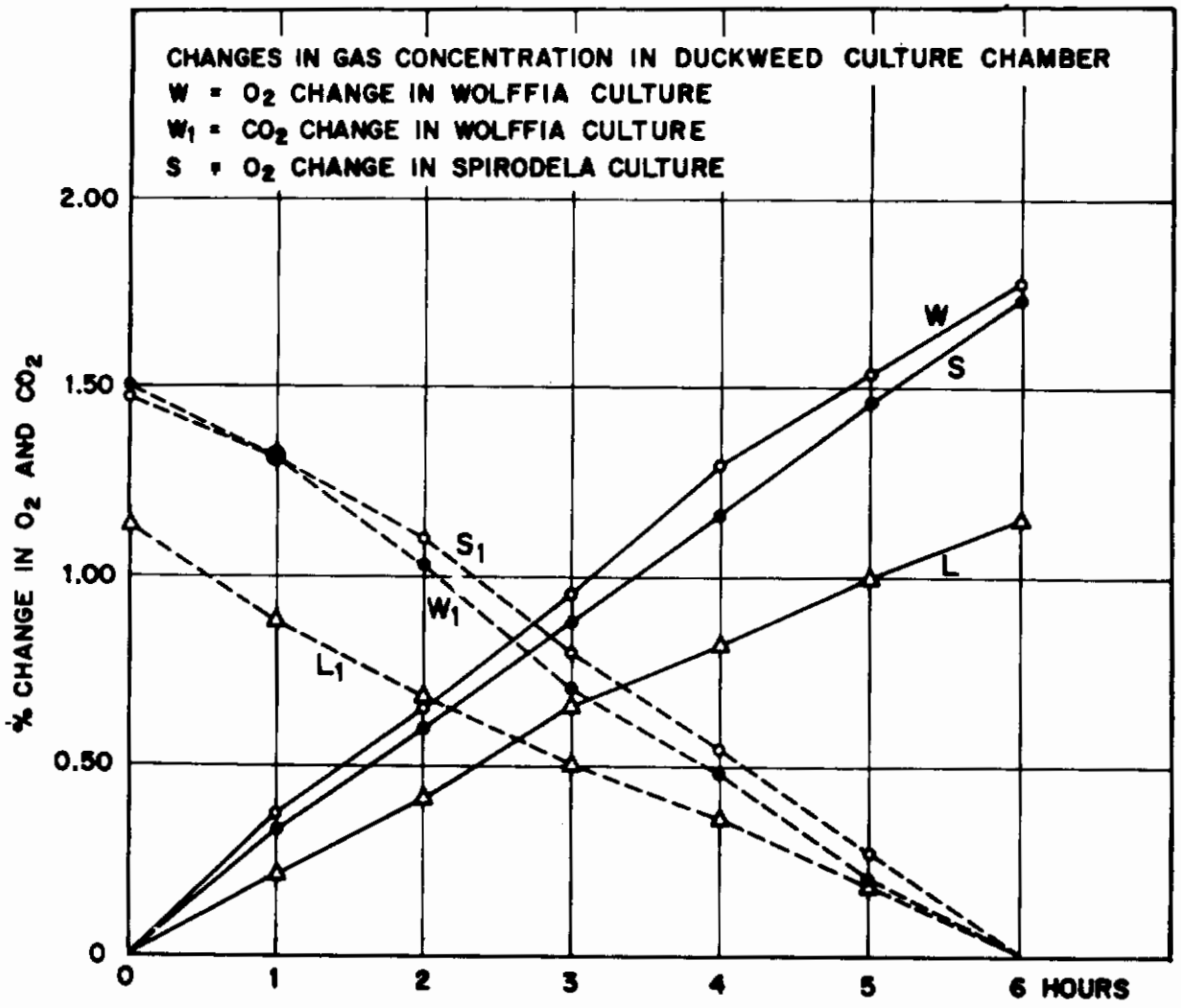

Figure 2. Changes in concentration of CO_2 and O_2 in the cylindrical growth chamber. Values obtained were averages of twelve 6-hour periods.

Lemna is somewhat lower. It will also be noticed that the AQ values for Wolffia and Spirodela approximate the RQ of man (with a balanced diet). The AQ value of Lemna under similar conditions was approximately unity. Of the three types of duckweed grown in the chamber, the greatest degree of success has come in the culture of Spirodela. At one time, a culture of this plant was maintained for a period of 90 days. During this period, approximately 300 grams of dry duckweed was obtained. The culture medium was replaced with fresh medium at 15-day intervals.

In a more recent study with controlled temperature (Fig. 3), a gas exchange rate of 19.2 liters/day was obtained. The curve shows that the exchange rate became light-limited since the addition of a 500 watt tungster lamp caused a sudden increase in the rate of oxygen release. This light could be used for only short periods because of inadequate cooling facilities. Under optimum conditions of light and temperature, it should not be difficult to obtain 25 liters or more of O_2 per day from a unit of this size.

GROWTH RATE STUDIES

The following table (#1) shows the division time for Lemna and Spirodela under continuous illumination of 200 foot-candles and temperatures of 30-39°C and atmospheric CO_2.

Table 1
Average Division Time of Lemna and Spirodela in Six Different Cultures of Clone Specimen

	Lemna	Spirodela
Minimum division time	12.00 hrs.	20.12 hrs.
Average division time	37.71 hrs.	34.15 hrs.
Maximum division time	51.75 hrs.	64.75 hrs.

These values could obviously be altered with optimal light intensity, temperature and CO_2 concentration. The above results were obtained from four separate cultures of each speciman at the end of a period of 14 days.

Growth of the Lemnaceae under filtered light.

Table 2 shows that maximum growth occurred in the plastic plates which had no light filter. The growth, when light was transmitted through plain window glass, was significantly lower than the controls. Of the 11 Corning glass filters used, only five showed an appreciable increase in the number of fronds; and of these five filters, only number six showed as much as 25 percent of the growth of the controls. This filter was a C.S. #3-70 with transmission between 500 and 4500 μm. Some frond development was observed with:

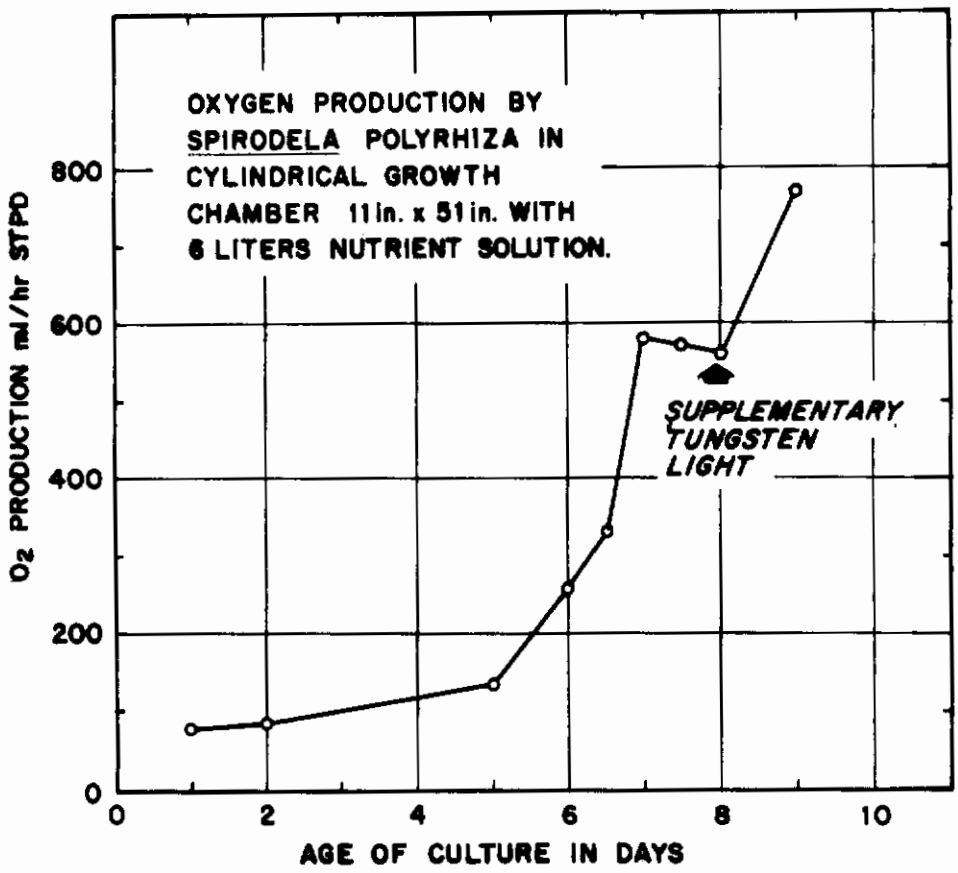

Figure 3 (left)

Rates of oxygen accumulation in cylindrical chamber with culture of Spirodela. At the end of the ninth day, the surface coverage by the plants was approximately 90 %.

Growth Rate of Wolffia with Light Transmitted through Corning Narrow Band Filters

FILTER No.	No. FRONDS 7th. DAY	No. FRONDS 19th. DAY	No. FRONDS 23rd. DAY
CONTROL (PLASTIC PETRI DISH)	20	98	220
WINDOW GLASS	25	60	151
CS # 9 - 54	18	22	26
CS # 0 - 54	12	17	17
CS # 3 - 80	12	15	19
CS # 2 - 61	7	12	14
CS # 3 - 70	16	33	54
CS # 7 - 60	3	3	3
CS # 5 - 56	3	5	5
CS # 7 - 69	4	4	4
CS # 7 - 57	3	4	4
CS # 7 - 54	4	4	4
CS 4 - 96	5	10	13

C.S. 9-54	220-4500 μm
C.S. 0-54	220-4500 μm
C.S. 3-80	480-4500 μm
C.S. 2-61	600-3500 μm
C.S. 3-70	490-4500 μm
C.S. 4-96	350-600 μm

Very little or no growth occurred with the filters:

C.S. 7-60	300-400 μm
C.S. 5-56	750-4250 μm
C.S. 7-59	720-1100 μm
C.S. 7-57	700-4250 μm
C.S. 7-54	230-420 μm

It is interesting to compare the growth when using filters C.S. 2-61 and C.S. 30-70. At 23 days, there were 54 fronds in the culture with the latter filter, whereas there were only 14 fronds with the C.S. 2-61 filter. The primary difference in filter characteristics is that C.S. 3-70 transmits 490 μm while C.S. 2-61 filter starts transmitting at 608 μm. Poor growth was obtained with all filters in which the 500-600 μm band was missing.

Growth of Lemnaceae under different light exposure periods.

Figure 4. shows a significantly greater number of fronds developing when the plant culture (Wolffia) was grown under 15 hours of light alternated with nine hours of darkness. With a reversal of these phases, the growth was considerably less. Those cultures grown with 15 hours of dark and nine hours of light show increased growth over the continuously illuminated cultures but less than that obtained with 15 hours of light and nine hours of darkness. In contrast to Wolffia, the Spirodela and Lemna, Figure 5, grew best under continuous illumination. In this experiment, averages were taken of the total fronds which were grown in triplicate for each condition of illumination. These cultures were grown under approximately 50 foot-candles of illumination and temperatures of 25-35°C and atmospheric gas concentration.

Nutritional Studies.

Even though this experiment was purely exploratory, it is suggestive that, for mice, a strict duckweed diet will sustain normal physiological activity for indefinite periods. At the end of the first week, the mice lost 8-10 percent body weight but this was regained during the second and third week of feeding. There were no visible signs or evidence of malnutrition or malfunction at the end of the 31 days on the diet.

DISCUSSION

Ney (8) has reported a very high gas exchange rate with the duckweeds. Using small cultures under controlled conditions of temperature, light (600-1000 foot-candles), and CO_2 concentration, this investigator

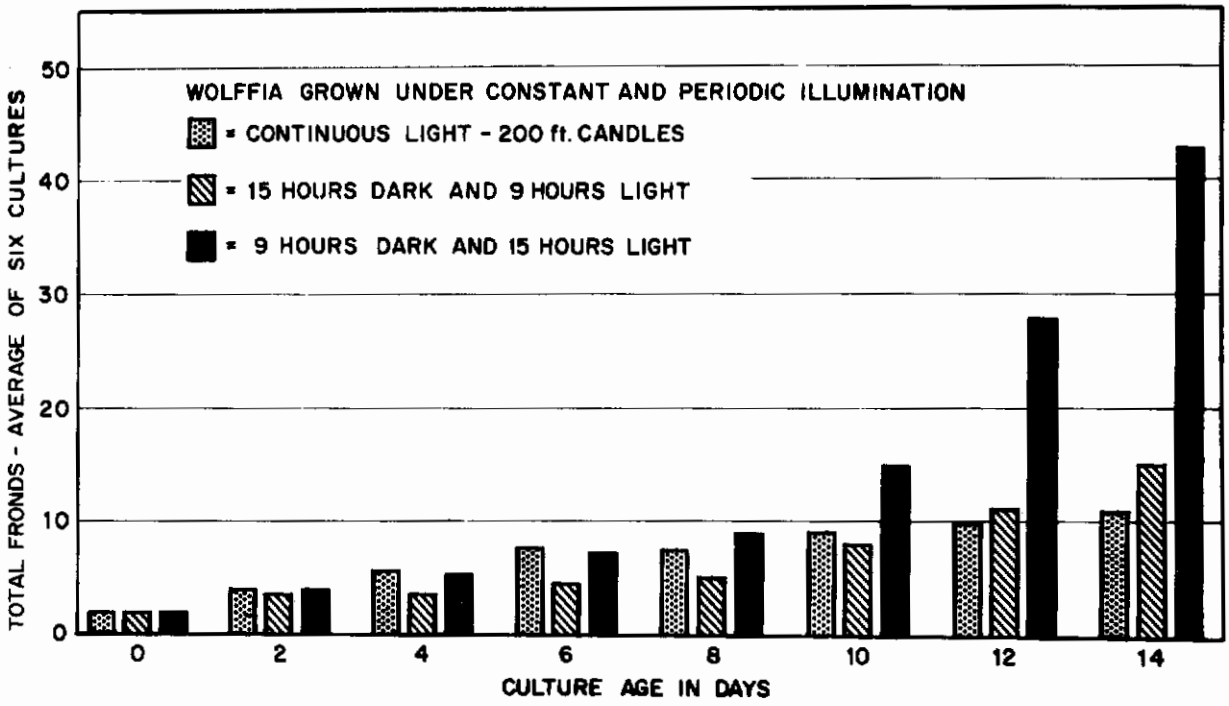

Figure 4. Growth of Wolffia under different periods of light and dark.

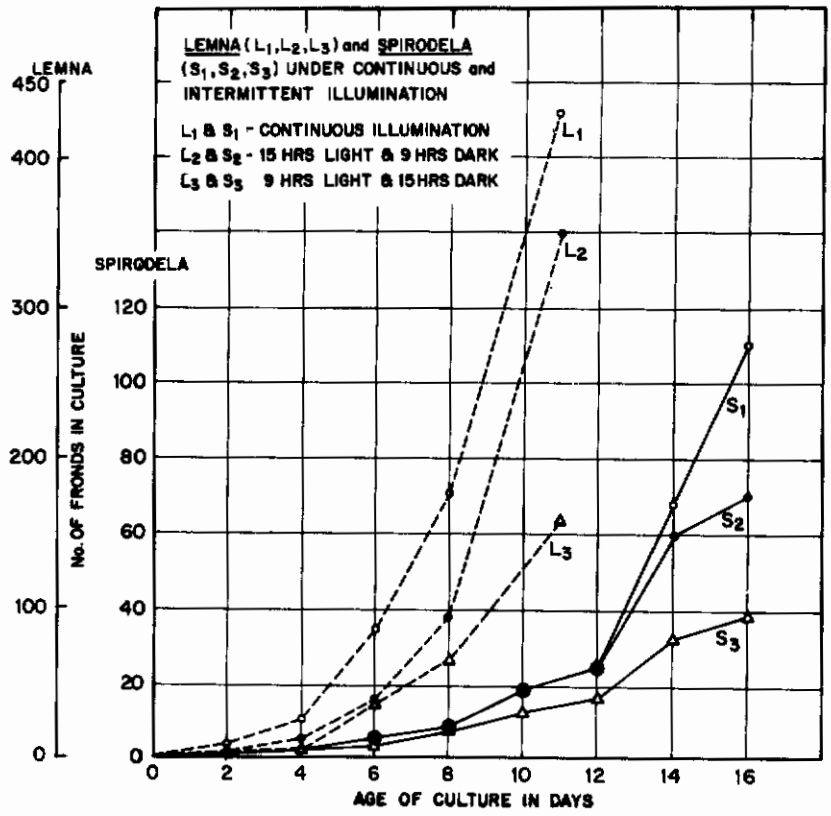

Figure 5. Growth of Lemna and Spirodela under different periods of light and Dark.

states that 25 square feet of frondal surface of duckweed, grown under optimum conditions, should have a gas exchange rate of one $L/ft^2/hr$. At this rate, 25 ft^2 of illuminated plant surface would provide sufficient gas exchange for one man. In his report Ney (8) states that a duckweed (Lemna) area of 2.5M^2 (25 ft^2) illuminated to an intensity level of 1000 foot-candles will exchange 25 L $CO_2 \longrightarrow O_2$/hour. In terms of plant growth, this would approximate 25 grams of dry plant material per hour. In our laboratory, Ward 10, with a thermophilic chlorella - Sorokin TX 71107, has obtained growth rates of 9 grams dry/L/day or .375 gm/L/hr. The culture system consisted of a rectangular plastic chamber .5M^2 and a one cm space between the plastic sides, illuminated on each side to an intensity of 4000 foot-candles (cool-white). Expressing the gaseous exchange in terms of area, this value of .375 gm dry algae/L/hr would become 3.00 gm dry/M^2/hr. Again, this growth could be expressed as 3.00 L O/M^2/hr. To produce 25 L O_2/hr, an area of 8-1/3 M^2 (85 ft^2) would be required. In comparing efficiency of light utilization, we take the ratio:

$$\frac{\text{fixed energy in plant material}}{\text{total incident light energy}}$$

In the current studies, we have been able to obtain an exchange greater than 100 $ml/ft^2/hr$. Data in this study are not comparable with the algae data above because of great differences in the light intensity (500 vs 8000 foot-candles) as well as the concentration of CO_2 (1% vs 2-5%). Other studies are in progress to better optimize the conditions of temperature and light control, increasing surface area, continuous replenishment of nutrients. We may, however, compare the efficiency of light utilization in the two systems:

$$\text{Efficiency of duckweed system from data of Ney} = \frac{\text{wt dry duckweed} \times 3.5 \text{ cal in mg/ft candle} \times 100}{\text{hrs to produce} \times \text{illuminated} \times 2.8 \times 10^{-3}}$$

Caloric value of a foot-candle/hr/Cm is 2.8×10^{-3}

$$\frac{25{,}000 \times 3.5 \times 100}{1 \times 1{,}000 \times 23{,}200 \times 2.8 \times 10^{-3}} = 135\%$$

Algal efficiency - data from Ward (10)

$$\frac{25{,}000 \times 3.5}{1 \times \frac{8000}{2} \times 83{,}000 \times 2.8 \times 10^{-3}} \times 100 = 11.4\%$$

Efficiency of the duckweed from this study at 500 foot-candle

$$\frac{19{,}000 \times 3.5}{24 \times 500 \times 10{,}000 \times 2.8 \times 10^{-3}} \times 100 = 19\%$$

Nutritional studies on the duckweeds is very meager. Nakamura (7) has considered the duckweed as a possible source of food for space travel. According to this author, Wolffia contains much starch. Vitamins A, B_2, B_6 and C were detected in the plant, with C the most abundant. Analysis

of the whole plant showed:

Carbohydrates	60-25%
Protein	8-10%
Fat	18-20%
Minerals	6-8%
Chlorophyll	1-2%

Since one of the biggest gains in the algal system is the extremely high growth rates achieved by thermophilic strains of Chlorella, under optimum conditions, it might not be amiss to search for a possible variant among the Lemnaceae which has similar characteristics. One of the desirable features of a duckweed system is that the gas exchange is direct between the atmosphere and the plant and does not require an elaborate system of dissolving the respiratory gases in a bulky fluid system which introduces special engineering difficulties in zero or sub-gravity conditions.

In the design of equipment for closed ecological system studies, careful consideration should be given to the material used in the construction of the unit. Most plastic materials are subject to photo-oxidative degeneration, with carbon monoxide as one of the products. This author has found that air, recirculated through plastic systems, including the common brands of plastic tubing and transparent rigid plastics in the presence of light, will give off considerable quantities of carbon monoxide. With high intensity illumination such as sunlight, a CO buildup of several hundred parts per million is not uncommon. Also plant pigments such as the carotenoids and chlorophylls will react similarly when exposed to light of high intensity. If the plants die, then the CO is released quite rapidly.

REFERENCES

1. Bates, H., "Recent Aspects in the Development of a Closed Ecologic System," Aerospace Medicine, 32: 12-24, 1961.

2. Hoagland, D.R., "Environmental Control of Plant Growth," F.W. Went Chronica Botanica Company, Waltham, Massachusetts

3. Landolt, E., "Physiologische und Okologische Unter suchungen an Lemnaceen," Ber. Schweiz. Bot. Gen., 67: 271-410, 1957.

4. Myers, J., "Study of a Photosynthetic Gas Exchange as a Method of Providing for the Respiratory Requirements of the Human in a Sealed Cabin, Part I," Report to the Air Research and Development Command, USAF School of Aviation Medicine, Randolph Air Force Base, Texas, 1955.

5. Myers, J., Ibid, Part II, 1957.

6. Myers, J.E. and Brown, A.H., "Gas Regeneration and Food Production in a Closed Ecological System," National Academy of Sciences, National Research Publication, 839, Washington, D. C., 1961.

7. Nakamura, H., "A Study of Wolffia as a New Food," Reports from the Microalgal Research Institute of Japan," 1: 7-13, 1960.

8. Ney, L.F., "Gas Exchange by the Duckweed Family," ONR Contract No. NOnr-2887(00), Stanford Research Institute, Menlo Park, California, 1960.

9. Ward, C.H., USAF Aerospace Medical Center, Brooks Air Force Base, Texas, Unpublished data.

10. Zuraw, E.A., Christiansen, G.D., Kippax, D.L., Benoit, R.J., Richards, N.L., Huppert, I.N., Leone, D.L., Gaucher, T.A., Trainer, F.R., Noel, F.F., "Photosynthetic Gas Exchange in the Closed Eco-System for Space," NASA Contract No. NASW-95, General Dynamics Corporation, Electric Boat Division, Groto, Connecticut, 1960-1961.