OBSERVATIONS ON GAS EXCHANGE AND ELEMENT RECYCLE WITHIN A GAS-CLOSED ALGAL-MOUSE SYSTEM

David T. Smernoff, Robert A. Wharton Jr.
and Maurice M. Averner
Complex Systems Research Center
University of New Hampshire
Durham, N.H. 03824

ABSTRACT

Life support systems based on bioregeneration rely on the control and manipulation of organisms. Algae are potentially useful for a variety of CELSS functions including the revitalization of atmospheres, production of food and for nitrogen fixation. We report the results of experiments conducted with a gas-closed algal-mouse system designed to investigate: 1) gas exchange phenomena under varying algal environmental conditions, and 2) the ability of algae to utilize oxidized mouse solid waste.

Inherent instabilities exist between the uptake and release of carbon dioxide (CO_2) and oxygen (O_2) by the mouse and algae in a gas-closed system. Variations in light intensity and cell density alter the photosynthetic rate of the algae and enable short-term steady-state concentrations of atmospheric CO_2 and O_2. Different nitrogen sources (urea and nitrate) result in different algal assimilatory quotients (AQ). Combinations of photosynthetic rate and AQ ratio manipulations have been examined for their potential in stabilizing atmospheric gas concentrations in the gas-closed algal-mouse system.

Element cycling experiments include wet oxidation of system waste materials for use as an algal nutrient source. Oxidized waste products demonstrate inhibitory properties although dilution has been shown to allow normal algal growth. Characterization of the nature of the inhibitory material has begun.

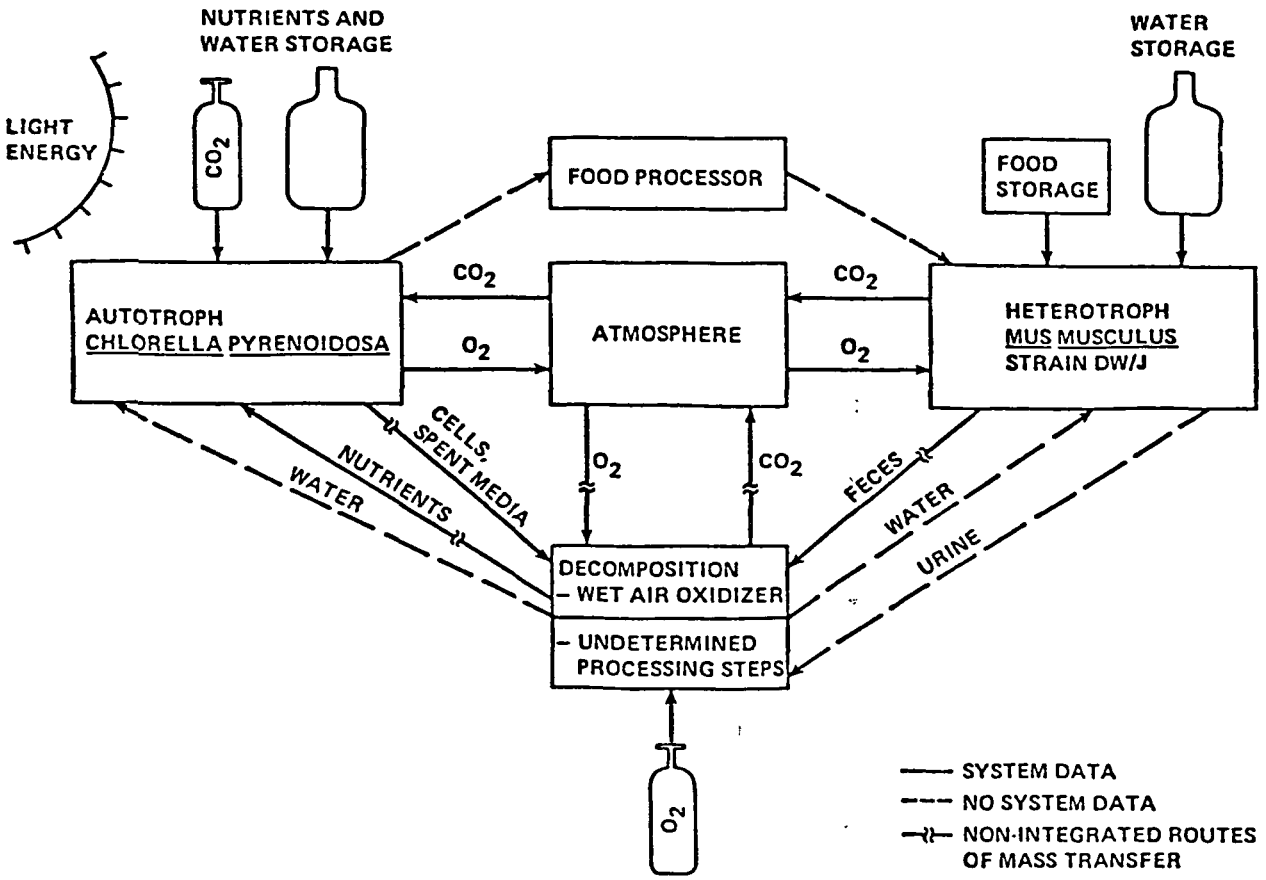

FIGURE 1. EXPERIMENTAL GAS-CLOSED MOUSE-ALGAL-WET OXIDIZER SYSTEM

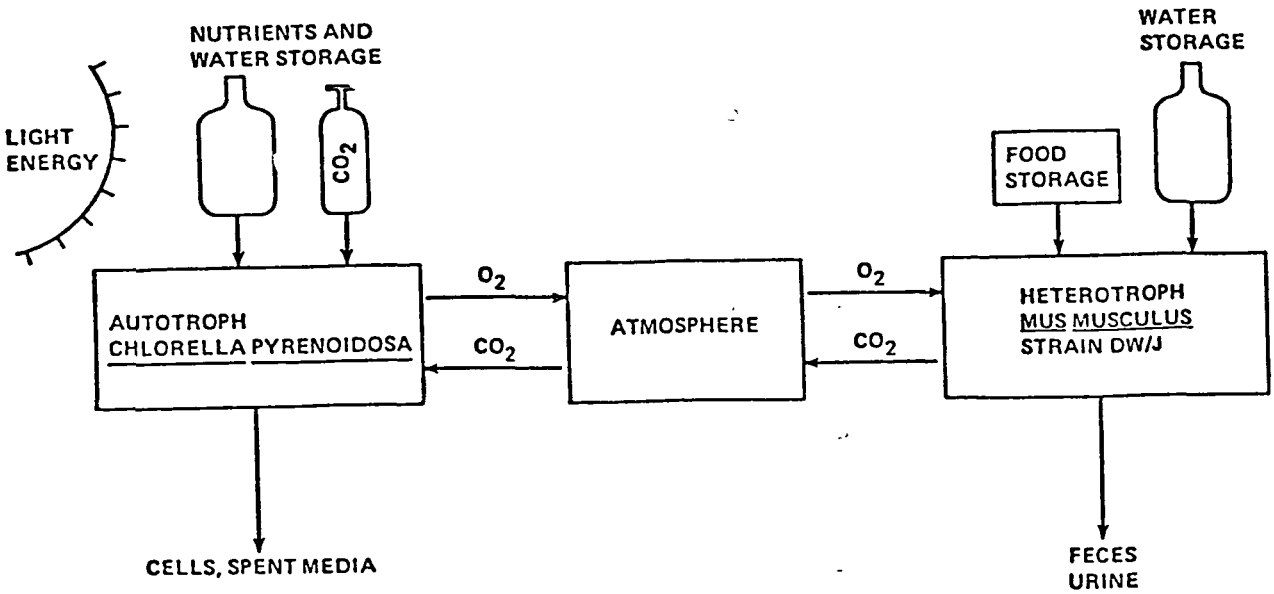

FIGURE 2. EXPERIMENTAL GAS-CLOSED MOUSE-ALGAL SYSTEM

INTRODUCTION

The development of controlled ecological life support systems (CELSS) relies, in part, on the ability to manipulate and control the organisms which are a part of the system. Algae are considered useful for several CELSS functions including the revitalization of atmospheres, production of food and for nitrogen fixation. Techniques to accomplish these functions using algae enhance the development of an operational CELSS by addressing control issues, and enhancing reliability and stability by increasing the available options.

The research reported is directed at understanding how an integrated CELSS might function and particularly to identify any problems which may occur. The strategy has been to control a small model CELSS based on algae and mice and to study its behavior under different operating conditions. The major system components (Fig. 1) are; the green alga Chlorella pyrenoidosa, the dwarf mouse strain DW/J and a bench scale wet-air oxidation (WAO) reactor. Primarily we are concerned with the atmosphere behavior within the gas-closed algal-mouse system, additionally we have initiated studies to determine the degree of element recycle possible. As indicated in Figure 1 the waste processing subsystem is not physically coupled to the algal-mouse system. This limits the ability to determine the recyclability of the system although preliminary analysis of system interactions is possible.

METHODS

Figure 2 schematically represents the operation of the algal-mouse system without the incorporation of waste oxidation into the system. The majority of data presented in this paper is concerned with the dynamics of gas exchange between the algae and the mouse under a variety of algal growth conditions. Parameters varied are the optical density of the cultures, the light intensity which the algal reactors receive and the nitrogen source in the algal media. Carbon dioxide is supplied either from cylinders or from the mouse reactor. Mouse food and water are externally supplied and oxygen is supplied from cylinders or the algal reactors.

Figure 3 shows details of the experimental algal-mouse system. It is comprised of three gas-tight reactors, two of which support continuous algal growth and one which houses a mouse. The system is designed to operate in either a gas flow-through mode or in a gas-closed mode. Measurement of atmospheric concentrations of oxygen (O_2 and carbon dioxide (CO_2) is accomplished with paramagnetic and infrared analyzers, respectively. The algal reactors are operated as turbidostats with optical density of the cultures being controlled by the addition of fresh media. The operation of the system includes the use of a computer to collect data and to operate pumps and valves used to alter system states.

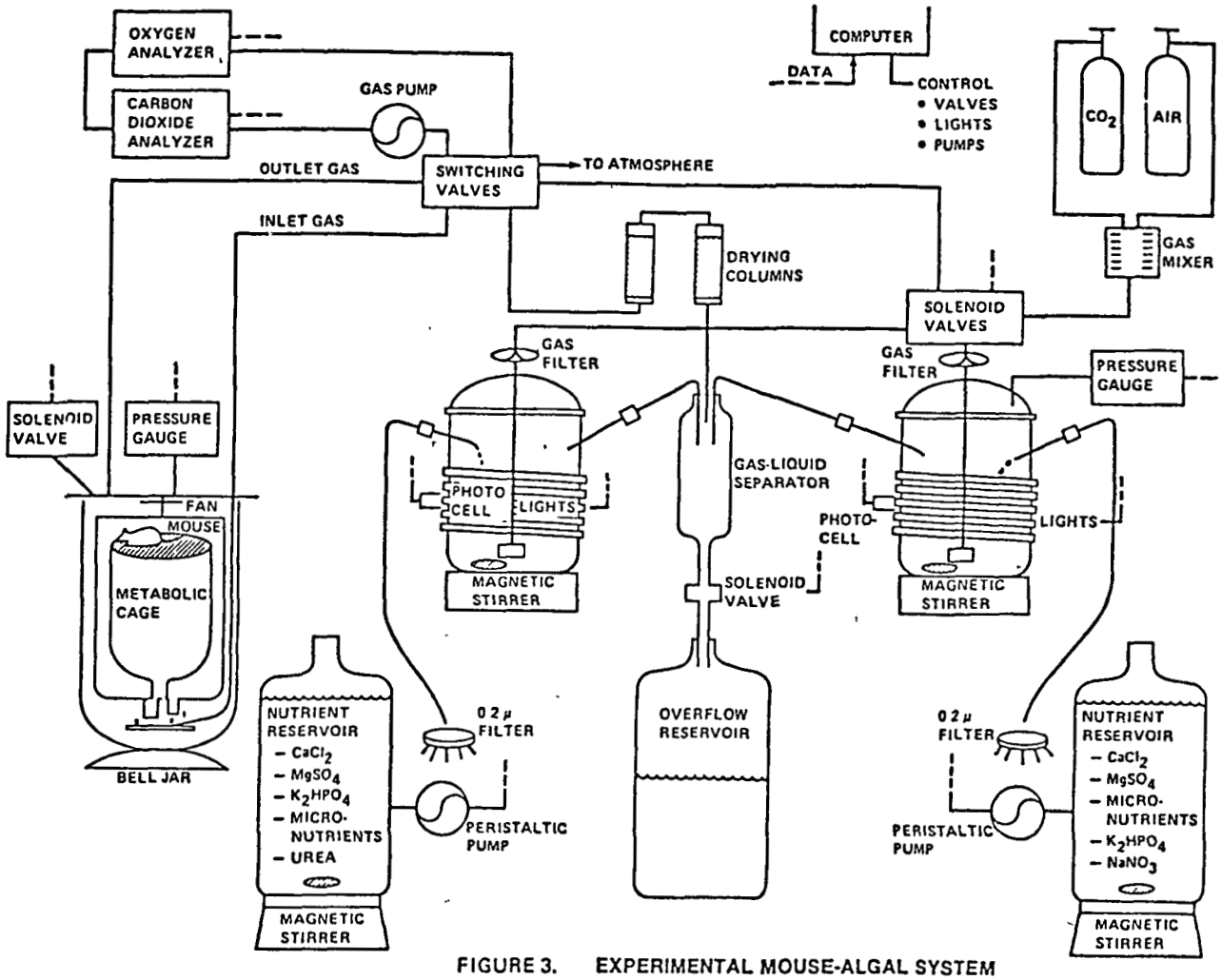

FIGURE 3. EXPERIMENTAL MOUSE-ALGAL SYSTEM

RESULTS AND DISCUSSION

Three types of gas exchange experiments are conducted using the system. Measurement of mouse respiratory quotients (RQ) is done by closing the mouse in the reactor with ambient atmospheric concentrations of CO_2 and O_2 and observing the changes in each gas concentration over time. The RQ is calculated from the change in CO_2 divided by the change in O_2 (RQ = moles CO_2 produced/moles O_2 consumed). Figure 4 graphically represents system behavior with a mouse only. Short-term measurements of mouse RQ have been determined to be 0.975 $\pm$0.06 (n=5).

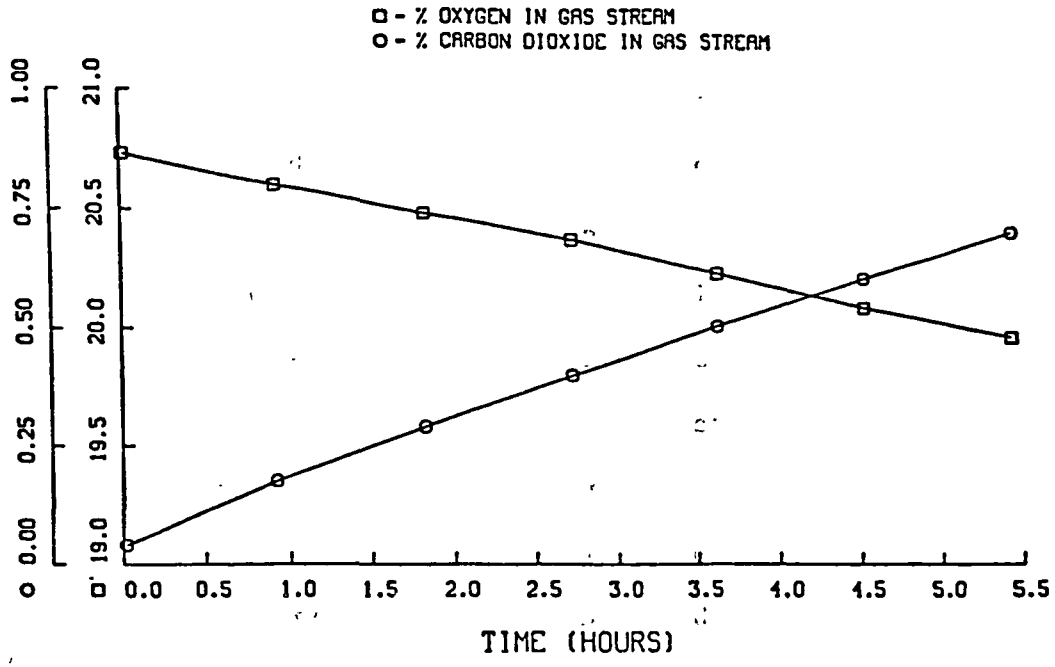

FIGURE 4. CO2-O2 RELATIONSHIP IN A GAS-CLOSED MOUSE SYSTEM
Measurement of Mouse Respiratory Quotients

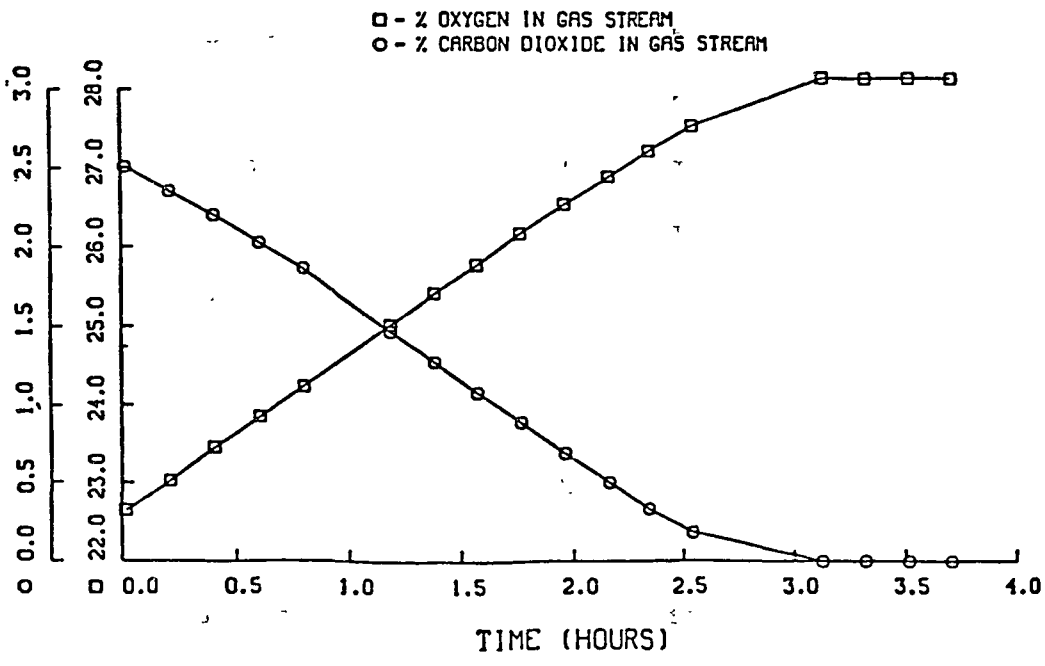

FIGURE 5. CO2-O2 RELATIONSHIP IN A GAS-CLOSED ALGAL SYSTEM
Measurement of Algal Assimilatory Quotients, Nitrate (OD 0.61)

The second type of experiments determine the algal assimilatory quotient (AQ) in a similar fashion. The algal cultures are normally supplied with CO_2 (2%) enriched air. When AQ measurements are made, the gas flow from the cylinders is stopped and the gas within the system is recirculated using a pump. The slopes of the CO_2 and O_2 concentrations are then used to calculate the AQ (AQ = moles CO_2 consumed/moles O_2 produced). Figure 5 exhibits the opposite set of responses from Figure 4, as would be expected. The third set of experiments, to be discussed later, are conducted with the mouse and algal reactors coupled.

Due to metabolic differences between the mouse and the algae the AQ and the RQ are not the same. This inherent mismatch will result in depletion of one atmospheric component. Therefore, in order to maintain stable concentrations of CO_2 and O_2 within a coupled mouse-algal system, it is necessary to control either the AQ or the RQ. Control of the RQ is possible but not a realistic option for use in a CELSS. Therefore, techniques to match the AQ of the algae to the RQ of the mouse are examined. One technique studied takes advantage of the observed difference in AQ between cultures grown on nitrate and those grown on urea. Table 1 exhibits the variation in AQ between nitrate and urea grown cultures.

TABLE 1: AQ as a Function of Nitrogen Source

	NITRATE	UREA
ASSIMILATORY QUOTIENT (AQ)	0.50 $\pm$ 0.07	0.77 $\pm$ 0.12
Sample size	(n=25)	(n=28)

The data in Table 1 was obtained from bacterially contaminated cultures. Our experience indicates that it is extremely difficult to maintain axenic algal cultures for long time periods. Therefore, it is valuable to establish AQ values for pure and contaminated cultures grown on nitrate or urea. Figure 6 exhibits the variation in AQ for pure and bacterially contaminated cultures grown on nitrate. The two uncontaminated points with AQ's near 0.45 were from a run which initially appeared to be pure, but which later showed bacterial contamination. From this information we can make the assumption that even low levels of bacterial contamination will have an effect on the apparent AQ of the algal culture. The large variation in AQ for contaminated runs is probably due to different bacterial population sizes. The uncontaminated points clustered around an AQ of 0.7 showed no bacterial

contamination, further supporting this hypothesis. Further data must be collected concerning the relative population sizes of bacteria and algae and how this affects the apparent AQ. Precise information on this interaction will allow greater control of algal gas revitalization systems.

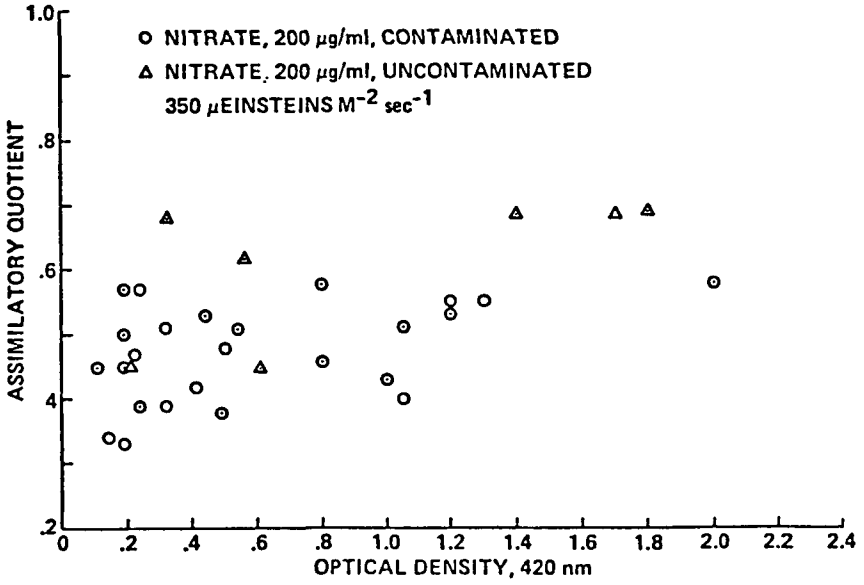

FIGURE 6. VARIATION OF ASSIMILATORY QUOTIENT AS A FUNCTION OF OPTICAL DENSITY

Another parameter which affects algal gas revitalization characteristics is the light intensity which the cultures receive. In combined algal-mouse runs variation in light intensity has been shown to allow control of gas exchange mismatches. Figure 7 demonstrates an initial system state which we refer to as a photosynthetic mode. In other words, the oxygen

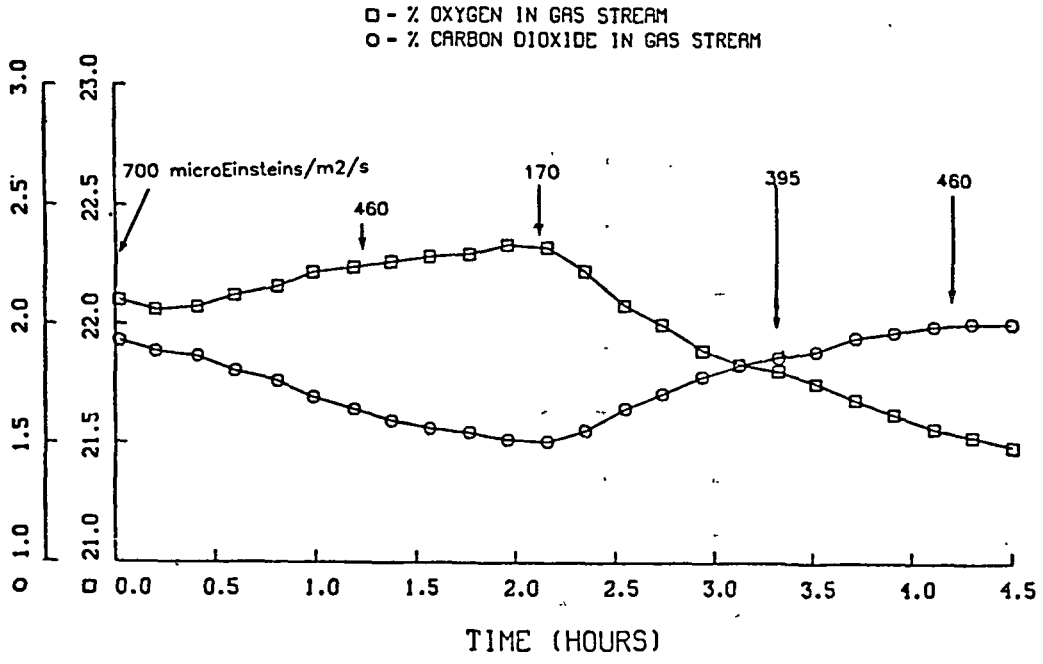

FIGURE 7. CO2-O2 RELATIONSHIP IN A GAS-CLOSED ALGAL-MOUSE SYSTEM
Effect of Variation in Light Intensity (Nitrate R1 OD 2.1 R2 OD 1.7)

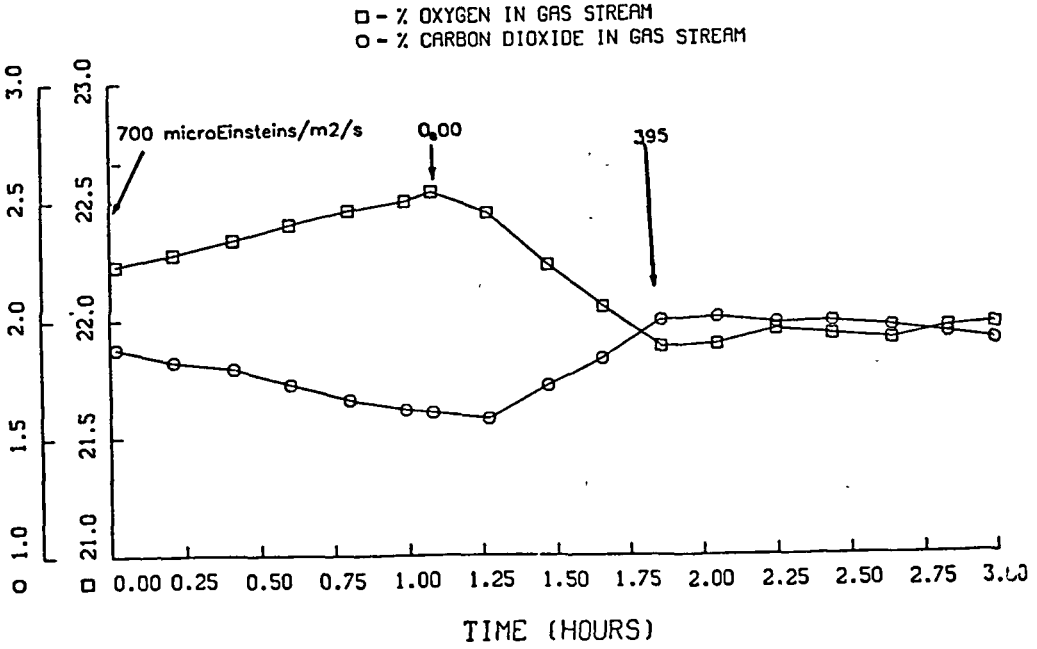

FIGURE 8. CO2-O2 RELATIONSHIP IN A GAS-CLOSED ALGAL-MOUSE SYSTEM
Effect of Variation in Light Intensity (Urea R1 OD 0.77, R2 OD 0.81)

production of the algae exceeds the oxygen uptake of the mouse. By lowering the light intensity (at 1.1 hours) we reduce the photosynthetic rate of the culture and lower the oxygen output. Further reductions in light intensity at 2.0 hours shifts the system state to a respiratory mode (i.e. oxygen consumption of the mouse exceeds oxygen production by the algae). Further changes in light intensity at 3.0 and 4.0 hours restore the system to a photosynthetic mode. Although figure 7 does not demonstrate a gas-stable system it does show that control can be achieved by photosynthetic rate manipulations. Figure 8 shows a system state in which variation of light intensity results in a relatively stable system state.

Figures 9 and 10 show what we refer to as the crossover area between photosynthetic and respiratory modes. The shaded area indicates the uncertainty associated with the data. The difference in algal AQ between nitrate and urea grown cultures is also evident in the crossover area curves. Refinement of this data will allow predictions to be made concerning the system states for cultures maintained at selected optical densities, light intensities and nitrogen sources. The significantly different gas-exchange characteristics at selected operating regimes may then be exploited for control purposes. Multiple reactors, each with distinctive gas exchange characteristics may be used to operate the system in a stable fashion with minimal control requirements.

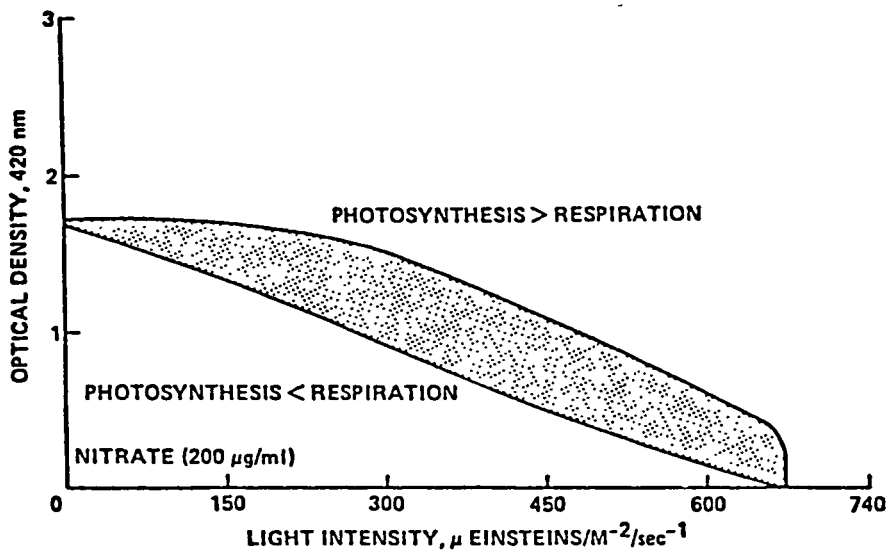

FIGURE 9. CROSSOVER AREA AS A FUNCTION OF OPTICAL DENSITY AND LIGHT INTENSITY

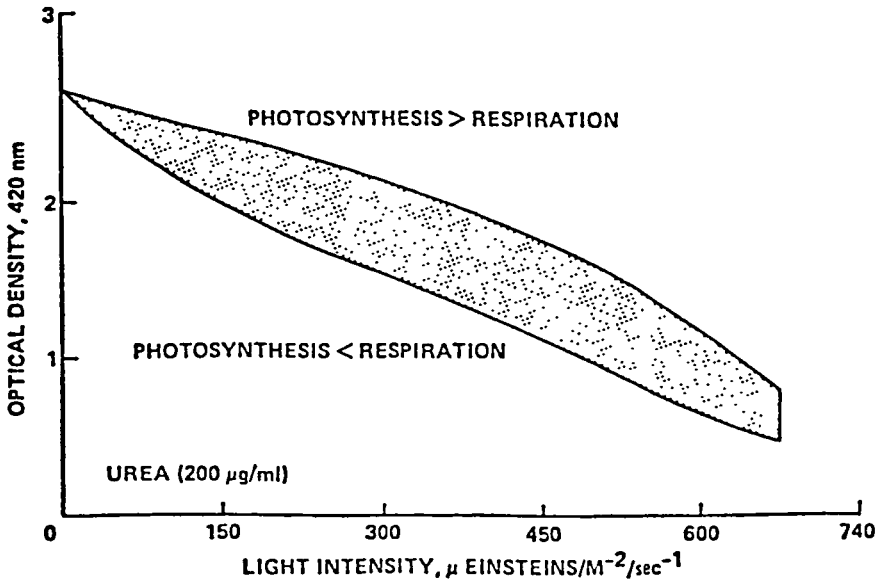

FIGURE 10. CROSSOVER AREA AS A FUNCTION OF OPTICAL DENSITY AND LIGHT INTENSITY

Figure 11 exhibits a system state in which gas concentrations show only slight variation. The operating characteristics involve only the manipulation of algal AQ by use of different nitrogen sources. The graph exhibits that two reactors, one growing on nitrate and the other on urea, can maintain stable atmospheric CO_2 and O_2 concentrations within a closed system. In order to show the feasibility of this technique for CELSS application it must be demonstrated that stability can be achieved for time periods much greater than 7 hours.

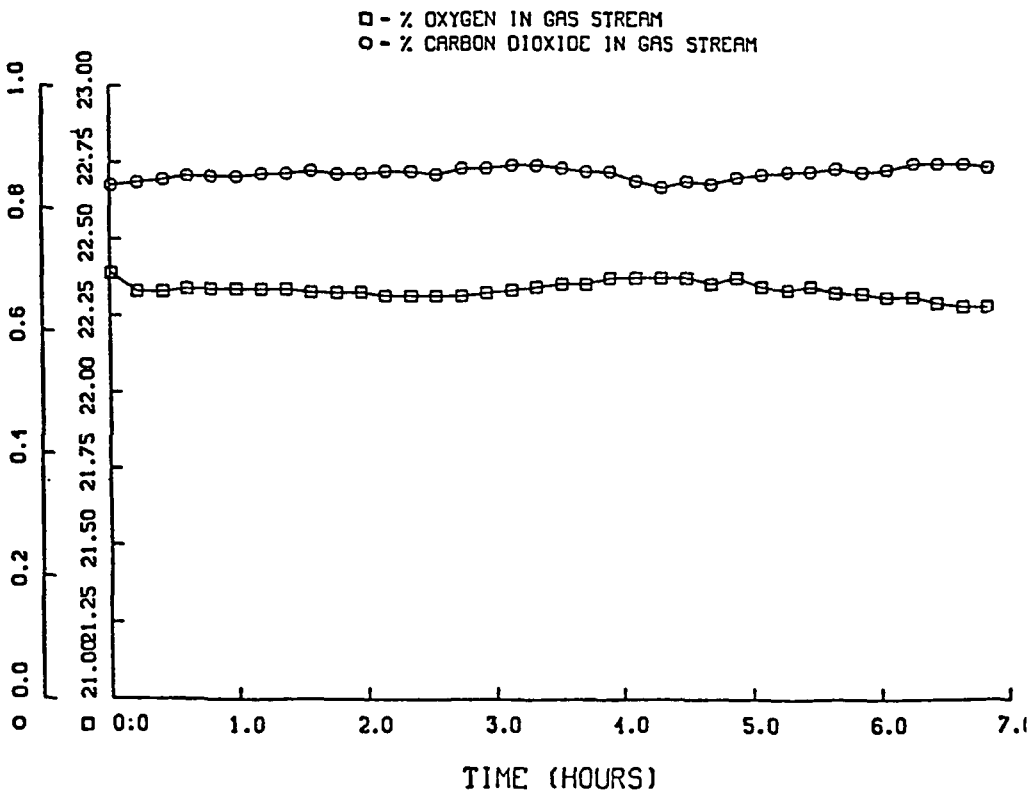

FIGURE 11. CO2-O2 RELATIONSHIP IN A GAS-CLOSED ALGAL-MOUSE SYSTEM
R1 Nitrate (OD 1.64): R2 Urea (OD 4.00): 700 microEinsteins/m2/s

The response of the system seen in Figure 11 does not agree with the response which would be predicted from the crossover area curves. The experimental conditions, as observed on the crossover area curves, would be predicted to lead to a system state in photosynthetic excess. However, a balance between photosynthesis and respiration is observed. In fact, at about 5.0 hours a respiratory trend is beginning to emerge which is directly in opposition to crossover area predictions.

There are several possible explanations for this discrepancy. First is the uncertainty of the crossover area curves. More data is required in order to refine the crossover area into a crossover point, this will allow more accurate prediction of system behavior. Secondly, the sum of subsystem behaviors (e.g nitrate and urea grown cultures) may not equal the sum of the overall system behavior. The crossover area curves were derived from urea and nitrate grown cultures operating independently, the crossover area for urea and nitrate cultures operating together may be different. To verify this hypothesis further data acquisition is required. A third possible answer may be due to the large mouse chamber volume which will significantly delay system response. Experiments conducted with a small volume mouse chamber show greater instability for combined urea-nitrate conditions, indicating that system volume is affecting observed system states. Comparison of small reactor to large reactor data and longer runs will be required in order to determine the

effect of large mouse reactor volume on system behavior.

Returning to Figure 1, we will now look at material recycling within the experimental system. As stated earlier, the system is not fully integrated which limits the ability to determine mass balance for the system. However, we have conducted a series of experiments to determine if algal growth can be supported by the fecal output of the mouse. Figure 12 shows the growth of algae on a wet-oxidized sample of mouse feces. A 1:1 dilution of the fecal wet-oxidate inhibits algal growth while a 1:15 dilution of the same material shows growth equal to a positive control.

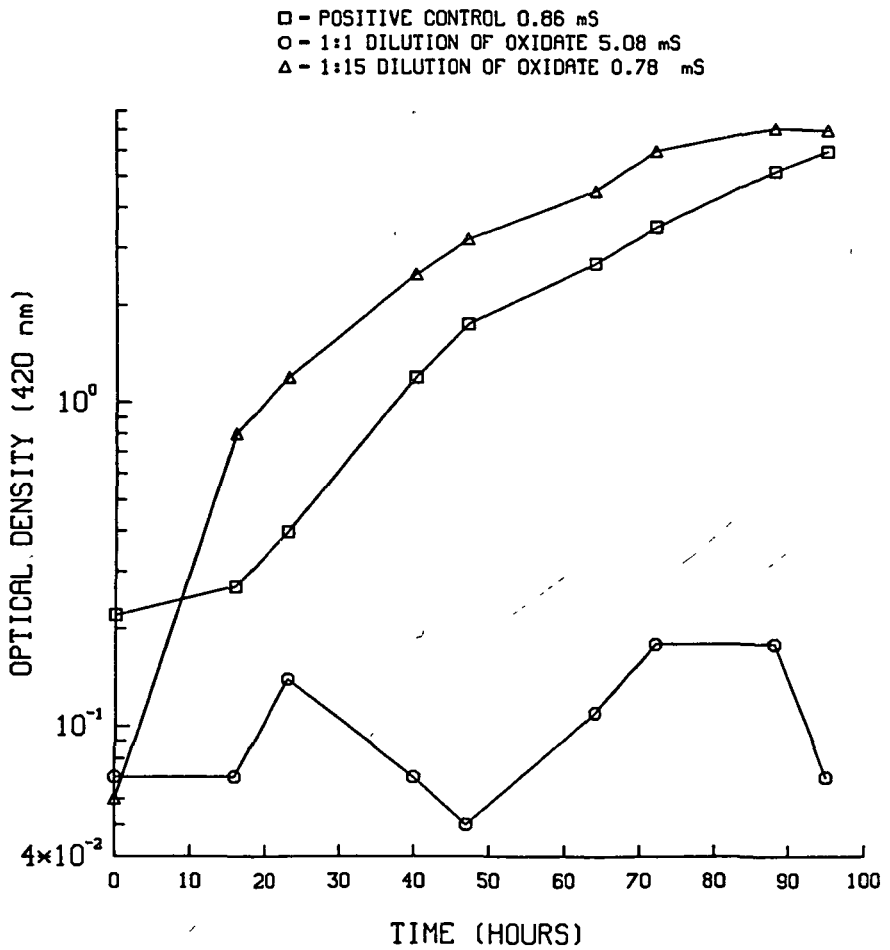

FIGURE 12. GROWTH OF ALGAE ON WET-OXIDIZED CHLORELLA

It was observed that the osmolarity of the 1:1 material was much greater than that of the normal control media. To test the hypothesis that the inhibitory effect was due to high salt concentration we ran an experiment with several different osmolarities of normal media. We found that the normal media with an osmolarity equal to the 1:1 fecal wet-oxidate showed normal algal growth. Therefore, it is unlikely that the inhibition observed was due to a high salt concentration effect. Another experiment tested wet-oxidized distilled water as an algal nutrient source. No inhibition of algal growth was observed, indicating that the wet oxidation reactor is not contributing an inhibitory substance. Therefore, we conclude that the feces is the source of the inhibition although at this time we have not determined what the inhibitory material is.

CONCLUSIONS

The use of algae in a CELSS will depend on many factors, including the ability to monitor and control the algal cultures. Work conducted under this research program has indicated that algae may be useful in the regeneration of the atmosphere within a closed spacecraft ecology. By taking advantage of the inherent characteristics of algae, endogenous control strategies have been developed which limit the amount of exogenous control energy which must be exerted on them. Production of large amounts of biomass, coupled with appropriate

processing techniques, fixation of nitrogen and minimal maintenance and control energy requirements make algae an attractive biological component for inclusion in a CELSS.

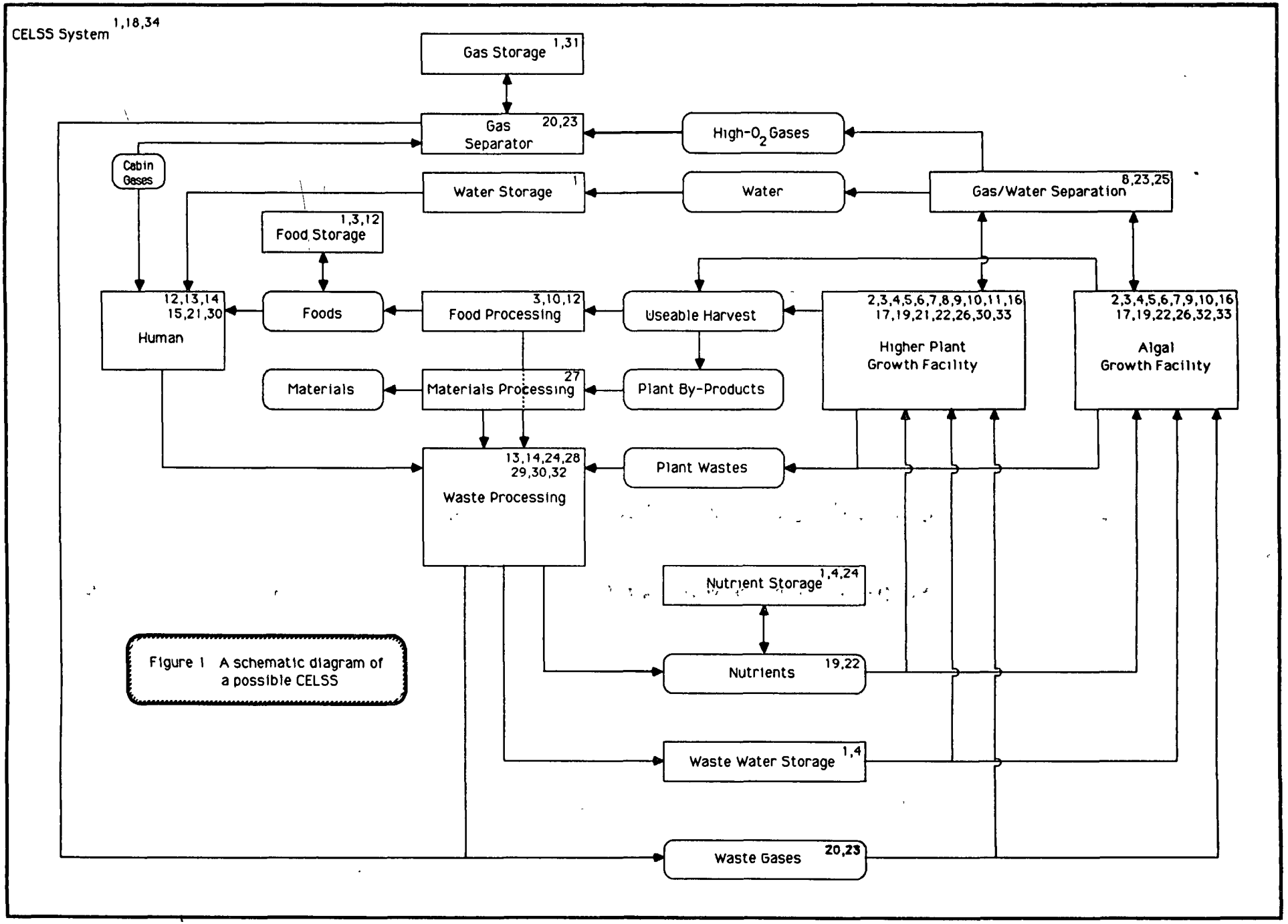

Figure 1 A schematic diagram of a possible CELSS