19 Sept 57

TITLE:

The selection and cultivation of microorganisms capable of carrying out essential functions in closed ecological systems.

JUSTIFICATION:

For a number of reasons space medicine experts have concluded that man's ventures beyond the earth's atmosphere will require that he be contained in a sealed enclosure entirely isolated from his surrounding environment. This concept of the "sealed cabin" brings with it a number of problems. Most of these, when examined from a fundamental point of view, result from the fact that on earth man is a tiny link in a very complicated bio-ecological system. As a consequence, when he moves himself out of this system he removes himself from the environment which is capable of taking his waste products and recycling them to reproduce his food and respiratory requirements. Major parts of the systems which recycle carbon, nitrogen, and oxygen are composed of microbial forms. All the biological functions performed in support of human life can be accomplished by microorganisms.

Space medicine research has proceeded to the point where simulated flights in experimental sealed cabins are being planned and conducted. These experiments lack the true "sealed cabin" nature for the very important reason that food substances are added and excreta must be removed either during or immediately after the flight. Thus true "sealed cabin" flights are not possible until such time as the ecological system within the cabin is closed and balanced.

INFORMATION WILL BE USED BY:

Space medicine, microbiologists, flight surgeons, flight (space) engineers.

IMPACT:

1. Beneficial -

It will result in the production of an important contribution in support of the "sealed cabin" concept. It will provide interested agencies with information regarding the possibility of establishing closed ecological systems for utilization in manned space vehicles.

2. Detrimental -

If this task is not initiated an important area of investigation will be neglected which might add considerably to the earlier successful accomplishment of manned space flight.

ESTIMATED COST:

FY 1958 - $900.00
FY 1959 - $450.00

TITLE:

The selection and cultivation of microorganisms capable of carrying out essential functions in closed ecological systems.

STATEMENT OF PROBLEM:

For a number of reasons space medicine experts have concluded that man's ventures beyond the earth's atmosphere will require that he be contained in a sealed enclosure entirely isolated from his surrounding environment. This concept of the "sealed cabin" brings with it a number of problems. Most of these, when examined from a most fundamental point of view, result from the fact that on earth man is a tiny link in a very complicated bio-ecological system. As a consequence, when he moves himself out of this system he removes himself from the environment which is capable of taking his waste products and recycling them to reproduce his food and respiratory requirements. Major parts of the systems which recycle carbon, nitrogen and oxygen are composed of microbial forms. All the biological functions performed in support of human life can be accomplished by microorganisms.

HYPOTHESIS:

All the essential functions conducted by other species of animals and plants in support of human life can be found to be accomplished by some member of the microbial world. Unicellular algae are efficient photosynthesizers. Certain of the fungi are excellent food producers. In nature practically all of the final steps in the reduction or oxidation of organic matter to the inorganic or elemental state is accomplished by microorganisms. Fixation of elementary nitrogen is accomplished by free living or symbiotic microorganisms. Autotrophic microbes are known which are capable of synthesizing all essential amino acids and all known vitamins of the B complex. In short, it is conceivable that a closed ecological system can be established in which the components consist of one or a select few microbial forms. Furthermore, it is proposed that the microbiological portion(s) of this ecology can be established and operated on an automatic or semiautomatic basis. The question of whether such a system can be devised that will be efficient enough to be of practical size can not be predicted.

BACKGROUND INFORMATION:

Meyers (1) has investigated very extensively the use of a green algae as a carbon dioxide - oxygen exchange system. His conclusions are equivocal from an engineering standpoint, but on a purely biological basis the system is entirely feasible.

Studies on the use of microbial forms, particularly algae as food have been reported. Chlorella and Scenedesmus have been found to contain sufficient protein to maintain human life, and important quantities of some vitamins (2, 3, 4). Geohegen (4) has found that rats thrive on a

mixed diet with algae as the sole source of protein. Jorgenson improved greatly the severe cases of malnutrition in lepers in Venzuela, using algal soups.

The use of microorganisms as productive agents in sewage disposal processes is routine. However, from an engineering viewpoint these systems may be less efficient than desired. A better method of waste disposal may be developed based on the incineration process, using the resultant ash and gaseous products to support the growth of food producing photosynthetic autotrophic microorganisms. Davis (5) found urea to be an excellent source of nitrogen for Chlorella algae. Fifty percent of the solid constituents of urine is urea. Feces contains minerals, nitrogen, trace elements, and organic matter which can be used in replacing some of the trace element requirements of algae.

Of particular interest to this proposed study is that class of microorganisms known as the blue-green algae. There are members of this group which are efficient photosynthesizers and which are capable, at the same time, of incorporating into their protein constitution molecular nitrogen as well as nitrate, ammonium, and urea nitrogen. Some members of the group are capable of growing at a temperature of over 80°C, an environmental condition which, to say the least, is extremely selective.

Another class of microorganisms which may be of value in these investigations are the photosynthetic bacteria. There are nitrogen-fixing members in this group and they are as a class good consumers of CO_2. The bacterial photosynthetic process has the obvious disadvantage that it uses some substances other than H_2O (such as H_2S, organic acids, H_2, etc.) as a hydrogen donor with the net result that molecular oxygen is not produced directly in the process.

PRESENT STATUS:

Work has been done on this proposed task on a pilot study basis.

Three all glass culture chambers for cultivating algae have been developed. Each chamber represents an improved modification of the previous model. The original design is based on the chambers developed by Dr. Meyers at the University of Texas. The chambers in use at this laboratory differ from the Meyers chambers in two important respects. Then do not incorporate a water jacket for temperature control and they are illuminated solely from their interior by use of fluorescent lamps. The most recent of these chambers, is now undergoing test in this laboratory. This is the largest of the glass chambers, having a capacity of approximately 500 ml.

A large Plexiglas chamber having a capacity of approximately 10.5 liters and a light input of 75 watts was designed by Dr. Gaume and built in the research shops.

Preliminary results of experiments conducted with one strain of blue-green algae (Anacystis nidulans) indicate that this chamber will probably be capable of providing gas exchange facilities for two mice.

The project has received and placed in operation a Beckman model H-2 pH meter. The equipment is so arranged that continuous or intermittent pH readings can be made either from the plastic chamber or the latest model glass chamber. A Klett-Summerson colorimeter has been received but not placed in operation as yet. Turbidity readings, as a measure of growth have been made on the Klett-Summerson colorimeter presently available in the Department of Microbiology. A Sharpless continuous centrifuge and a vacuum furnace have been received and will be used in harvesting algae from the large tank.

After a few weeks of familiarization and modification, the large Plexiglas chamber was put into operation on 12 July. The apparatus was sterilized with ethelene oxide gas, innoculated with approximately 300 ml of a heavy suspension of Anacystis nidulans obtained from one of the glass chambers, and charged with fresh, sterile medium. Throughout the entire experiment, growth was measured and recorded as optical density. Periodically, when the optical density reached a value indicating that a growth limiting condition had been achieved, the algae were harvested and fresh medium added. Flow rate of gas was measured with a water manometer. CO_2 uptake and O_2 evolution were measured with gas analysis equipment of the Department of Space Medicine. Microscopic examination and periodic plating on nutrient agar indicated that the culture was contaminated with bacteria almost from the outset. It was apparent that the bacterial count remained relatively low during the earlier portions of the experiment, but in the latter phases, the bacterial count became excessively high and the experiment was stopped. It should be noted that even at the terminal stage of this run the algae demonstrated excellent morphological characteristics upon microscopic examination. Furthermore, as evidenced by continued oxygen evolution, photosynthesis was apparently not adversely affected by the presence of the bacteria.

The bacterial role in this mixed culture may be considered to be that of a saprobe rather than that of a parasite. Since the presence of bacterial contaminants in algae cultures will be of considerable practical concern, further studies are planned to elucidate the precise relationships that exist in this type of mixed culture.

The data from the latter part of this one experiment with the large chamber have been plotted in figure 1. This figure represents the type of data which can be obtained with the present facilities. If does not represent the optimum conditions which we expect to attain.

When fresh medium is added to the chamber the pH is high, approximately 8.0. As the gas bubbles through the chamber, CO_2 is rapidly absorbed as indicated by the CO_2 uptake curve until equilibrium is reached at a pH of 7.3 to 7.4. Since CO_2 uptake is a function not only of the

photosynthetic activity of the algae, but also of the pH of the medium, oxygen evolution is taken as a better measure of photosynthesis.

The rate of flow of the gas through the chamber affects the photosynthetic rate. This is seen by the observation that O_2 evolution was increased when the flow rate was increased between 120 and 130 hours. The lack of further increase in O_2 evolution following a further increase in flow rate between 160 and 170 hours may be due to the fact that the culture had achieved light saturation (maximum optical density) and to the presence of the bacterial contaminants.

PROPOSED EXPERIMENTATION:

The Plexiglas chamber used in these experiments was accidentally briken while being sterilized. A new chamber of approximately the same capacity is under construction.

One more experiment will be run to determine optimum flow rate following which the chamber will be placed in steady state operation at an optical density of 0.8 to 0.9. This can be done by daily harvest and the addition of fresh medium.

When a steady state condition is achieved, the chamber will be put into a closed system with one or two mice, depending on the ultimate O_2 production rate achieved.

At the same time the algal harvest will be fed to mice to determine whether it will support their existence alone or whether it can be used as a food supplement.

One of the glass chambers will be placed in operation at reduced atmospheric pressure and high oxygen tension to determine the effect of these variables on the growth rate and photosynthetic ability of the algae.

As many strains of algae as can be obtained will be examined for their food value and possible toxicity.

Attempt will be made to grow algae on medium derived directly or indirectly from human excreta. In this connection, mixed cultures of bacteria and algae will be studied since the bacteria may contribute to the waste, disposal function of the system without excessive O_2 uptake.

Other microbial types (fungi, bacteria) will be investigated for their waste disposal and food producing abilities.

All information will be combined in the design of a closed ecological system in which one or several small mammals are totally supported by one or a series of microbial cultures.

MANAGEMENT:

1. Supplies

a. Routine and special chemicals	$ 50.00
b. Expendable supplies and standard glassware	200.00

2. Equipment *

a. Special continuous culture equipment	100.00
b. Gas handling and analysis equipment	500.00
c. Pumps, miscellaneous	500.00
TOTAL COST	$1350.00

3. Personnel

a. Principal investigator - 1500 man hours

b. Technician - 2000 man hours

4. Time

Estimated completion date - 24 months after initiation.

PROPOSED STUDY SEQUENCE:

The logical extension of this study is the establishment of a completely closed ecological system with a human component capable of indefinite continuity.

* Some major equipment items have been funded from FY 1957 funds of the Department of Space Medicine and surplus general funds.

REFERENCES

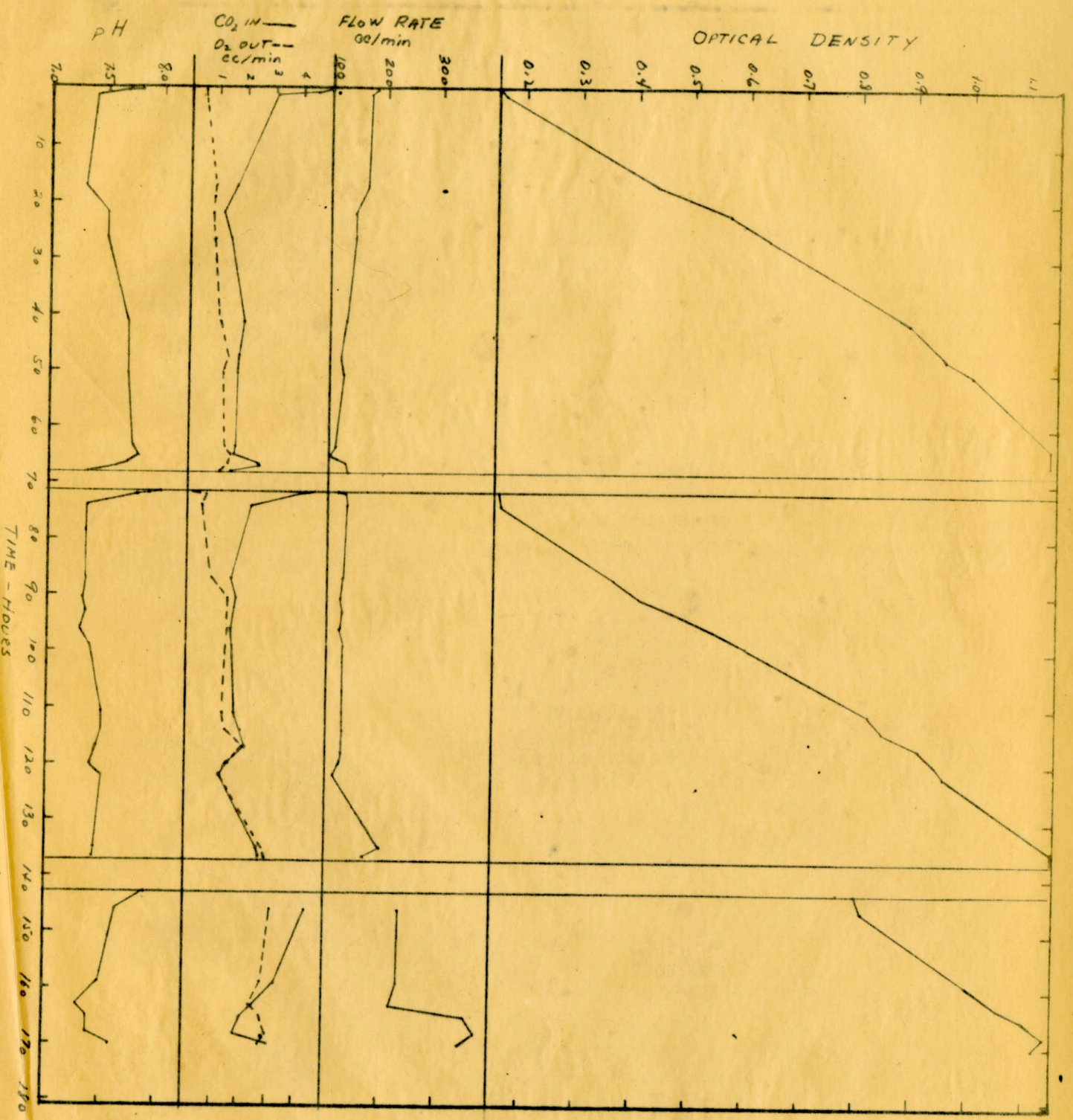
1. Myers, J. Basic remarks on the use of plants as biological gas exchangers in a closed system. J. Avn. Med. 25:407 (1954).

2. Schieler, McClure, and Dunn (U.C.L.A.). Amino acid composition of chlorella. Food Research 18:377, (1953).

3. Thomas, Wm. H., and R.W. Kraus. Nitrogen metabolism in scenedesmus as affected by environmental changes. Plant Physiol. 30:2 pp. 115-122, March 1955.

4. Gaffron, H. Food from algae. Research 6:222-230, June 1953.

5. Carnegie Report on algae cultures. Carnegie Inst. of Wash. Yearbook No. 51, pp. 99, 132, 1952.


pH
CO_2 IN ——
O_2 OUT - -
cc/min
FLOW RATE
cc/min
OPTICAL DENSITY
TIME - HOURS