# OPERATION OF AN EXPERIMENTAL ALGAL GAS EXCHANGER FOR USE IN A CELSS

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## ABSTRACT

Concepts of a CELSS anticipate the use of photosynthetic organisms (higher plants and algae) for air revitalization. The rates of production and uptake of carbon dioxide and oxygen between the crew and the photosynthetic organisms are mismatched. An aglal system used for gas exchange only will have the difficulty of an accumulation or depletion of these gases beyond physiologically tolerable limits (in a materially closed system the mismatch between assimilatory quotient (AQ) and respiratory quotient (RQ) will be balanced by the operation of the waste processor). We report the results of a study designed to test the feasibility of using environmental manipulations to maintain physiologically appropriate atmospheres for algae (*Chlorella pyrenoidosa*) and mice (*Mus musculus* strain DW/J) in a gas-closed system. Specifically, we consider the atmosphere behavior of this system with *Chlorella* grown on nitrate or urea and at different light intensities and optical densities. Manipulation of both the photosynthetic rate and AQ of the alga has been found to reduce the mismatch of gas requirements and allow operation of the system in a gas-stable manner. Operation of such a system in a CELSS may be useful for reduction of buffer sizes, as a backup system for higher plant air revitalization and to supply extra oxygen to the waste processor or during crew changes. In addition, mass balance for components of the system (mouse, algae and a waste processor) are presented.

## INTRODUCTION

A Controlled Ecological Life Support System (CELSS) is one option for maintaining human life during extended space flight. A CELSS uses energy to recycle matter through an integrated variety of biological and physical processes, thereby regenerating consumable supplies. The objective of the NASA-sponsored CELSS program is to investigate the feasibility of producing food and revitalizing atmospheres by growing algae, higher plants, and processing wastes by microbial or physical-chemical oxidation /1/.

Revitalizing the atmosphere within a spacecraft or planetary habitat requires that the crew be continuously supplied with oxygen ($O_2$) and that carbon dioxide ($CO_2$) be removed from the system. Physico-chemical methods are available to remove and reduce $CO_2$ and generate $O_2$ /2/. The presence of algae and higher plants in a CELSS allows the photosynthetic uptake of $CO_2$ and production of $O_2$. Therefore, physico-chemical methods of air revitalization are not envisioned as part of a CELSS except, possibly, as a backup system.

The use of biological processes to maintain physiologically appropriate concentrations of $CO_2$ and $O_2$ requires the development of strategies which maximize system reliability and stability while using the inherent characteristics of the organisms as control points. Previously we have reported on the use of environmental manipulations to stabilize the behavior of a gas-closed mouse-algal system /2/.

We report here the recent results obtained from the operation of this system (Figure 1). Specifically, we consider the atmospheric behavior of the gas-closed system using cultures of *Chlorella pyrenoidosa* and a dwarf mouse

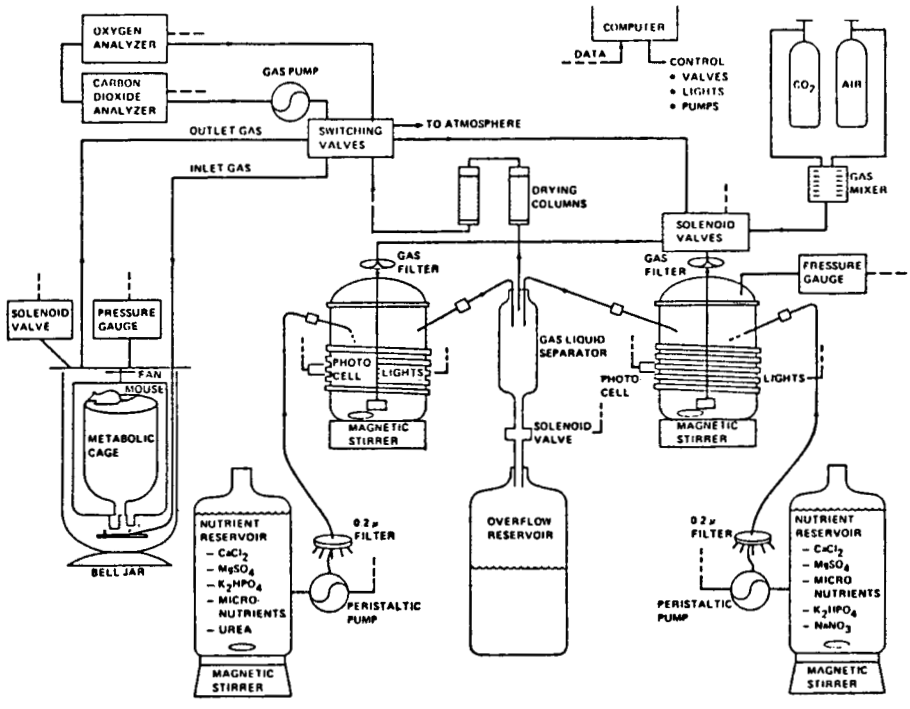


Fig. 1. Experimental mouse-algal system.

*Mus musculus dw/j*. Additionally the results of preliminary mass balance studies of the system are presented, including the use of an abiotic waste processor.

## MATERIALS AND METHODS

A variety of photosynthetic gas exchange experiments were conducted using the system (Figure 1). Gas analyzers measured changes in partial pressures of $CO_2$ and $O_2$ within the system. The system was operated with algae only, a mouse only, and with the algal and mouse reactors coupled.

Measurement of algal assimilatory quotients (AQ = moles $CO_2$ consumed/moles $O_2$ produced) were made by closing the algal reactor to the ambient atmosphere. The algal cultures are normally supplied with $CO_2$ (2%) enriched air. When AQ measurements were made, the gas flow from the cylinders was stopped and the gas within the system was recirculated using a pump. The slopes of the $CO_2$ and $O_2$ concentrations were used to calculate the AQ.

Measurement of mouse respiratory quotients (RQ = moles $CO_2$ produced/moles $O_2$ consumed) were accomplished by closing the mouse in the chamber with ambient atmospheric concentrations of $CO_2$ and $O_2$ and observing the changes in each gas concentration over time. The RQ was calculated from the change in $CO_2$ divided by the change in $O_2$. Respiratory quotients were measured for short time periods (one or two hours) which included awake, sleeping and eating periods.

Combined algal-mouse experiments were conducted with the algal reactor and the mouse chamber linked and closed to the exchange of gas with the ambient atmosphere. Two algal vessels were required to supply the $O_2$ requirements of the dwarf mouse. Algal growth conditions (light intensity and cell density) were varied to control the photosynthetic *rates* of each culture. Different nitrogen species (urea and nitrate) were used in the algal growth medium to change the *ratio* of $CO_2$ uptake to $O_2$ release. Combinations of both rate and ratio manipulations were used to determine the optimal operating conditions (*i.e.* nitrogen source, light intensity, optical density) for maintenance of atmospheric levels of $CO_2$ and $O_2$ within the physiological tolerances of both the algae and the mouse.

Bacterial populations within the algal cultures were monitored by plating samples onto trypticase soy agar (TSA) plates, incubated for 48 hours at 30 C. Plate counts for both bacteria and algae were made using the spread plate method and TSA plates.

Carbon, hydrogen and nitrogen percentage (by weight) of the algae, mouse food and feces were determined by running lyophilized samples on a Perkin-Elmer model 240B elemental analyzer. To determine percent oxygen, ash percent was determined and oxygen calculated by difference. The contribution of other elements (sulfur, phosphorus, etc.) was assumed to be negligible.

Mass balance determinations for the mouse were made by measuring the inputs to (food and water) and outputs from (feces, urine, unconsumed food and water) sets of three mice housed in a metabolic cage not connected to the gas exchange system. Elemental analysis of these materials was conducted and averaged for a series of 15 runs. Assumptions were made concerning the magnitude of exhaled water from the mice and values for percentages of water in feces and urine were obtained from the literature /4/. Mass balance of the wet-air oxidation of feces was determined from a set of runs conducted in a system physically distinct from the gas exchange system.

## RESULTS AND DISCUSSION

### Atmosphere Revitalization

Metabolic differences between the mouse and algae result in an inherent mismatch between AQ and RQ leading to depletion of either $CO_2$ or $O_2$, depending upon the direction and degree of the mismatch /3/. This mismatch is more evident in a materially-open system while in a materially closed system, the operation of a waste processor will reduce the impact of the AQ-RQ mismatch. Therefore, in order to maintain stable concentrations of $CO_2$ and $O_2$ within a coupled algal-mouse system, it is necessary to more closely control the AQ and the RQ. Control of the RQ is possible but not a realistic option for use in a CELSS because it would require excessive restrictions on crew diet and activity. Thus, techniques to match the AQ of the algae to the RQ of the mouse have been examined. One factor that influences AQ is the observed difference between nitrate and urea grown cultures /5/. The average AQ of axenic cultures of *Chlorella pyrenoidosa* grown on nitrate was 0.67 ± 0.02 (n=5) and on urea was 0.80 ± 0.04 (n=3) over a range of light intensities and optical densities.

The AQ of cultures grown on nitrate and urea differ because nitrate is a more oxidized form of nitrogen than urea. As urea is metabolized to ammonia, $CO_2$ is released, which raises the AQ with respect to nitrate. This can be shown by considering the composition of *Chlorella pyrenoidosa*, as determined by elemental analysis, $C_{5.77}$: $H_{9.30}$: $O_{2.47}$: $N_{1.00}$. The incorporation of $CO_2$, water and either nitrate or urea into *Chlorella pyrenoidosa* and $O_2$ can be expressed for nitrate:

$$5.77CO_2 + 4.65H_2O + NO_3^- = C_{5.77}H_{9.30}O_{2.47}N + 8.36O_2. \quad (1)$$

yielding a theoretical AQ of 5.77/8.36 = 0.69.

for urea:

$$5.27CO_2 + 3.65H_2O + 0.5(N_2H_4CO) = C_{5.77}H_{9.30}O_{2.47}N + 6.11O_2. \quad (2)$$

yielding a theoretical AQ of 5.27/6.11 = 0.86. This discussion demonstrates how the differing oxidation states of nitrate and urea affect the AQ. The theoretical values are close to the experimental values.

The presence of bacteria in the algal cultures was found to affect $CO_2$ and $O_2$ levels within the system. The bacteria present were contaminants and were not intended to represent the decomposer (waste processing) functions of a CELSS. The contamination could have occurred by several routes, specifically by failure of the media input filters, back-contamination from the mouse chamber or during sampling procedures. Bacterially contaminated cultures exhibited a decrease in the apparent algal AQ and an increase in the variation of AQ. Contaminated nitrate cultures grown under a range of light intensities and optical densities had an average AQ of 0.50 ± 0.07 (n=25) while uncontaminated nitrate cultures had an AQ of 0.67 ± 0.02 (n=5). Contaminated urea grown cultures had an AQ of 0.77 ± 0.12 (n=28) while the uncontaminated urea cultures had an AQ of 0.80 ± 0.04 (n=3). This decrease is due to bacterial respiration consuming $O_2$ and producing $CO_2$ at a different ratio than algal photosynthesis was producing $O_2$ and consuming $CO_2$ (*i.e.* bacterial RQ ≠ algal AQ). In an operational CELSS, in which bacterial contamination will occur, reduction in the apparent AQ must be compensated for by an increase in the photosynthetic rate of the algal cultures, or finding operating modes that yield AQ's greater than the crew RQ. Control of respiratory gas concentrations will be most effective if the algal systems bracket the crew RQ.

Bacterially contaminated cultures grown on nitrate exhibit a decrease in apparent AQ and a large AQ variation. The large variation in AQ is likely due to different bacterial populations as well as to population sizes. We concluded that even low levels of bacterial contamination affect the apparent AQ of the algal culture. To determine how a mixed bacterial-algal microbial community affects the apparent AQ, further data must be collected regarding the bacterial community diversity and relative population sizes of bacteria and algae within the system.

In a CELSS, the composition of mixed algal-bacterial cultures will need to be monitored in order to maintain defined communities which effectively regenerate atmospheres. Our experience indicates that it is difficult to maintain axenic algal cultures for long time periods (weeks) and to attempt this in a space-based system will be both impractical and unnecessary for long-duration (years) space missions. Large fermentors would be very difficult to sterilize and the equipment required would consume more power, volume and maintenance time and

have too large a launch mass to be practical. The cost of maintaining axenic cultures would be much greater than the advantage gained.

The RQ of the dwarf mouse was 0.97 ±0.06 (n=5), this agrees with values up to 0.97 for dwarf mice reported by Eley and Myers /6/. The mean RQ for humans is 0.82 /7/. Variation of diet and/or activity levels results in different RQ values. The dietary ratio of carbohydrate, lipid and protein are known to affect RQ because of different oxygen requirements for the oxidative metabolism of foods. Activity levels affect RQ because the nature of oxygen utilization varies with the metabolic requirements of the animal. Since the air revitalization system must be responsive to normal variation in crew diet and activity levels there were no restrictions on the diurnal activity or diet of the mouse. Since the mouse RQ is higher than the human RQ, changes in the algal operating conditions will need to be explored for human-scale experiments.

A CELSS air revitalization system will be designed to minimize the RQ-AQ mismatch. However, because the range of AQ control is limited, control will also depend on the ability to manipulate rates of photosynthesis. In combined algal-mouse experiments, variation of light intensity controls gas exchange mismatches by affecting photosynthetic rates through culture density. Decreasing or increasing incident light intensity directly affects the concentrations of $CO_2$ and $O_2$ within the system atmosphere by changing the photosynthetic rate of the culture.

Figure 2 illustrates the crossover area (where photosynthesis ~ respiration) between photosynthetic mode (*i.e.* oxygen production of the algae exceeds the oxygen uptake of the mouse) and respiratory mode (*i.e.* oxygen consumption of the mouse exceeds oxygen production by the algae). The crossover area curves exhibit the difference in algal AQ between nitrate (Figure 2a) and urea (Figure 2b) grown cultures. Predictions can be made with this data regarding the overall system state for cultures maintained at selected optical densities, light intensities, and nitrogen sources. Consequently, it is possible that multiple reactors, each at selected operating regimes and with distinctive gas exchange characteristics, may be used to provide flexibility for control of the system.

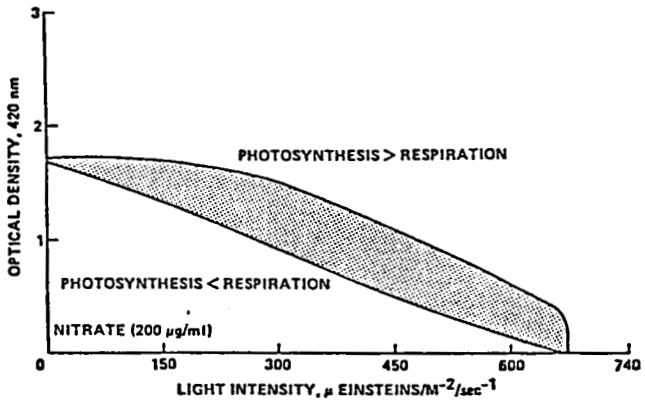


Fig. 2a. Crossover area as a function of optical density and light intensity.

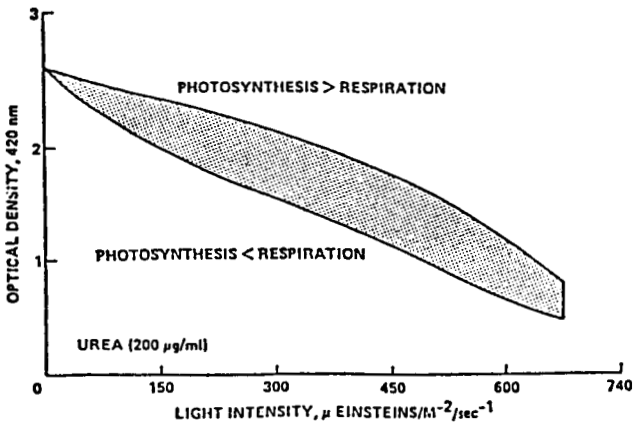


Fig. 2b. Crossover area as a function of optical density and light intensity.

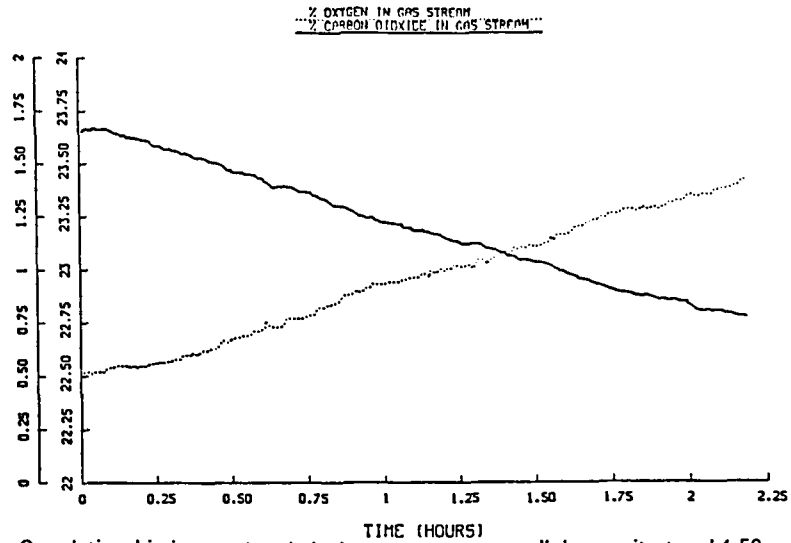


Fig. 3. $CO_2$-$O_2$ relationship in gas-closed algal-mouse system, small dome, nitrate od 1.50, urea od 4.50.

Combined algal-mouse runs were conducted using both the large and small mouse chambers coupled to the two algal reactors, one growing on nitrate and the other on urea. Figure 3, a small mouse chamber run, shows the concentration of $O_2$ increasing with a corresponding decrease in the $CO_2$ concentration. This photosynthetic mode can be explained by considering the operating conditions of each algal reactor. The high optical densities of the two cultures led to an overall system state in photosynthetic excess as predicted in Figure 2.

A large mouse chamber run (Figure 4) exhibits a system state in which the concentration of $O_2$ is decreasing with a corresponding increase in the $CO_2$ concentration. Similarly, the reactor conditions of Figure 4, as seen in Figure 2, would lead to a system state in respiratory excess which is demonstrated experimentally. As can be seen in Figure 3, the timescale for significant changes to occur in the gas composition of the small chamber is between 1 and 2 hours. Since the timescale will scale as the system volume, significant changes in the large chamber require a timescale of roughly 18 to 36 hours. Thus, Figures 3 and 4 demonstrate that system states can be predicted from the crossover area curves and that these predictions are independent of the buffer volume of the mouse chamber.

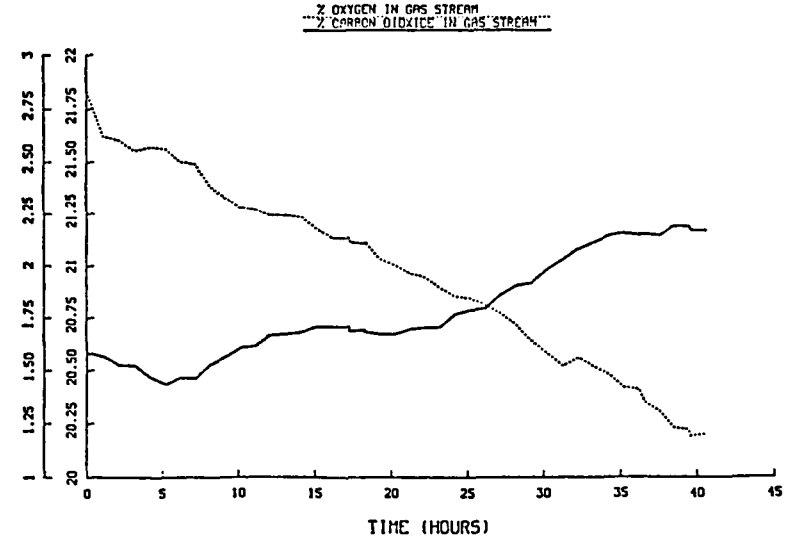


Fig. 4. $CO_2$-$O_2$ relationship in gas-closed algal-mouse system, large dome, nitrate od 0.95, urea od 1.45.

The observed buffering capacity of the large chamber might appear useful as a control point. However, responsiveness of the system decreased with the larger system volume which effectively increased the time required to recover from perturbations. Furthermore, minimization of reservoir sizes reduces the weight, volume and hence cost of a CELSS and consequently large buffers are not practical.

Figure 5. which illustrates the system operating in a stable fashion over 2-3 system timescales, demonstrates that long-term stability is possible by combinations of rate and ratio manipulations. The initial photosynthetic mode was mitigated by lowering the optical density of the urea reactor at 3.0 hours. At about 10.0 hours the decreasing $CO_2$ and increasing $O_2$ slopes showed a decrease in magnitude. However, a further optical density decrease of both reactors was necessary to change the slopes to a value close to zero. Maintenance of these conditions allowed the system to remain in a stable state.

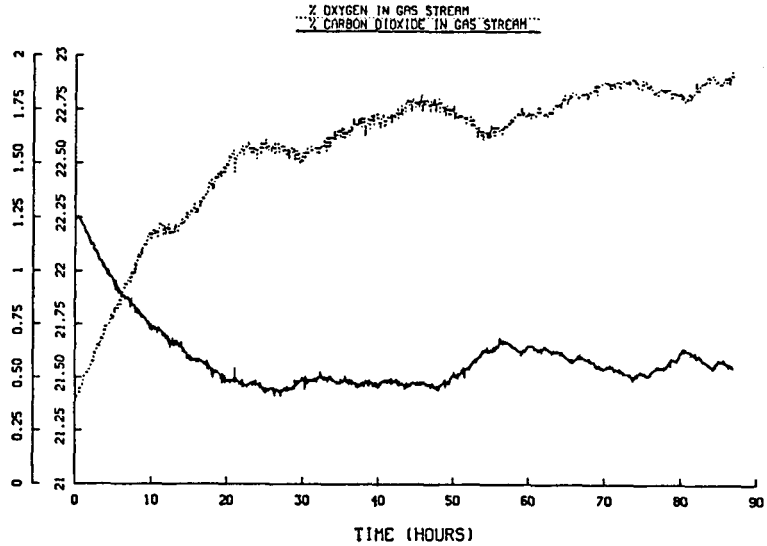


Fig. 5. $CO_2$-$O_2$ relationship in gas-closed algal-mouse system, large dome, nitrate od 0.80, urea od 0.90.

The initial photosynthetic mode observed is contrary to crossover area curves predictions (Figure 2). Variation of bacterial community diversity and population size will alter the photosynthetic capability of the system and lead to disparity between predictions and observations. Additionally, differences in RQ's between mice may account for part of the variability. Further, combined nitrate, urea system behavior may not be the same as separate nitrate or urea system behavior from which the crossover area curves of Figure 2 were derived.

We conclude that environmental manipulations of algal growth conditions can significantly reduce the inherent mismatches in respiratory gas requirements of a photoautotroph-heterotroph system. The use of environmental controls has the advantage of limiting the amount of intensive control or external devices required to maintain stability. Our data indicate that photosynthetic rate is easily manipulated by variation of light intensity and optical density. AQ may be manipulated by choice of nitrogen source. The effects of bacterial contamination can be minimized by aseptic culture techniques and monitoring of population size and diversity. The ultimate goal will be to maintain well defined algal-bacterial communities. The effects of buffer size must also be taken into account in the design and operation of an algal gas exchanger in order to allow realistic timescales for system control and to minimize cost.

Further experimental work must be completed to determine the precise operating conditions required to maintain atmospheric stability within a closed system. Once such equilibrium conditions are established, an algal gas exchanger will be able to operate reliably, with minimal external manipulations, and be able to respond appropriately to the respiratory gas requirements of a human crew. Given a scaled version of the algal system described, with the same surface to volume ratio and equivalent light intensity, we calculate that 300 liters of algae would be required per person per day. Improvements in light use efficiency and reactor design will decrease the volume of culture required to support a human crew.

Mass Balance

The maintenance of concentrations of $CO_2$ and $O_2$ within acceptable physiologic ranges indicates that endogenous control of the photoautotrophic (algal) component allows atmospheric stability to be sustained. To determine the degree of closure through the system, quantification of elemental flows through system compartments is required. In addition to the respiratory gases, carbon and oxygen cycle through living tissue, feces, urine, water, mouse food and algal nutrients. The rates and concentrations are measured in order to more accurately assess the stability of the system.

The system elemental flows (carbon, oxygen, nitrogen, hydrogen (C,O,N,H)) are quantified as they pass between three compartments: mouse, algal and waste processor (Figure 6). Elemental analysis of mouse food, feces,

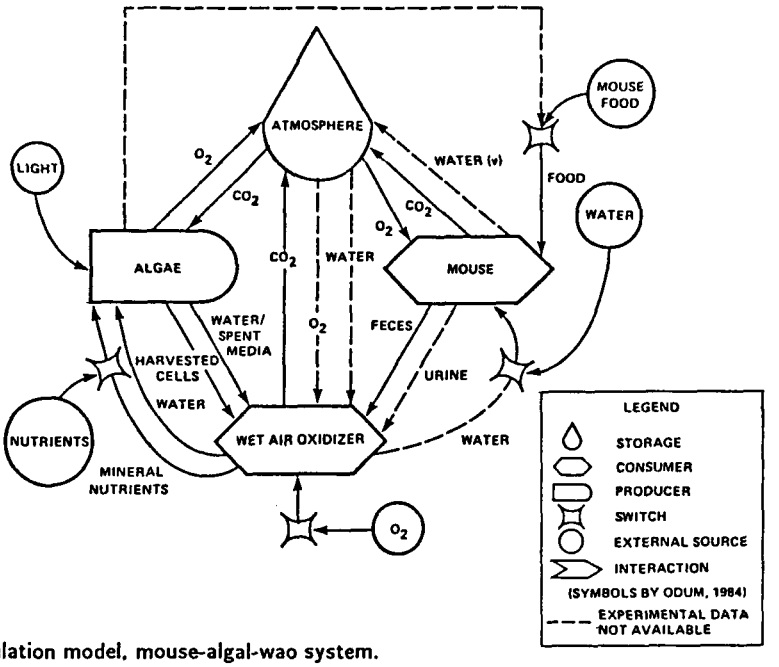


Fig. 6. Simulation model, mouse-algal-wao system.

urine and algal cells and the known elemental composition of algal media, waste processor inputs and measured waste processor outputs are used to calculate mass balances through and between system compartments.

Several assumptions are made within this approach. To achieve system closure the algal cells must be used as mouse food, although this has not been done experimentally. Given the known composition of the mouse food and algal cells, it would be possible to theoretically "replace" the mouse food with algal biomass. However, the nutritional quality and the response of mouse metabolism to an algal diet is not well defined. The composition of spent algal media is not directly measured, but it may be calculated from the known initial composition minus the measured algal cell uptake values. The exhaled water vapor from the mouse is not directly measured so it was calculated by balancing oxygen output. These assumptions allow a preliminary analysis of system mass closure but a more thorough analysis must await refinement of system capabilities.

To analyze mass flow through the mouse-algal-wet oxidizer system a simulation model has been developed, an adaptation of a related mass flow model based on humans, wheat and a wet air oxidation reactor (WAO). Elaboration of the model into algorithms has not been completed although the development of model structure has enhanced analysis of experimental data and, once completed, will be useful for predicting the behavior of the experimental system. Initially, the experimental data will be used to validate the model. Once validated, the model can be used in place of the experimental system to more rapidly determine the flow of mass under a variety of operating conditions. The basic structure of the model (Figure 6) is explained in detail below.

An important consideration in the development of the model has been the degree of complexity incorporated into it. There are several levels of complexity which have been considered. These include the compartment level (i.e. mouse and its environmental conditions), the organism level, the organ system level, the cellular level, the biomolecular level and the atomic level. Increasing the detail of the model to include the atomic interactions associated with biochemical reactions is considered to be too detailed. Similarly the compartment level alone is too general to yield useful information on the flow of materials through the system. It was determined that the compartment level containing the biomolecular level as a subsystem is most appropriate. The biomolecules that are included are carbohydrates, proteins and lipids. Other than accounting for C,O,N,H in such biomolecules, there may be no need to distinguish these compounds further in terms of detailed biochemical pathways. In fact, some areas of the model may not include detail at this level. For example, for certain segments of the model it may be necessary to include only the living component as a whole and not to break it down into specific molecules.

Model structure will be illustrated by tracing the flow of carbon. The model has been broken into segments to clarify the interactions occurring within each compartment. Figure 7a shows the flow of carbon through the mouse compartment. Carbon is introduced into the system from either mouse food or algal cells in the forms of carbohydrate, lipid and protein. Once eaten by the mouse, the metabolism of carbohydrate and lipid yields energy by conversion of simple sugars to $CO_2$ and $H_2O$ in the presence of $O_2$. Additionally, the fats and sugars can be stored by the mouse. The carbon in proteins is used in maintenance of the mouse, the net flow may

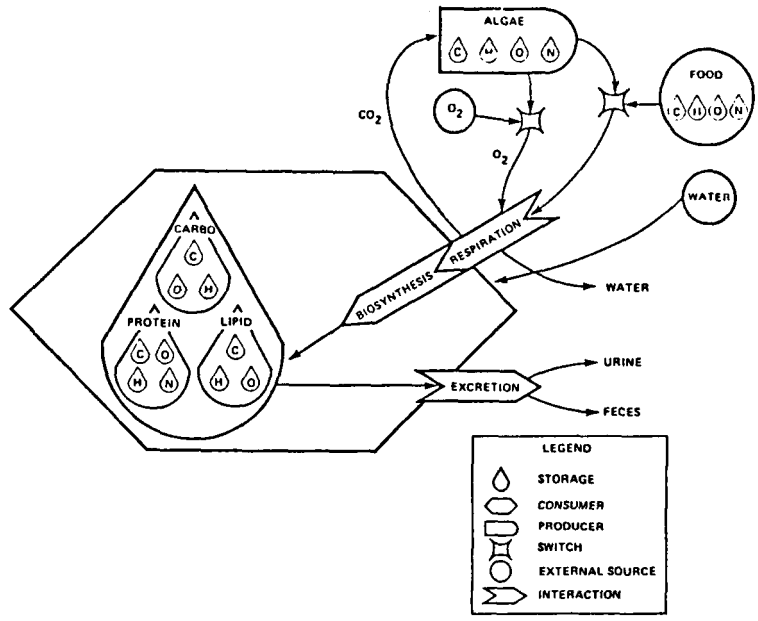


Fig. 7a. Simulation model, mouse compartment.

be set to zero or to whatever value experimental data suggests. Thus carbon introduced to the mouse is 1) metabolized to $CO_2$ and exhaled, 2) excreted in feces and urine or 3) retained in the mouse.

Table 1 shows the mouse mass balance which has been calculated manually from information contained within the model and from experimental data. The data was collected in two ways; values for $CO_2$ consumption and $O_2$ production were calculated from the slopes of RQ measurements made within the gas-closed mouse system, values for food and water consumption and fecal and urine production were obtained from metabolic cage experiments conducted outside the bell jar. The value for water vapor exhalations was not measured and thus was calculated from the O, H values remaining after all other inputs and outputs were balanced. The ratio of O to H was very close to the expected 8:1 ratio and the assumption was made that this remaining mass must be the water vapor content.

TABLE 1 Mass Balance of a *Dwarf Mouse*
(All values in grams/mouse/day)

| | TOTAL | CARBON | HYDROGEN | OXYGEN | NITROGEN |
|---|---|---|---|---|---|
| OUT | | | | | |
| Urine Solids | 0.1319 | 0.0267 | 0.0070 | 0.0250 | 0.0357 |
| Urine Water | 0.7300 | - | 0.0803 | 0.6497 | - |
| Fecal Solids | 0.1911 | 0.0684 | 0.0101 | 0.0591 | 0.0088 |
| Fecal Water | 0.3549 | - | 0.0390 | 0.3159 | - |
| Water Exhaled | 2.162 | - | 0.2393 | 1.914 | - |
| $CO_2$ Exhaled | 2.19 | 0.597 | - | 1.592 | - |
| Subtotal | 5.7671 | 0.6921 | 0.3757 | 4.556 | 0.0445 |
| Storage (in mouse) | 0.1260 | 0.0306 | 0.0133 | 0.079 | 0.0032 |
| Total Out | 5.8931 | 0.7227 | 0.3890 | 4.635 | 0.0477 |
| IN | | | | | |
| Food | 1.684 | 0.7447 | 0.1031 | 0.6399 | 0.0074 |
| Water | 2.680 | - | 0.2948 | 2.385 | - |
| $O_2$ Consumption | 1.610 | - | - | 1.610 | - |
| Total In | 5.974 | 0.7447 | 0.3979 | 4.6349 | 0.0074 |
| Difference | 0.081 | 0.022 | 0.0089 | 0.0 | 0.0197 |
| Percent Recovery | 98.65 | 97.05 | 97.8 | 100.0 | 70.8 |

The high percent recovery seen in Table 1 indicates that the mouse system demonstrates closure for the primary bioelements. Carbon and hydrogen flows show a 97% recovery; the 100% recovery of oxygen is found because the excess oxygen was all placed in the water vapor column and a stoichiometric amount of hydrogen was added to the hydrogen value. The 71% recovery of nitrogen is lower than expected. Possible sources of loss include the bacterial denitrification of urine and fecal urea to $N_2$, the loss of urine in the collection process by either volatilization or adherence to surfaces, and variation of solids contents in both urine and feces between different experimental runs.

Returning to the carbon trace through the system, the $CO_2$ exhaled by the mouse is introduced to the algae as its sole source of carbon (Figure 7b). Algal photosynthesis then converts the $CO_2$ into simple sugars and $O_2$ in the presence of water and light. Biosynthesis of the sugars to lipids and proteins may be incorporated into

the model, or the model can be run using only an algal biomass pool in place of the more specific biomolecules. Theoretically the algal biomass may then be recovered for use as a mouse food. Waste algal biomass may also be directed to the waste oxidizer.

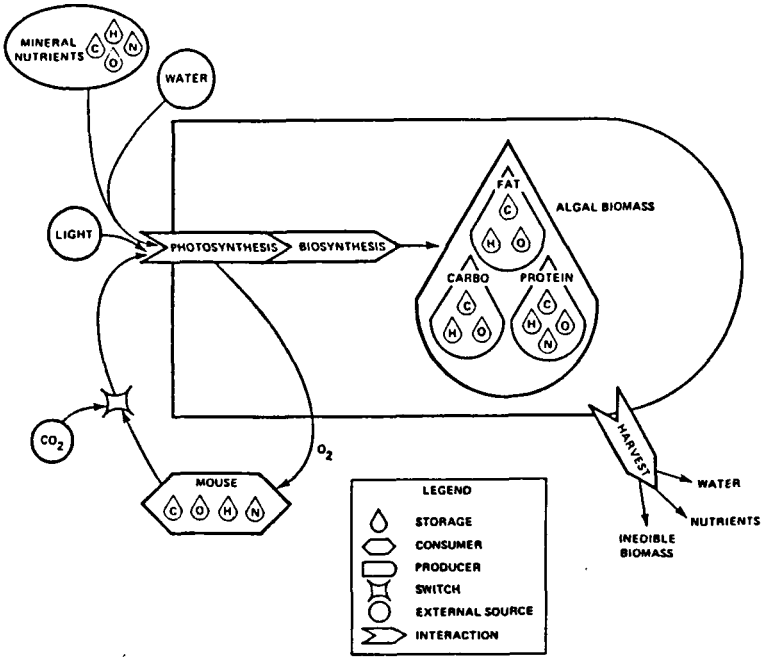


Fig. 7b. Simulation model, algal compartment.

The WAO receives carbon from the mouse feces and oxidizes it into $CO_2$, low molecular weight organic acids (primarily acetic) and CO in the presence of $O_2$ (Figure 7c). $O_2$ was supplied from a gas cylinder although theoretically it could be supplied from the algal compartment. When the carbon from mouse feces has been oxidized to $CO_2$ and this $CO_2$ is then metabolized to algal biomass, the cycle of carbon through the system is complete. Similar paths may be traced for oxygen, nitrogen and hydrogen so that an overall system balance for the major biomolecules may be observed.

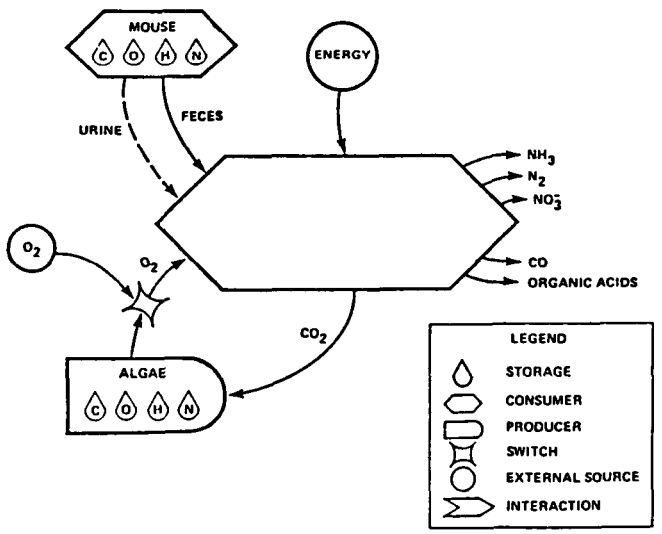


Fig. 7c. Simulation model, wet air oxidizer compartment.

Table 2 shows the mass balance of a single wet-air oxidation run using mouse feces. The excess carbon probably results from either variations in the percent carbon in feces or inaccuracies in the measurement of carbon streams in WAO effluent. The excess hydrogen in the system was assumed to be forming combustion water and thus was set to full recovery. The oxygen recovery is deceiving because of the large excess of oxygen used in the WAO process. Looking at the total oxygen used in the oxidation (1.80 g) and the recovery of only 1.03 g indicates a sink for oxygen which is not yet explained. Once again, nitrogen exhibits a low recovery (86%) which may due to difficulties in measuring small quantities of nitrogen compounds or variation in the nitrogen content of feces.

TABLE 2 Wet Air Oxidation Mass Balance
(All values in grams)

| | TOTAL | CARBON | HYDROGEN | OXYGEN | NITROGEN |
|---|---|---|---|---|---|
| OUT | | | | | |
| Oxygen (g) | 22.53 | - | - | 22.53 | - |
| Carbon Dioxide (g) | 0.7806 | 0.2129 | - | 0.5677 | - |
| Carbon Dioxide (aq) | 0.0118 | 0.0032 | - | 0.0086 | - |
| Organic C (aq) (acetic acid) | 0.1720 | 0.0688 | 0.0115 | 0.0917 | - |
| Carbon Monoxide | 0.2000 | 0.0857 | - | 0.1143 | - |
| Nitrogen (g) | 0.00444 | - | - | - | 0.00444 |
| Ammonia (aq) | 0.0361 | - | 0.0064 | - | 0.02968 |
| Nitrate (aq) | 0.00155 | - | - | 0.0012 | 0.00035 |
| Organic N(aq) | 0.00241 | - | ? | ? | 0.00241 |
| Combustion Water | - | - | 0.0311 | 0.2488 | - |
| Precipitate (as Calcium Phosphate) | 0.0807 | - | - | 0.045 | - |
| Total Out | 23.82 | 0.3706 | 0.049 | 23.61 | 0.0369 |
| IN | | | | | |
| Feces | 0.934 | 0.334 | 0.049 | 0.289 | 0.043 |
| Oxygen | 24.33 | - | - | 24.33 | - |
| Total In | 25.26 | 0.334 | 0.049 | 24.62 | 0.043 |
| Difference | 1.44 | -0.0366 | 0.0 | 1.057 | -0.0061 |
| Percent Recovery | 94.3 | 110.9 | 100.0 | 95.7 | 85.8 |

To change the steady-state flow balance of Figure 7 to a dynamic model, equations must be developed that express the various flows in terms of the state variables, i.e., the various storages of algal and mouse biomass, atmospheric $CO_2$, $O_2$, $H_2O$, light and nutrients. The simulation model being developed allows elemental balances to be calculated from experimental data. When the model is validated it will be useful to observe the changes in mass flow as conditions are varied. Thus, for example, the effects on the system of a doubling of the $CO_2$ input from the mouse compartment may be done completely by the model without having to actually add a mouse to the experimental system. The storage and flow of elements will be much easier to study with a well validated model than with the experimental system upon which it is based. As the model predicts interesting system behaviors, experiments may be conducted to verify model results and observe actual system behavior.

Table 3 shows one example of an overall system mass balance used to determine the accumulation and/or depletion of bioelements. The lack of complete experimental data, compounded by the non-integrated nature of the experimental system, makes it difficult to make accurate conclusions about element balance within the system. Data required includes; measurement of water vapor exhalations, recycling of urine, measurement of combustion water produced in the WAO and use of algal biomass as mouse food.

TABLE 3 Mass Balance of the Mouse-Algal-WAO System

| | | |
|---|---|---|
| MOUSE | | |
| | RQ = 0.975 | |
| | Start with: | 0.5460 g feces |
| | which equates to: | 2.190 g carbon dioxide produced |
| | | 1.610 g oxygen consumed |
| WAO | | |
| | Requires: | 0.602 g oxygen (to oxidize feces) |
| | Yields: | 0.4560 g carbon dioxide |
| | | 0.1838 g carbon monoxide |
| | | (+ 0.0668 g oxygen = 0.1838 g carbon dioxide) |
| | | 0.03633 g nitrogen |
| | | (+ 0.1246 g oxygen = 0.1609 g nitrate) |
| | Total Oxygen Consumption: | 2.40 g oxygen (1.610 + 0.0668 + 0.1246 + 0.602) |
| ALGAE | | |
| | Receives: | 2.829 g carbon dioxide (2.190 + 0.4560 + 0.1838) |
| | Produces: | 3.070 g oxygen (AQ = 0.67) experimental |
| | | 2.110 g oxygen (AQ = 0.975) theoretical |

The data shown in Table 3 indicates that if the AQ is less than the RQ there will be a net increase of $O_2$ in the system. This agrees with theoretical calculations /3/. If the AQ is equal to the RQ there appears to be a net loss of $O_2$ although a balance would be expected. The reason for this discrepancy is probably due to the formation of combustion water in the WAO. There is a significant loss of $O_2$ from the WAO mass balance

which, if completely accounted for, would probably balance the $O_2$ in the AQ equal to RQ scenario of Table 3. When looking at the whole system mass balance, the algal AQ must actually balance with the mouse RQ as well as the WAO RQ (which would equal the ratio of $CO_2$ produced/$O_2$ consumed).

The data in Table 3 exhibits the amount of $NO_3^-$ which would be obtained from the oxidation of the specified amount of feces, assuming the complete reduction of all nitrogen to $NO_3^-$. This value could then be compared to the amount of $NO_3^-$ required by the algal cultures to support one mouse for one day. Again, data is lacking to make this complete comparison because of the non-integrated nature of the system. However, it is clear that such analyses could be made with a combination of the simulation model and system modifications. Calculation of such balances at this time is not useful because of the inaccuracies introduced by the nature of the system. More accurate analysis will be possible with the stoichiometric equations contained in the simulation model and data acquired from integrated subsystems.

## CONCLUSIONS

The operation of this experimental system has helped to elucidate issues associated with the development of bioregenerative life support (BLS) systems. The issues raised are of two varieties. One is the analysis of the role which algae will play in BLS systems. The second variety is concerned with the way in which algal systems will be designed and operated. The development of a functional BLS system will rely on numerous iterations of subsystems, the pre-prototype air revitilization system described here is one step in that process.

Both the positive and negative attributes of the role which algae may play in BLS systems have been outlined in /8/. Operation of this system demonstrated that algae can; be controlled by manipulation of inherent characteristics of the organisms, and satisfy respiratory gas requirements. We also encountered some of the same difficulties anticipated of space-based systems. The inability to use algal-derived food prevented mass closure of the experimental system and the presence of bacterial contaminants raised questions about the long-term stability and reliability of algal cultures.

The operation of the experimental system demonstrated that algae are capable of providing air revitalization under controllable conditions and by use of endogenous algal characteristics. Problems associated with bacterial contamination can be dealt with by understanding relative population dynamics and compensating for bacterial respiration by increased algal photosynthesis. Determination of appropriate material reservoirs can be explored experimentally and will lead to proper scaling of buffers. Development of the simulation model will aid in examining issues related to mass balance, subsystem integration and overall system performance (stability and reliability).

Application of these principles to a human-scale system will require further experimentation to; define optimal algal environmental conditions, engineer functional systems which require minimal human attention and produce sufficient algal biomass to satisfy the respiratory and nutritional requirements of humans.

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