Atmosphere Behavior in Gas-Closed Mouse-Algal Systems:
An Experimental and Modelling Study.

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ABSTRACT

Concepts of biologically-based regenerative life support systems anticipate the use of
photosynthetic organisms for air revitalization.  However, mismatches in the rates of
production and uptake of oxygen or carbon dioxide between the crew and the plants will lead
to an accumulation or depletion of these gases beyond tolerable limits.  One method for
correcting these atmospheric changes is to use physicochemical devices.  This would conflict
with the constraint of minimal size and weight imposed upon the successful development of a
competitive bioregenerative system.  An alternate control strategy is based upon reducing
the gas exchange mismatch by manipulation of those environmental parameters known to affect
plant or algae gas exchange ratios.  We have initiated a research program using a dual
approach of mathematical modelling and laboratory experimentation aimed at examining the gas
exchange characteristics of artificial animal/plant systems closed to the ambient
atmosphere.  Our goal is to develop control techniques and management strategies for
maintaining the atmospheric levels of carbon dioxide and oxygen at physiological levels.  A
mathematical model simulating the atmospheric behavior in these systems has been developed
and an experimental gas-closed system has been constructed.  These will be described and
preliminary results will be 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 though an integrated
variety of biological and physical processes, thereby regenerating consumable supplies.  The
primary alternative to recycling is storage and/or resupply wherein all consumable materials
are brought on board and waste products are discarded.  Between these two extremes, various
hybrid systems are possible.  Although more difficult to design, the CELSS alternative is
under consideration because it is more cost effective than storage and resupply for
long-term space missions /1,2/.

The NASA-sponsored CELSS program has as objectives investigating the feasibility of
producing food and revitalizing atmospheres by growing plants and algae, and processing
wastes by microbial or physical-chemical oxidation.

A CELSS can be envisioned as a rigorously controlled, materially closed system (or nearly
so), recycling matter to provide a habitable atmosphere, a dependable supply of potable
water, and a nutritionally balanced diet.  Such a system would still fall short of its
goal--to support human life--if it were not capable of being controlled and managed to
provide these materials and functions at the proper time and at the proper rates.  To meet
these two criteria of material closure and controllability, research directed at the
development of a CELSS may be divided into two areas: Closure of the Nutrient Loop, which
seeks to establish mechanisms by which wastes can be recycled into usable forms, and System
Control and Management, which examines the behavior of individual components, their
interactions as a system, and how these behaviors can be integrated into a viable control
strategy.  The focus of this paper will be on the latter, the development of alternative
control techniques and management strategies to stabilize the behavior of systems in which
biological components are integrated with physical-chemical processes.

An example of a critical systems control problem in a bioregenerative life support system is the development of techniques regulating the rate of production and uptake of carbon dioxide and oxygen so that no changes in their atmospheric concentrations occur beyond allowable limits. This problem is complicated by the fact that the biological cycling of carbon dioxide and oxygen are coupled. Manipulating the rate of photosynthesis and/or respiration will change the rates of oxygen and carbon dioxide exchange simultaneously. Thus, attempting to balance the rate of oxygen exchange can disrupt the balance of carbon dioxide exchange and _vice versa_.

Ideally, in a simple animal-plant system, closed to the exchange of carbon dioxide and oxygen with the ambient external atmosphere, respiration by the animals should be equivalent to plant photosynthesis, thereby maintaining a fixed concentration of atmospheric oxygen and carbon dioxide. An equilibrium based upon the equivalence of photosynthesis and respiration is usually not possible, however, because the RQ (respiratory quotient--moles carbon dioxide produced/moles of oxygen consumed) of animals generally does not match the AQ (assimilatory quotient--moles of carbon dioxide consumed/moles of oxygen produced) of plants. The RQ of mammals is a function of their diet, and for humans has a mean value of about 0.85 /3/, whereas the AQ for plants has a mean value of about 0.95 /4/. Because of this mismatch, the atmospheric concentration of oxygen and carbon dioxide in a gas tight system containing only animals and plants will not be stable.

For example, in the gas-closed system illustrated in Figure 1, the mouse uses 1.0 volume of oxygen for each 0.85 volumes of carbon dioxide produced (RQ=0.85). The algae takes up 0.85 volumes of carbon dioxide and produces 0.89 volumes of oxygen (AQ=0.95). Thus, about 0.11 volumes of oxygen are lost from the atmosphere during each complete cycle. In gas-closed systems where AQ exceeds RQ the stability of the atmosphere will be disrupted by the continual loss of oxygen. In natural ecosystems, the concentration and relative stability of atmospheric oxygen and carbon dioxide is maintained by the buffering capacity of the atmosphere and the oceans. This buffering capacity is provided both by the enormous size of these compartments as well as certain chemical equilibria. For example, there are about 8300 cubic meters of ocean water and 1260 cubic meters of atmosphere for each square meter of land /5/. The atmospheric and water volumes enclosed in a reasonably sized CELSS however, will not be large enough to provide for the dilution or chemical transformation required to maintain a steady-state concentration of oxygen and carbon dioxide.
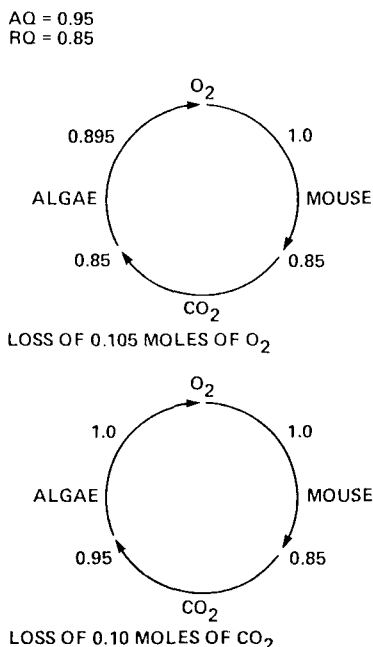


Fig. 1. Stoichiometry of gas exchange in a gas-closed, mouse-algal system in which either carbon dioxide exchange is balanced (top) or oxygen exchange is balanced (bottom).

Within a reasonably sized CELSS, attempting to compensate for this loss of oxygen solely by increasing the rate of respiration or decreasing the rate of photosynthesis may temporarily stabilize the concentration of carbon dioxide or oxygen, but this type of manipulation alone will not produce a stable atmosphere. This is because varying the rates of photosynthesis or respiration will not effect the value of AQ or RQ which are ratios.

Because of this RQ:AQ mismatch, the major premise of this study is that free-running animal/plant systems closed to exchange with external carbon dioxide and oxygen will have an unstable atmosphere /6,7/. This instability stems from two levels: rate instability caused by a mismatch between the rates of photosynthesis and respiration, and ratio instability caused by the mismatch of RQ and AQ. Manipulating the source of rate instability cannot bring about a stable atmospheric concentration of oxygen and carbon dioxide in a gas-closed animal-plant system because of ratio instability, but controlling ratio instability is a prerequisite for any strategy that seeks to limit the effects of rate instability.

### CONTROL STRATEGIES

NASA has, of course, successfully developed the technology required to maintain spacecraft atmospheres within the limits imposed by human well-being. These techniques, however, have been geared to short-term manned missions and, in general, rely upon large reservoirs of liquid oxygen and large stores of chemicals for carbon dioxide removal. These approaches to air revitilization are characterized by an increase in both weight and volume directly proportional to flight duration. For long-duration manned missions such "brute force" techniques must be replaced by more sophisticated methods, which will minimize the requirements for massive amounts of stored life-support supplies by utilizing appropriate regenerative processes, and effective system control and management techniques.

An alternate approach to atmosphere control is to minimize the RQ:AQ mismatch by manipulating those environmental parameters known to effect animal RQ e.g., diet, or, more realistically, known to effect photosynthetic AQ, e.g., the type and concentration of inorganic nitrogen species added to the algal medium. The research described herein is part of a program aimed at investigating the feasibility of stabilizing the atmosphere in a gas-closed, mouse-algal system by varying the algal AQ through environmental manipulation.

Control strategies fall into many categories. Chamber volume, algal biomass, light flux density, temperature of the algal culture, the concentration and species of nitrogen in the algal medium, are all variables that can prompt stabilizing system responses. Increasing chamber volume will modulate the effects of biological processes on the atmosphere. The amount of algal biomass determines the maximum chamber rate of carbon dioxide removal from the atmosphere. The photosynthetic rate shows a saturating response to light flux density and by manipulating this factor one can limit the rate of photosynthesis. The algae shows an optimum temperature for both photosynthesis and respiration, and by shifting the chamber temperature both above and below the intersection of these two curves one can limit these rates. Low nitrogen concentrations in the algal medium limit N uptake and subsequent protein synthesis. This shifts biosynthesis to either increased lipid production, which lowers the AQ, or increased carbohydrate synthesis, which can raise the AQ. At the extreme, nitrogen starvation can prevent carbon dioxide fixation and growth. Additionally, the use of nitrate as the nitrogen source lowers the AQ. By shifting from urea to nitrate one can bracket the RQ of the mouse with the AQ of the algae.

The strategy and techniques required to manage the system and the need to project the effects of present decisions on future operation of the system, has led to an approach emphasizing the parallel and complementary use of mathematical models and experimental systems. Computer simulations have been developed for each of the system components. The development and feasibility of possible experimental systems and control strategies will be evaluated based upon the linked operation of these models. As experimental systems are run, information will be extracted from the experimental systems and added to the models for future generations of systems and strategies.

### MODEL STRUCTURE

The major components of the model are the autotroph (alga) and heterotroph (mouse), their respective chambers, and their input and output storage pools (Fig. 2). Biomass is broken into three classes of molecules: carbohydrates, lipids, and proteins. The atmospheres of the chambers are described in terms of their $CO_2$, $O_2$, and water vapor content. Heterotroph inputs include drinking water and food storage. Output includes solid and liquid waste, followed as carbohydrate, lipid, protein, stool water, urea and waste water. The autotroph input and output storage include new and old medium and harvested algae.
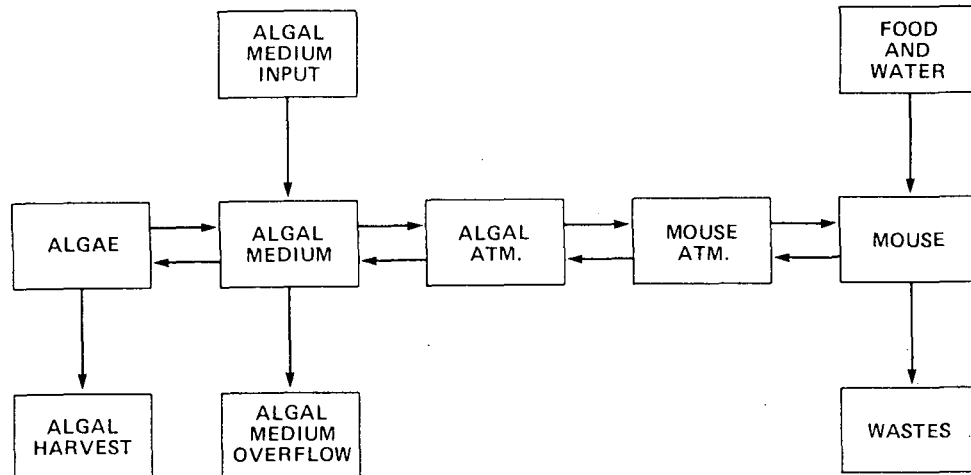
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│ MEDIUM  │                                              │ AND     │
│ INPUT   │                                              │ WATER   │
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│ ALGAL   │   │ ALGAL   │                              │         │
│ HARVEST │   │ MEDIUM  │                              │ WASTES  │
│         │   │ OVERFLOW│                              │         │
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Fig. 2.  Model structure of a gas-closed, mouse-algal system.


## The Autotroph

Presently, carbon dioxide fixation is described as function of carbon dioxide and oxygen concentration, temperature, light flux density, and chlorophyll concentration.  In biosynthesis, the apportioning of fixed carbon to carbohydrates, lipids, and proteins determines the AQ.  Algal respiration includes both maintenance respiration in darkness and light respiration.  Growth is described in terms of packed cell volume, cell number, and chlorophyll content.


## The Heterotroph

The respiratory demand of the mouse is derived from a daily caloric input and output.  The waste stream is a function of chamber environmental conditions and the daily caloric output. Daily caloric output is distributed to represent a range of metabolic rates.  Respiration of reserves of carbohydrate, lipid, and protein provides the caloric requirements and creates oxygen demand.  Differential oxidation of carbohydrates, lipids and proteins determines the rate at which carbon dioxide is evolved in respiration.


## The Physical System

Carbon dioxide, oxygen, and water vapor are exchanged between the two chambers at a rate determined by the air flow rate and their respective concentrations.  Solid waste from the mouse is generated as a percentage of the food intake rate.  The production of metabolic water in respiration modulates the output of waste water from food water and drinking water. Urea production is determined by rate at which proteins are respired.  The algal chamber is maintained by a continuous culture system that can run in either a chemostat or turbidostat mode.


## Model Results

The model has been run to simulate the behavior of a gas-closed system containing a mouse alone or in the presence of algal cultures continuously grown under varying environmental conditions.  Figure 3 depicts the behavior of the system when it contains only a mouse.  As expected, the atmospheric level of carbon dioxide increase while the oxygen level decreases. The volume of the closed system is such that at approximately ten hours, the concentrations of these gases had exceeded acceptable levels and the system "died".  If under the same conditions an algal culture, manifesting an AQ of 0.95 is added to the closed system, the mouse will survive for a longer period of time;  approximately fifty hours.  However, due to the mismatch of gas exchange quotients, there is a continual loss of oxygen from the atmosphere (Fig. 4).  If the AQ is lowered to 0.80 the system will survive for more than 150 hours.  However, the mismatch in quotients will cause a continual increase in oxygen

concentration until it will eventually be above some allowable limit (Fig. 5 ). If the AQ is equivalent to the RQ the system will be stable.
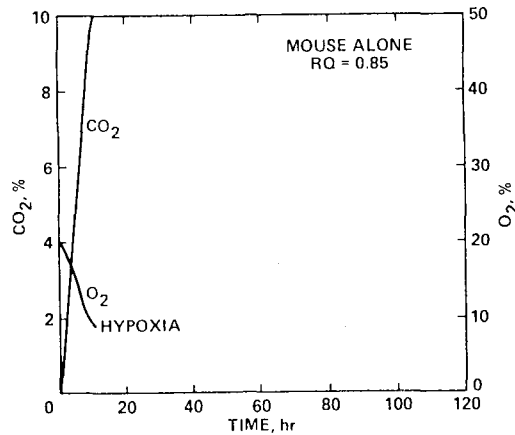


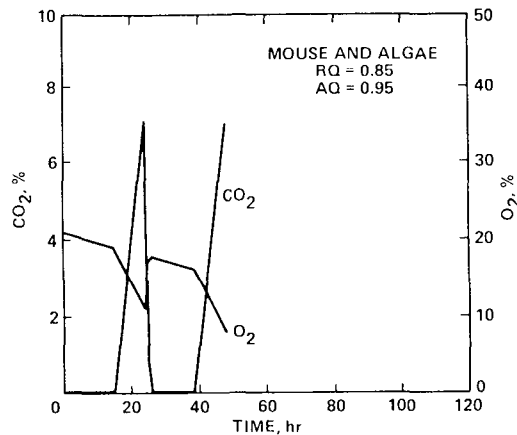Fig. 3. Atmospheric behavior in a simulated gas-closed, mouse-algal system containing only a mouse, RQ=0.85.



Fig. 4. Atmospheric behavior in a simulated gas-closed mouse-algal system containing a mouse, RQ=0.85, and an algal culture, AQ-0.95.

To attempt to maintain stability by the continuous matching of the gas exchange quotients would be extremely difficult. An alternate strategy. would be to bracket the mouse RQ by using several algal cultures, either simultaneously or in sequence, each culture manifesting an AQ either higher or lower than the mouse RQ. Increases or decreases in the oxygen concentration could be countered by increasing the growth of the appropriate culture. Growth on nitrate will lower the AQ and increase the atmospheric oxygen concentration, while growth on urea will raise the AQ and reduce the oxygen concentration.

continuous growth either as a chemostat or as a turbidostat. The chambers, either singly or together can be attached to a gas delivery and measurement system so constructed as to allow for operation either in a gas flow-through or gas-closed mode. The atmospheric concentrations of oxygen and carbon dioxide are determined by paramagnetic and infra-red analysis respectively. Tests of the algal reactor run as a chemostat indicate that, after initial transients, steady-state algal growth is achieved as measured by dry weight, turbidity and cell counts. Measurements of gas exchange by mice or algae are carried out by closing the system and determining the change of gas concentration in the closed chamber atmosphere. During the measurement period the kinetics of gas production or uptake are linear. Using this system we have obtained initial data on algal (Chlorella pyrenoidosa) assimilatory quotients as a function of nitrogen source and concentration. As well, mouse respiratory quotients under short-term resting conditions have been determined.
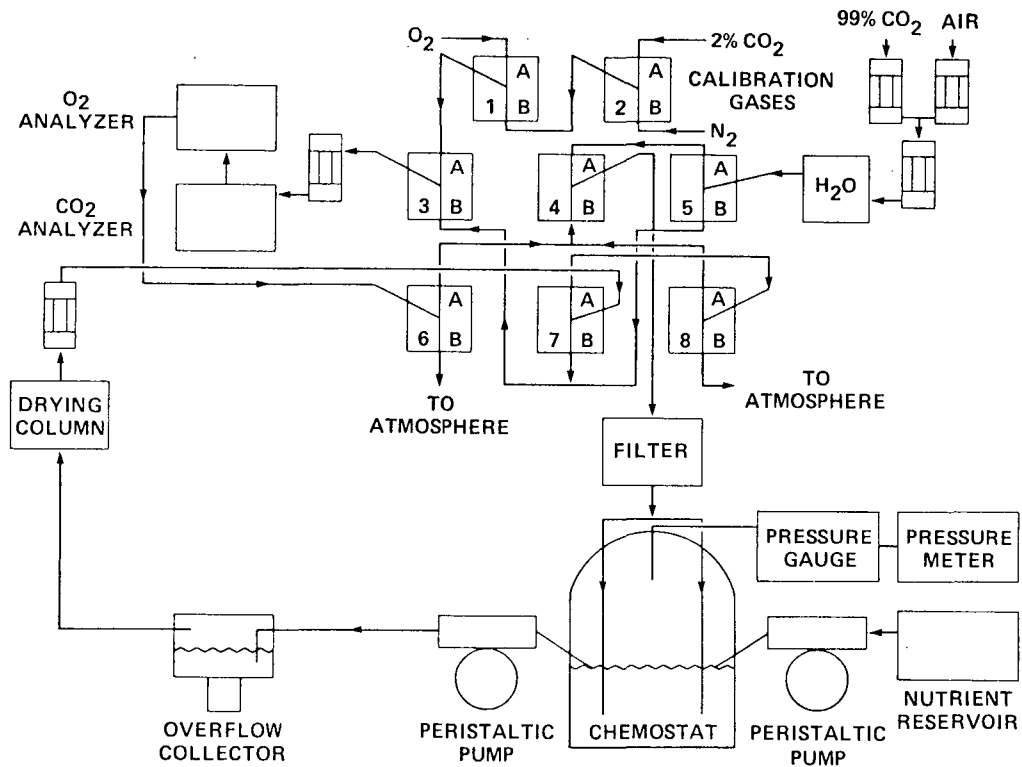


Fig. 7. Experimental apparatus for determining the atmospheric concentrations of carbon dioxide and oxygen in flow through and gas-closed systems.

The respiratory quotient of non-eating mice as measured in our system is 1.04 (SD=0.07) which agrees well with the expected value of 1.0. Assimilatory quotients measured during algal growth on nitrate and urea were qualitatively correct but were quantitatively lower than predicted.

Analysis of these data indicated that the AQ of the algal cultures might vary as a function of the cell concentration. Measurements of the AQ of steady-state algal cultures grown to different cell densities on urea and turbidometrically controlled were carried out. The expected AQ for Chlorella grown on urea is 0.82 /8/. The AQ's observed in our growth conditions were consistently lower than 0.82 and were a function of the optical density of the culture (Fig. 8). As algal cultures increase in optical density without any compensatory increase in illumination, the rate of photosynthesis will decrease due to shadowing effects. This decrease in photosynthetic rate is depicted in Figure 9. This figure depicts the carbon dioxide uptake rate and the oxygen production rate normalized by the optical density of the culture and plotted as a function of the optical density of the culture at which the measurement was made. As expected the normalized rates decrease as a function of the optical density of the cultures. The slopes of the curves, however, are different. This difference in slope will result in varying AQ's. Only at the intercept of

the curves at the ordinate does the ratio of the curves appraoch that expected for urea (0.82). The basis for this effect of cell concentration on AQ is presently under investigation.
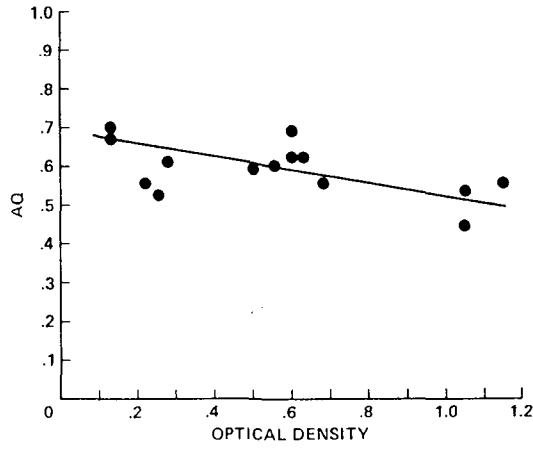
Fig. 8. AQ's of urea grown, steady-state cultures of _Chlorella_ _pyrenoidosa_ as a function of culture optical density.
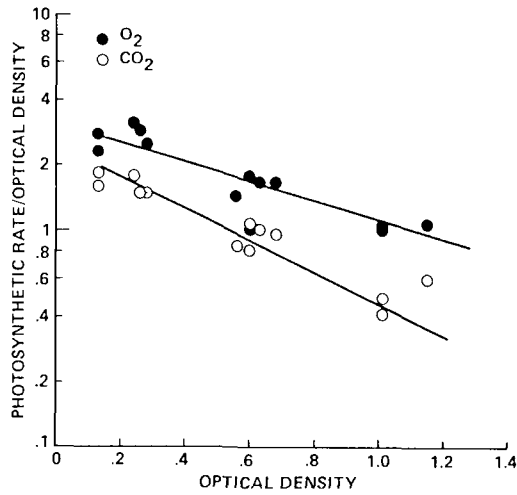
Fig. 9. AQ's of steady-state cultures of _Chlorella_ _pyrenoidosa_, normalized by the optical density, as a function of optical density.

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